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Chromatin Immunoprecipitation Cloning Identifies KLF11 as a Novel Progesterone Receptor Target Gene That Regulates Proliferation in Uterine Leiomyoma. Ping Yin, Zhihong Lin, Scott Reierstad, Youhong Cheng, Erica E Marsh, Hiroshi Ishikawa, Joy Innes, Julie Kim, Serdar E Bulun. *Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL, USA.*

Objective: Uterine leiomyomas are the most common tumors of the female genital tract and the leading cause of hysterectomy. While antiprogestins (e.g., RU486) reduces the size of leiomyomas and progesterone stimulates the proliferation of uterine leiomyoma cells in vivo and in vitro, the mechanism of progesterone action is not well understood. The goal of this study was to identify direct target genes of progesterone receptor (PR), which mediates the activity of progesterone and RU486, in uterine leiomyoma.

Methods: Leiomyoma tissue was obtained from 20 premenopausal women not on hormonal treatment (age range 32-47) and undergoing hysterectomy. Primary smooth muscle cells (SMCs) were isolated and cultured. After treatment with progesterone, these cells were subjected to an unbiased procedure, namely chromatin immunoprecipitation (ChIP)-cloning using an anti-PR antibody. All immunoprecipitated DNA fragments were cloned, sequenced and mapped to the human genome to identify PR-binding sequences genome-wide in primary leiomyoma cells.

Results: We identified a total of 18 novel PR binding sites that could be regulatory regions for nearby genes. One of the PR-regulatory modules identified is located 20.5 kb upstream of the transcriptional start site of KLF11. ChIP assays confirmed that PR is recruited both to the basal promoter region immediately upstream of the KLF11 transcription start site and to this distal module in a progesterone-dependent manner. Recruitment of the transcription factors Sp1 and RNA polymerase II was demonstrated at the distal module and the basal promoter of KLF11 by ChIP. The mRNA level of KLF11 was downregulated by progesterone and significantly upregulated by RU486. SiRNA knockdown of PR also dramatically increased KLF11 expression. *In vivo*, KLF11 expression was significantly reduced in leiomyoma tissues compared with adjacent myometrial tissues. SiRNA knockdown of KLF11 markedly increased leiomyoma cell proliferation and abolished the inhibitory effect of RU486 on leiomyoma cell proliferation.

Conclusions: We uncovered KLF11 as a novel gene targeted by PR, progesterone and RU486 in leiomyoma SMCs. KLF11 mediated the therapeutic (anti-proliferative) effects of RU486 in leiomyoma cells.

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2

Essential Nutrient Supplementation Preventing Adult Metabolic Disease & Metabolomic Profiling. James Cox,[3] Robert McKnight,[2] Michael Varner,[4] Robert Lane,[2] Kjersti Aagaard-Tillery.[1,2,3] *[1]MFM, Baylor College of Medicine, Houston, TX, USA; [2]Pediatrics, University of Utah, Salt Lake City, UT, USA; [3]Metabolomics Core Facility, University of Utah, Salt Lake City, UT, USA; [4]MFM, University of Utah, Salt Lake City, UT, USA.*

Objective: Chromatography-mass spectrometry (C-MS) profiling provides "metabolic spectral footprints" . We have shown that a constrained *in utero* environment alters fetal one-carbon metabolism, and supplementation (ENS) along this pathway prevents (i) adult metabolic disease, and (ii) lifelong abrogated expression of epigenetically-modified fetal genes. We thus sought to characterize the spectral footprint, and determine if metabolomic profiling in newborn pups can identify biomarker(s) associated with the development of adult disease.

Methods: Sprague-Dawley P1 dams underwent bilateral uterine artery ligation (e19) or sham surgery; resultant F1 yielded IUGR or sham lineages which were allocated to ENS or control diet. Comprehensive spectral footprints among resultant F2 offspring were generated using LC-MS (lipid quantification) and GC-MS (polar metabolites) at d21,160&400 (n=48). Data were visualized in reduced planar space via principle component analysis and projection onto latent structures-discriminate analysis (PLS-DA). D21 metabolites contributing to the PLS-DA model were further analyzed as potential biomarkers.

Results: Adult F2 phenotypic differences (*eg,* obesity, insulin resistance, dyslipidemia) are accompanied by significant altered spectral footprints of over 23 known metabolites (Fig. 1); ENS abrogates this effect. Moreover, an altered profile with a non-identified metabolite of characteristic retention time and mass (RT_m/z 16.24) was uniquely absent in those IUGR F2 pups at risk for obesity and insulin resistance later in adult life (bottom panel Fig. 2).

Conclusions: Essential nutrient supplementation with unaltered caloric content prevents adult obesity and insulin resistance and is accompanied by significant alterations in the F2 spectral metabolic footprint. Moreover, potential metabolite biomarkers may predict the development of the aberrant adult phenotype.

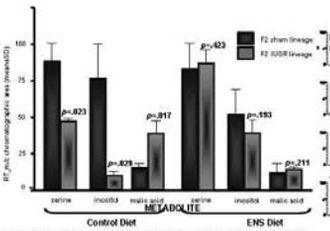

Figure 1. Example summary spectral data for serine, inositol and malic acid.

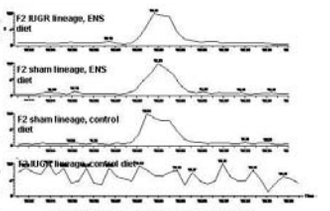

Figure 2. Spectral footprint for RT_m/z 16.24.

3

Relationship between Endoglin, Placental Growth Factor, Soluble VEGF Receptor-1 (sFlt-1) and Oxygen Concentration in the Placenta and Coelomic Fluid in Early Pregnancy. Shanthi Muttukrishna, Sangeeta Suri, Eric Jauniaux. *UCL Institute for Women's Health, University College London, London, United Kingdom.*

Objective: In early pregnancy, the placenta develops under low oxygen concentration and produces various proteins for effective implantation. Recent studies suggest that pro and anti angiogenic proteins are altered in pregnancy complications such as pre eclampsia. Therefore, the objective of this study is to measure the oxygen tension in the placenta, coelomic cavity and relate it to levels of PlGF, VEGF, Endoglin and sFlt-1 in early pregnancy.

Method: In a prospective study, pregnant women undergoing surgical termination of pregnancy for psychosocial reasons were recruited at 6-12 weeks gestation. Placental bed blood (PB) and coelomic fluid (CF) samples were obtained by transvaginal needle aspiration under ultrasound guidance. The oxygen concentration was measured immediately using an IRMA blood gas monitor. Placental extracts, placental bed serum, maternal serum (MS) and CF samples were analysed for Endoglin, PlGF, sFlt-1 and VEGF using ELISAs. This study was approved by the UCLH ethics committee.

Results: Data were analysed in three separate groups according to gestation; 6-8 weeks (n=8), 8-10 weeks (n=14), 10-12 (n=16) weeks. ANOVA showed a significant increase in placental bed oxygen concentration ($P<0.001$) and a significant decrease in CF oxygen tension ($P=0.01$). PLGF levels increased by almost 3 fold ($P=0.001$) in MS. Levels of sFLT-1 significantly decreased by 75% ($P=0.01$) with increasing gestation in PB. There is a negative correlation between sFLT-1 and oxygen concentration in the PB ($P=0.03$) and a positive correlation ($P<0.01$) between PlGF, Endoglin and VEGF levels in the placental extracts. In the PB, sFLt-1, PlGF and Endoglin were positively correlated ($P<0.05$). In the AF sFlt-1 and Endoglin was positively correlated ($P<0.001$).

Conclusion: The increase in PlGF and decrease in sFlt-1 with gestation and the negative correlation between sFlt-1 levels and oxygen concentration in the PB between 6-12 weeks suggests that sFLT levels may be controlled by oxygen concentration in vivo. The significant association between PlGF, Endoglin and VEGF in the placenta suggests these pro and anti angiogenic factors are tightly controlled for the development of placental vasculature in early pregnancy.

4

A Murine Genetic Model of Metastatic Granulosa Cell Neoplasms. Xiaohui Li, Stephanie A Pangas. *Pathology, Baylor College of Medicine, Houston, TX, USA.*

Objective: Granulosa cell tumors are metastatic ovarian neoplasms with no known germline mutations. We have recently shown that female mice genetically engineered to specifically delete the transcription factors Smad1 and Smad5 from ovarian somatic cells develop granulosa cell tumors with 100% penetrance. Furthermore, the majority of Smad1/5 double conditional knockout (cKO) mice bear peritoneal metastases of increasing frequency with age. The objectives of this study were to analyze the hormonal-dependent profile and gene expression changes that occur during primary and metastatic tumor development in Smad1/5 cKO mice, and to determine the similarity of these changes to that known for human granulosa cell neoplasms.

Methods: Granulosa cell-specific deletion of Smad1/5 were generated by crossing mice with floxed alleles of Smad1/5 to those expressing Cre-recombinase from the Amhr2 locus. Smad1/5 dKO tumors and visible peritoneal metastases were analyzed using Affymetrix Mouse Genome 430 2.0 oligonucleotide arrays. Serum was collected from control and cKO adult females and assayed for FSH, estradiol, LH, inhibin A and B. Quantitative PCR was performed for candidate genes expressed in mouse cKO tumors based on (1) known expression in human granulosa cell tumors, and (2) tumor-specific microarray analyses.

Results: Serum levels of follicle stimulating hormone (FSH) were significantly decreased in Smad1/5 cKO mice ($p<.05$), while LH and estradiol were unchanged from controls. Known markers of metastasis (inhibin A and B) were significantly upregulated in Smad1/5 cKO females with metastatic tumor development ($p<.01$), consistent with observed decreases in circulating FSH. When Smad1/5 dKO tumors were compared to wild type preovulatory granulosa cells by quantitative PCR, significant ($p<.05$) overexpression of FSH receptor (Fshr) and cyclin D2 (Ccnd2) and underexpression of luteinizing hormone receptor (Lhcgr) were observed.
Conclusions: Our data are of dual potential importance. First, they comprise a novel murine genetic-based model of granulosa cell cancer and metastasis. Second, the observed relationship between circulating hormonal ligand and receptor changes and metastatic disease mimic the human condition and hence provide a framework for further studies focusing on potential therapeutic and diagnostic targets.

5

Neurogenic Differentiation of Human Endometrial Stem Cells and Murine CNS Transplantation. Erin F Wolff, Zane B Andrews, Xiao-Bing Gao, Katherine V Yao, Hugh S Taylor. *Obstetrics, Gynecology and Repoductive Sciences, Yale University, New Haven, CT, USA.*
Objectives: Previously we have demonstrated multipotentcy of stem cells derived from human endometrium. Here we induced neurogenic differentiation of human endometrial stem cells and demonstrate the potential for transplantation using a mouse model.
Methods: Human endometrial stromal cells (ESC) were characterized using flow cytometry. Neurogenic differentiation into a dopaminergic pathway was then induced using a two step method: cells were first cultured in differentiation medium I for 48 hours then differentiation medium II up to 96 hours. Whole cell patch clamp recording was performed for evidence of G-protein coupled inwardly rectifying potassium (GIRK) channels characteristic of central neurons. Nestin expression was identified using anti-human nestin followed by FITC-labeled IgG. A Parkinson's disease model was induced in six B6 mice by treatment with methyl-phenyl-tetrahydropyridine (MPTP) while 2 were treated with vehicle control. Human ESC were transplanted into the striatum of 4 MPTP treated mice. Two mice treated with MPTP and 2 saline treated animals were injected with PBS as controls. After 5 weeks, striatal dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) concentrations were measured. Human nestin antibody was used to identify human cell engraftment.
Results: Human ESC displayed the following distribution: 0.3% CD45+, 1.4% CD30+, 99.6% CD90+, 99.7% PDGFRß+, and 99.7% CD146+. Cells cultured in the dopaminergic conditions displayed neurogenic morphology with axon like projections and pyramidal cell bodies. Voltage clamping revealed the presence of barium sensitive potassium channels, which were lacking in undifferentiated cells. ESC induced to a dopaminergic pathway expressed nestin, a neural stem cell marker. Striatal DA concentrations in unlesioned, ESC transplanted, and PBS injected mice were 100, 51, and 44 ng/mg respectively. DOPAC concentrations were 5.0, 3.4, and 2.6 ng/mg respectively. DOPAC/DA ratios were 0.70, 0.59, and 0.50 respectively.
Conclusions: Human ESC were differentiated into dopaminergic cells. These cells display neural markers and voltage characteristics consistent with dopaminergic neurons. Human ESC can be transplanted into the striatal region of mice, where they increase dopamine concentrations. Endometrial stem cells show therapeutic potential for use in Parkinson's disease.

6

Long-Term Increases in Vasorelaxation and Blood Flow to the Pregnant Sheep Uterus Following Local Delivery of Adenovirus VEGF to Uterine Arteries. Anna L David,[1] Belen Torondel,[2] Victoria Wigley,[1] Khalil Abi-Nader,[1] Ian Zachary,[2] John Martin,[2] Michael Boyd,[3] Terry Cook,[4] Charles Rodeck,[1] Donald Peebles.[1] *[1]Institute for Women's Health, University College London, London, United Kingdom; [2]Department of Cardiovascular Medicine, University College London, London, United Kingdom; [3]Biological Sciences Unit, Royal Veterinary College, London, United Kingdom; [4]Department of Pathology, Imperial College, London, United Kingdom.*
Introduction: The long-term aim of this study is to treat fetal growth restriction by increasing utero-placental perfusion. Previous data show that uterine blood flow (UBF) was increased 4-5 days following adenovirus vector mediated over-expression of VEGF in the uterine artery. The aims of the current study were to 1) determine whether increased vascular relaxation and UBF could be sustained for more than 4 weeks and 2) investigate the site and amount of VEGF production.
Methods: Ultrasonic flow probes (Transonic Inc, USA) were implanted around both uterine arteries at laparotomy in mid-gestation pregnant sheep (n = 6, 90 days of gestation, term = 145 days) and UABF measured via telemetry. Baseline UABF was obtained over 5 days, prior to injection of adenovirus vector (5 x 10^{10} particles) containing the VEGF (Ad.VEGF-A) gene into one uterine artery and β-galactosidase (Ad.lacZ) into the other. UABF was monitored until term. At post-mortem sections were taken from the uterine arteries at 4 levels and studied in an organ bath. Short term VEGF expression was assessed using VEGF ELISA and immunohistochemistry in the uterine artery 5 days after vector injection in a further 6 sheep.
Results: By term Ad.VEGF-A transduced vessels were significantly less responsive to phenylephrine (E_{max} 135 SE:12.1 vs E_{max} 156.1 SE:23.3, $p< 0.05$) than Ad.lac injected vessels. There was no significant difference in the relaxation response to bradykinin. 30 days post injection UABF increased by 33.3% from baseline on the VEGF-A injected side compared with 16.9% on the LacZ injected side. VEGF expression was quantifiable using ELISA and was observed in the perivascular adventitia of uterine arteries by immunohistochemistry.
Conclusions: Adenovirus mediated over-expression of VEGF results in increased uterine artery blood flow and relaxation of the uterine arteries long term. These results suggest it may be possible to develop a therapeutic intervention to increase placental perfusion by increasing VEGF expression.

7

Vascular Endothelial Growth Factor Polymorphisms Are Associated with Congenital Heart Defects. Huberdina PM Smedts,[1] Aaron Isaacs,[2] Andre G Uitterlinden,[3] Cornelia M van Duijn,[2] Dominique de Costa,[1] Wim A Helbing,[4] Eric AP Steegers,[1] Regine PM Steegers-Theunissen.[1,2,4,5] *[1]Obstetrics and Gynecology/Div of Obstetrics and Prenatal Medicine, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Epidemiology, Erasmus MC; [3]Internal Medicine, Erasmus MC; [4]Pediatric Cardiology, Erasmus MC - Sophia Children's Hospital; [5]Clinical Genetics, Erasmus MC.*
BACKGROUND: The tight regulation of vascular endothelial growth factor expression (VEGF) is essential for endocardial cushion formation. Disturbances in VEGF synthesis can result in congenital heart defects (CHDs), particularly outflow tract defects (OTDs). Three functional single nucleotide polymorphisms (SNPs) in the VEGF gene, -2578 C/A and -1154 G/A in the promoter region, and -634 G/C in the 5'UTR region are associated with increased VEGF expression for the -2578C and -1154G allele respectively.
OBJECTIVE: To investigate whether 3 functional SNPs in the VEGF gene are associated with congenital heart defects.
METHODS: A case-control study was conducted in an ethnically homogenous population. At around 16 months post partum, blood samples were collected from 294 case triads, including 194 OTDs, and 317 control triads. Genotyping was performed by TaqMan discrimination assay. Genotype and haplotype frequencies were compared between case- and control triads by χ^2 test. The family based association test (FBAT-o) was used to establish linkage and association for the VEGF alleles and haplotypes.
RESULTS: Allele frequencies of the three VEGF polymorphisms were similar in case and control triads. The genotypes were in Hardy-Weinberg equilibrium. VEGF alleles -2578C and -1154G were transmitted more frequently to CHD cases, $P = 0.02$ and $P = 0.04$, and in particular to OTDs, $P = 0.003$ and $P = 0.002$. The VEGF -2578A/-1154A/-634G haplotype was significantly less transmitted to OTDs ($P = 0.002$). There was no evidence for a parent of origin effect.
CONCLUSIONS: The VEGF -2578C and -1154G alleles were overtransmitted to CHD cases, particularly those with OTDs. Replication of these findings in other study populations is needed.

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Identification and Hormonal Regulation of a Novel Form of NKp30 in Human Endometrial Epithelium. Anna P Ponnampalam, Caroline E Gargett, Peter AW Rogers. *Centre for Women's Health Research, Monash Institute of Medical Research and Department of Obstetrics & Gynaecology, Monash University, Melbourne, Victoria, Australia.*
Introduction: NKp30 is a member of the immunoglobulin super family and one of three existing natural cytotoxicity triggering receptors. NKp30 is a glycosylated protein and is thought to be selectively expressed in resting and activated natural killer cells. We have found that NKp30 mRNA levels in the endometrium change significantly during the menstral cycle.[1] The aims of the present study were to fully characterize the expression and localization of NKp30 mRNA and protein in human endometrium, and to investigate the hormonal regulation of NKp30.
Methods: NKp30 mRNA expression was quantified by real-time PCR, and

protein expression by immunohistochemistry and western blotting. Clonally derived endometrial epithelial cells (CEEs) employed in this study were phenotyped by flow cytometry. Hormonal regulation of NKp30 transcription and translation was investigated using an endometrial explant system.

Results: NKp30 mRNA was significantly up-regulated in fresh tissues during the late secretory phase of the menstrual cycle. Interestingly NKp30 mRNA was also present in CEEs in comparable amounts to those found in fresh tissue. NKp30 protein was predominantly found in the endometrial glands and luminal epithelia of the secretory phase endometrium. Western blotting and de-glycosylation studies showed that a novel glycosylated form of NKp30 is present in endometrial epithelium and CEEs, and that it can dimerize. The epithelial origin of the CEEs used was confirmed by the presence of epithelial cell markers CK8 and CD49f, and by the absence of pan-leukocyte marker CD45 and natural killer cell marker CD56, as determined by flow cytometry. Dual parameter flow cytometry also showed that the majority of the cells co-expressed CD49f and NKp30. Transcription and translation of the novel form of NKp30 was induced in endometrial explants *in vitro* by 48 hours treatment with progesterone.

Conclusion: This is the first report to show the presence of both NKp30 mRNA and a novel glycosylated form of NKp30 protein in endometrial epithelial cells. Although further studies are needed to elucidate the function of NKp30 in the endometrium, the known characteristics of the protein suggest that it may be involved in endometrial immune tolerance mechanisms during implantation of the embryo.

[1] Ponnampalam et. Al. (2004), *Mol. Hum. Reprod.* **10**: p879-893.

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A Novel Extranuclear Mechanism of Estrogen Action in the Human Pregnancy Myometrium. Toni Welsh,[1] Aisling Murphy,[2] Amy Merlino,[1] LiJuan Yi,[1] HuiQing Tan,[1] Sam Mesiano.[1,2] *[1]Reproductive Biology, Case Western Reserve University, Cleveland, OH, USA; [2]Obstetrics and Gynecology, University Hospitals Case Medical Center, Cleveland, OH, USA.*

Objective: Our objective was to determine how estradiol affects myometrial function in human pregnancy and how its actions are controlled during parturition. We hypothesized that parturition-related changes in myometrial estrogen responsiveness are mediated by changes in estrogen receptor-α (ERα) expression and that ERα functions in the classic transcriptional pathway and also in an extranuclear mode via the direct stimulation of the ERK MAPK signaling pathway.

Methods: Myometrial biopsies from preterm and term c-sections were obtained before and after the onset of labor. Immunoblotting was used to identify the ERα isoforms expressed. ERα isoform transcriptional activity (in response to 100 nM estradiol for 16h) was assessed in the hTERT-HM myometrial cell line using transient transfection of specific ERα splice variants and an estrogen responsive luciferase reporter plasmid. Immunoblotting was used to determine whether estradiol affects ERK1/2 phosphorylation (and therefore activity) in hTERT-HM cells.

Results: The principal ERα detected in the human pregnancy myometrium was a 46kDa variant (ERα46); the full-length 66kDa ERα (ERα66) was only barely detectable in 4 out of 20 biopsies. ERα46 levels were not affected by advancing gestation but increased 1.8-fold in association with labor onset at term ($P<0.05$). As expected, estradiol increased ERE-luciferase activity in ERα66-transfected hTERT-HM cells ($P<0.03$). ERα46 failed to induce transcription at the ERE-luciferase reporter in response to estradiol in hTERT-HM cells, but instead repressed the transcriptional activity of ERα66. Estradiol stimulated ERK1/2 phosphorylation in hTERT-HM cells within 60 minutes ($P<0.05$) and this effect was inhibited by tamoxifen.

Conclusions: Our data suggest that estrogenic actions on the human pregnancy myometrium are mediated by a truncated ERα splice variant and that this receptor does not act as a classic ligand activated transcription factor but instead acts via an extranuclear pathway by directly activating the ERK MAPK signaling cascade. These novel data suggest that the estrogenic drive on the human pregnancy myometrium is mediated by extranuclear pathways via a truncated ERα splice variant. Support: March of Dimes Birth Defects Foundation and NICHD Obstetric-Fetal Pharmacology Research Network.

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The Effect of IL-1β on PR-B Expression and Function in Human Myocytes. Mark Tattersall,[1] Suren Sooranna,[1] Amanda Tattersall,[1] Peta Grigsby,[2] Leslie Myatt,[2] Mark Johnson.[1] *[1]Academic Obstetrics and Gynaecology, Imperial College, London, United Kingdom; [2]Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH, USA.*

Introduction: Circulating progesterone levels remain high with the onset of labour in humans, unlike most non-primates. It has been suggested there is a functional progesterone withdrawal, possibly mediated by changes in progesterone receptor (PR) isoform expression or phosphorylation status, that leads to a decrease in progesterone sensitivity of the myometrium. PR has two main isoforms - PR-B (main activator of progesterone-responsive genes) and PR-A (modulator or repressor of PR-B). Regression analysis of myometrial PR mRNA expression and gestational age showed a significant decline in PR-B mRNA levels. In addition, western blot analysis indicated PR-B levels are decreased in term early labour compared to non-labour samples. We have previously reported that interleukin-1β (IL-1β) levels increase significantly in term labour. The objective of this study was to determine if IL-1β alters PR isoform expression and function.

Methods: Samples of lower segment myometrium were taken from pregnant women undergoing elective LSCS at term. Myocytes were isolated using collagenase digestion and cells grown to confluency. Protein and mRNA analysis was performed on cells that were serum starved for 24h before exposure to 1ng/ml IL-1β for 24 or 6h, respectively. Luciferase assays were perfomed on cells transfected with the progesterone response element (PRE),TK-Renilla ± PR-B, serum starved and then incubated with 1μM MPA ± 1ng/ml IL-1β for 24h.

Results: IL-1β significantly decreased PR-B mRNA levels and inhibited the progesterone-induced increased in PRE activity in myocytes co-transfected with PR-B and PRE. Western blot analysis showed increased phosphorylation of PR-B at Ser-294 in response to IL-1β treatment. Inhibition of JNK activity by SP600125 (20μM) attenuated this response and the ERK inhibitor U0126 (10μM) also partially decreased the effect of IL-1β.

Conclusion: IL-1β significantly decreases PR-B mRNA expression and PR-B-mediated PRE activity. IL-1β increases the level of PR-B phosphorylation at Ser-294 which is reported to be involved in ligand-dependent down-regulation of PR-B by signaling it for degradation by the 26S proteosome. Thus IL-1β may reduce myometrial progesterone sensitivity prior to the onset of labour through a variety of mechanisms.

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The Role of the mTOR Signaling Pathway in Myometrial Differentiation. Shabana N Jaffer,[1,3] Oksana Shynlova,[1] Stephen J Lye.[1,2,3] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [2]Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON, Canada; [3]Physiology, University of Toronto, Toronto, ON, Canada.*

INTRODUCTION: The adaptive growth of the uterus during gestation is a critical event that involves gradual changes in cellular phenotypes from early pregnancy until the onset of labour, including an early proliferative phase, an intermediate synthetic phase of cellular hypertrophy, and a third phase in which the cells assume a contractile/labour phenotype. The mTOR (mammalian Target Of Rapamycin) signaling pathway regulates cell growth by linking nutrient sensing to cellular growth. In this study, the role of the mTOR pathway in the induction of myometrial cell proliferation was investigated.

OBJECTIVES: (1) Analyze the protein expression and phosphorylation levels of mTOR (P-mTOR) and its downstream effectors (P70S6 kinase & 4E-BP1) throughout normal pregnancy in rats; **(2)** Use an ovariectomized rat model to analyze the modulation of the mTOR pathway by estrogen; **(3)** Analyze the effects of both estrogen and IGF-1 growth factor on mTOR signaling using primary cultures of rat myometrial smooth muscle cells (SMC).

METHODS: The expression of total and Phospho (P)-mTOR, P-P70S6Kinase, and P-4E-BP1, was assessed by western blot analysis in myometrial tissue from non-pregnant (NP), pregnant and ovariectomized (OVX) rats. OVX animals were treated with estrogen for 6, 24 & 48 hours. To obtain primary myometrial SMC, uterine tissue was enzymatically digested. After 4 days in culture, primary myocytes were treated with different concentrations of IGF-1 (1, 10, & 100 ng/ml) and mTOR pathway activation was assessed by immunoblotting.

RESULTS: Results demonstrate that P-mTOR, P-p70S6kinase and P-4E-BP1 proteins are expressed in the rat myometrium throughout gestation with significant upregulation during the proliferative phase of pregnancy. Estrogen treatment of OVX rats for 6 hours caused a transient increase in IGF-1 mRNA expression followed by an up-regulation of P-mTOR protein expression.

Treatment of the myometrial SMC with IGF-1 stimulated the phosphorylation of mTOR. These data support a link between estrogen, IGF-1, and mTOR in the regulation of myocyte proliferation during pregnancy.
(This study was funded by the Canadian Institute for Health Research: 37775).

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Cervical Ripening: Release of the Brake? Doan Y Dao, Xiang-Hong Li, Ruth A Word. *Obstetrics and Gynecology, UT Southwestern Medical Center, Dallas, TX, USA.*
Objective: Microphthalmia-associated transcription factor (MiTF) belongs to a highly specialized class of transcription factors that dictates cell-specific differentiation. Recently, we cloned a unique isoform of MiTF (MiTF-CX) that is greatly expressed in human cervical stromal cells during pregnancy but is down-regulated 12-fold in the ripened cervix. Since the final pathway of cervical ripening is characterized by dissolution of cervical extracellular matrix (ECM) and signaling through the pro-inflammatory cytokine IL-8, we tested the hypothesis that IL-8 gene expression is regulated by MiTF-CX and determined the molecular mechanism of this regulation. **Methods and Results**: Transfection of human cervical stromal cells with MiTF-CX resulted in significant suppression of IL-8 mRNA and protein (from 770 ± 20 to 415 ± 25 pg/ml, $P \leq 0.01$). Moreover, mutations in MiTF-CX that impair its DNA-binding (mutMiTF-CX) resulted in dramatic increases in IL-8 production (from 770 ± 20 to 3285 ± 145 pg/ml, $P \leq 0.001$). Gel shift assays were conducted with oligonucleotides corresponding to 10 Eboxes in the human IL-8 promoter. One strong binding Ebox ($^{-397}$CACATG^{-391}) was identified. Specific binding was established using oligonucleotides with or without mutations in this Ebox, supershift with MiTF antibodies, competition assays, and absence of binding to mutMiTF-CX. Further, specific binding was confirmed in nuclear extracts from cells that overexpressed MiTF-CX, but not control or mutMiTF-CX. The physiologic relevance of MiTF binding to this Ebox was demonstrated in vivo. Specifically, reporter gene studies using the full length IL-8 promoter revealed that MiTF-CX decreased IL-8 promoter activity significantly (from 4.3 ± 0.12 to 1.7 ± 0.15 U/bgal, $P \leq 0.05$). In contrast, repression of IL-8 promoter activity by MiTF-CX was abolished by mutations in Ebox^{-397}(CACATG to CACACA) (from 3.97 ± 0.06 to 3.96 ± 0.07 U/bgal) **Conclusions**: In cervical stromal cells, MiTF-CX returns IL-8 production to baseline levels. MiTF binding to Ebox^{-397} in the IL-8 promoter is crucial for MiTF-CX suppression of IL-8 gene expression. We suggest that maintenance of cervical competency during pregnancy is an active process maintained through suppression of IL-8 by the transcription factor MiTF-CX. During cervical ripening, loss of MiTF-CX would result in significant up-regulation of IL-8 mRNA and protein synthesis and thereby recruitment and activation of leukocytes within the cervix and dissolution of the ECM.

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G Protein-Coupled Receptor Kinase (GRK) 6 Selectively Regulates Oxytocin Receptor Responsiveness in ULTR Human Myometrial Smooth Muscle Cells. Jonathon M Willets,[1,2] Hayley Shaw,[2] RA John Challiss,[2] Justin C Konje.[1] *[1]Reproductive Sciences Group, Cancer Studies & Molecular Medicine, University of Leicester, Leicester, United Kingdom; [2]Cell Physiology & Pharmacology, University of Leicester, United Kingdom.*
Background: Oxytocin receptors (OTR) play an important role in parturition stimulating myometrial contractions via production of inositol 1,4,5-trisphophate (IP_3) and increasing intracellular Ca^{2+}. Continuous agonist exposure leads to rapid OTR desensitization however the molecular mechanisms responsible are not fully understood, but likely involve GRK-mediated phosphorylation of agonist occupied receptors.
Objective: To assess OTR responsiveness and the role that GRKs play in OTR desensitization in ULTR cells.
Methods: We used confocal imaging of the fluorescent biosensor eGFP-$PH_{PLC\delta}$ to assess the regulation OTR phospholipase C-stimulated signaling in ULTR transfected cells. To deplete GRK expression ULTR cells were transfected with siRNAs against GRK2 (5'-GGCAGCCAGUGACCAAAAAtt-3'), GRK6 (5'GGACACAAAAGGAAUCAAGtt-3'), or a negative control siRNA using Interferin transfection reagent. GRK expression was determined 48 h later by Western blotting.
Results: Short applications (30sec, termed R1) of oxytocin (100nM) produced rapid transient translocation of eGFP-$PH_{PLC\delta}$ from plasma membrane to cytoplasm, indicative of IP_3 production. A second oxytocin challenge (100nM, 30sec termed R2) 5min after initial exposure resulted in reduced translocation of the IP_3 biosensor (73±3.8%, n=12 cells ≥6 separate experiments; data are expressed as means ± SEM for the % change in R2 relative to R1), indicative of receptor desensitization. Extending the wash period between oxytocin challenges indicated OTR re-sensitization was complete after 15min. Transfection with anti-GRK2 or anti-GRK6 siRNA resulted in depletion of endogenous GRK2 or GRK6 expression (80±2%, n=4 and 83±5% n=4, respectively $p<0.01$ one-way ANOVA), whilst negative control siRNA had no affect. Co-transfection of ULTR cells with eGFP-$PH_{PLC\delta}$ and anti-GRK6 siRNA inhibited OTR desensitization by 50±9% (means ± SEM, n=17 cells from 8 separate experiments, $p<0.05$ one-way ANOVA). Anti-GRK2 and negative control siRNAs were ineffective.
Conclusions: Our data suggest that endogenous GRK6 plays a leading role in the regulation of OTR signaling in ULTR human myometrial cells. Moreover, identifying OTR desensitization mechanisms may lead to novel strategies to prevent inappropriate myometrial contraction.

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Prostacyclin Receptor Signaling through cAMP/PKA and The Transcription Factors GATA-6 and Myocardin Upregulates Contraction-Associated Proteins and Contractile Proteins in Pregnant Human Myometrium. Kristina M Fetalvero,[1,2] Roger C Young,[3] Kathleen A Martin.[1,2] *[1]Surgery, Dartmouth-Hitchcock Medical Center, Hanover, NH, USA; [2]Pharmacology, Dartmouth-Hitchcock Medical Center, Hanover, NH, USA; [3]Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA.*
Objective: To determine the effects and mechanisms of action of prostacyclin receptor stimulation in pregnant human myometrium.
Methods: Following informed consent, myometrial tissue was obtained from the lower uterine segment of term pregnant women at the time of cesarean delivery. Myometrial strips were organ cultured, or cells isolated by enzyme digestion were monolayer cultured, in DMEM with 10% FBS. siRNA was transfected in both culture systems using a Nucleofector (Amaxa). Contraction-associated protein (CAP) and contractile protein expression were analyzed by western blotting or RT-PCR. The stable prostacyclin receptor (IP) agonists iloprost or cicaprost were used.
Results: Both agonists induced expression of COX-2, connexin-43, and the oxytocin receptor, as well as the contractile proteins smooth muscle-myosin heavy chain (SM-MHC), h-caldesmon, and calponin in organ culture and/or cell culture. IP-receptor antagonist inhibited these inductions. COX-2 activity was functionally upregulated as measured by increased production of $PGF_{2\alpha}$. IP agonists activated cAMP and PKA. siRNA against PKA reduced COX-2, connexin-43, and contractile protein induction by iloprost, while 8-Br-cAMP mimicked the induction. siRNA against the transcription factors GATA-6 or myocardin inhibited iloprost-induced SM-MHC and COX-2.
Conclusions: Prostacyclin receptor agonists increase CAPs and contractile proteins in human myometrium. cAMP/PKA signaling contributes to this mechanism, perhaps by ultimately regulating the transcriptional effectors GATA-6 and myocardin. Because COX-2 expression and activity is upregulated by one of its own products, prostacyclin may provide a positive feedback mechanism linking several CAPs and contractile proteins. These mechanisms may be involved in the conversion of the human uterus from quiescent to the actively contracting phenotype of labor.

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Expression Profiles of KCNQ and KCNE Genes in Late Pregnant Mouse Myometrium and Fetal Membranes and the Contribution of K_v7 Channels to Myometrial Contractility. Laura A McCallum,[1] Iain A Greenwood,[2] Rachel M Tribe.[1] *[1]Division of Reproduction and Endocrinology, King's College London, London, United Kingdom; [2]Division of Basic Medical Sciences, St George's University of London, London, United Kingdom.*
Background: Voltage-gated potassium (K_v) channels contribute to the regulation of spontaneous myometrial contractions in vitro. K_v7 channels, encoded by KCNQ 1-5, are implicated as they suppress membrane excitability/contraction in other smooth muscles. K_v7 channels can form multimers with other KCNQ encoded proteins and/or β-accessory subunits encoded by KCNE genes (1-5). They could also play a role in fetal membrane physiology. We have examined the expression of KCNQ and KCNE genes in late pregnant myometrium and fetal membranes and investigated their potential role in myometrial contractility using pharmacological agents.
Methods: Myometrial tissue and fetal membranes were obtained from late pregnant (day 17-18) C57/BL6 mice. Total RNA was extracted using Trizol and cDNA synthesised with Superscript III. qRT-PCR for KCNQ1-5 and KCNE1-5 was quantified using a standard curve and data expressed relative to the geometric mean of GAPDH and β2Microglobulin. Myometrial strips were

used for *in vitro* tension measurement and activity measured as mean integral tension (MIT) ± XE991/Chromanol (K_v7 inhibitors) or retigabine/flupirtine (K_v7 activators).

Results: All KCNQ and KCNE isoforms were detected in late pregnant myometrium and fetal membranes. KCNQ1 had significantly higher expression ($p<0.01$) than other KCNQ genes and KCNE4 was the most abundant accessory subunit ($p<0.001$) in the myometrium. In fetal membranes, KCNQ1 and KCNE4 were the most highly expressed genes ($p<0.01$), however KCNQ5 was more abundant than KCNQ2 and KCNQ3 ($p<0.01$, $p<0.05$). XE991 significantly increased spontaneous myometrial contractions (1-10 μM $p<0.01$), however the $K_v7.1$ channel inhibitor chromanol 293B had no significant effect. Retigabine and flupirtine (20 μM) significantly reduced spontaneous contractions ($p<0.001$).

Conclusions: K_v7 channels regulate uterine contractility in late pregnant mice. Myometrial KCNQ and KCNE expression profiles are distinct to those found in other smooth muscles, indicating potentially different channel conformations. Fetal membrane expression profiles also diverge from those reported in other epithelia and warrant further investigation into their potential role in fluid balance.

Funded by Tommy's the baby charity (Reg Charity No: 1060508).

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Calcium-Activated Chloride Channels Contribute to the Plateau Potential of the Tissue-Level Action Potential and Provide Counter Currents for Bulk Transmembrane Calcium Flux in Pregnant Rat Myometrial Strips.

Roger C Young, Adam R Bemis. *Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA.*

Objective: 1. To determine if calcium-activated chloride (Ca-act Cl) channels contribute to the mechanism for generation of the plateau potential; 2. To determine if inward Ca-act Cl currents counteract outward calcium currents during normalization of intracellular calcium

Methods: Thin strips of myometrium from day 21 timed pregnant rats were studied. Strips were placed in a horizontal isometric muscle bath using Krebs solution. Forces were recorded with a Grass FT-03 tension transducer. DC coupled microelectrode data were obtained by direct impalement, and electrical activity was recorded simultaneously with phasic contractions. In parallel studies, two contact electrodes were applied and bioelectrical signals were recorded simultaneously with contractions. Each contact electrode was AC coupled to ground. Contact electrode recordings show spiking signals that reflect action potential spikes, and were used to measure the spiking frequency and duration. Motion artifacts were controlled by subtraction of the background signals of the two electrodes. This dual contact electrode technique proved a simple and reliable method to observe the onset and duration of the plateau phase of each tissue-level action potential. Function of Ca-act Cl channels was modulated by exposure of the tissue to niflumic acid, 9-AC or chlorotoxin.

Results: Microelectrode data confirmed that under normal conditions (no Ca-act Cl current inhibition), phasic contractions were directly related to the expression of spiking action potentials over an envelope of a plateau depolarization. Contact electrodes showed the frequency of spike generation at the beginning of each contraction was reduced when niflumic acid, 9-AC or chlorotoxin was added to the bath. These Ca-act Cl channel blockers also variably shortened the duration of the spiking activity and prolonged the relaxation of the tissue after cessation of spiking activity.

Conclusions: Our data indicate two physiological functions for Ca-act Cl channels. First, they significantly contribute to the generation of the plateau depolarization of the tissue-level action potential. Second, they are capable of supplying counter currents for bulk transport of calcium out of the cell, although the variability of the effects of the blocking agents suggest there are other mechanisms for generating inward current.

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Vitamin C Prevents Placental and Circulatory Indices of Pre-Eclampsia and Alterations in Fetal Growth in Hypoxic Pregnancy.

BN Modi,[1] T Cindrova-Davies,[1] C Dunster,[2] FJ Kelly,[2] L Poston,[2] GJ Burton,[1] DA Giussani.[1] *[1]Physiology, Cambridge University; [2]King's College London.*

The aetiology of pre-eclampsia is unknown. Markers of oxidative stress in affected women suggest that this is a major cause of the disease (Redman & Sargent.*Science* **308:**1592,2005). However, recent negative findings from clinical antioxidant trials have questioned its involvement, highlighting that preeclampsia is a heterogeneous disorder (Poston et al.*Lancet* 367:1145,2006). Because pre-eclampsia is increased at high altitude (Zamudio.*Front Biosci* **12:**2967,2007), this study tested the hypothesis that in pregnancy complicated by reduced placental oxygenation, hypoxia-induced oxidative stress is a predominant mechanism. We investigated the effect of hypoxic pregnancy, with and without antioxidant treatment, on placental and maternal circulatory indices of oxidative stress in rats.

Methods: On pregnancy day 6 , Wistar rats were randomised into: Normoxia (21% O_2 7 litters), Hypoxia (14% O_2 8 litters) and Hypoxia + vit C (14% O_2 + 500mg.100 ml^{-1} vit C in water, 6 litters). On day 20, dams were anaesthetised, maternal blood taken, pups measured and weighed, placentae weighed and frozen. Only placentae from two male pups from any one litter were investigated. Blood was processed for ascorbate, urate, L-Cysteine and glutathione (GSH) measurement. Placental protein was analysed for heat shock protein 70 (HSP70; Western).

Results: Hypoxia ± Vit C did not affect maternal food or water intake. Vit C elevated maternal ascorbate by 70 % of baseline; a similar increment to human trials (Poston *et al.* 2006). Hypoxia elevated placental HSP 70 and maternal plasma urate and L-Cysteine, but decreased GSH. Vit C in hypoxic pregnancies prevented all stimulated effects, but the reduction in GSH persisted. Hypoxic pups had a reduced ponderal index and elevated head diameter: body weight ratio; effects also prevented by Vit C.

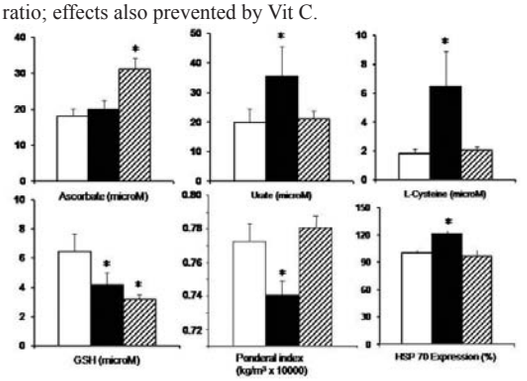

Fig. 1. Normoxic (white), hypoxic (black) and hypoxic+Vit C (hatched). *P<0.05 vs. normoxia, ANOVA.

Conclusions: Maternal treatment with Vit C prevents indices of pre-eclampsia and alterations in fetal growth in hypoxic pregnancy.

BBSRC and The BHF.

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Uric Acid Inhibits Trophoblast Integration into Endothelial Monolayers.

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Hyperuricemia develops as early as 10 weeks of gestation in women who later develop preeclampsia (PE). During this time the invasive trophoblast cells are actively remodeling the uterine spiral arterioles, integrating into and finally replacing the vascular endothelial lining. In the non-pregnant population uric acid has been shown to have several pathogenic effects on vascular endothelium. We propose that elevated concentrations of uric acid have detrimental effects on uterine vascular endothelium and may compromise trophoblast remodeling of the spiral arterioles.

Objective: Examine the effects of uric acid on trophoblast integration into endothelial cell monolayers.

Methods: Uterine microvascular endothelial cell (UtMVEC) monolayers were labeled with cell tracker green and then primed with uric acid (8.2 mg/dL), PE serum (5%) or PE serum (5%) + uricase for 2 hours. Immortalized first trimester extravillous cytotrophoblast cells (HTR8-svNeo), labeled with cell tracker red, were subsequently introduced on top of the endothelial cell monolayers. The integration of trophoblast cells into the endothelial cell monolayers over 24 hours was assessed using immunofluorescent microscopy and Image J software, with the ratio of trophoblast to endothelial cells calculated from counts of red versus green cells. Data were analyzed using one-way ANOVA with Fisher's post-hoc analysis and presented as means ± SD.

Results: Uric acid attenuated trophoblast integration into UtMVEC monolayers by 34% ±5.8% ($p<0.05$) compared to control. Trophoblast integration into monolayers was substantially reduced with exposure to PE serum and this reduction was partially reversed when the serum was initially treated with uricase (68% vs. 38% reduction compared to control, n=3).

Conclusions: Exogenous uric acid, at concentrations measured in women with PE, is capable of compromising trophoblast integration into a UtMVEC monolayer in a fashion similar to PE serum. Additionally, removal of uric acid

from PE serum with uricase partially rescues the ability of trophoblast cells to integrate into this endothelial monolayer. Taken together these data support the notion of uric acid as a pathogenic agent in the uterine microvasculature and identifies uric acid as a potential mediator of compromised spiral arteriole remodeling in PE pregnancies.

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Differences in Placental Glutathione Peroxidases in Normal and Pre-Eclamptic Pregnancies. Hiten Mistry, Paula Williams, Margaret Ramsay, Michael Symonds, Fiona Broughton Pipkin. *CREL/Division of Obstetrics & Gynaecology, University of Nottingham, Nottingham, United Kingdom.*

Background: In pre-eclampsia (PE) there is impaired placentation, oxidative stress and endothelial damage. Glutathione peroxidases (GPx1, 3, 4) are selenoproteins and have a critical role in regulating antioxidant status; they reduce hydrogen peroxide products, so dampening down the propagation of reactive oxygen species thus protecting the endothelial lining of placental spiral arteries. We know of no systematic study of placental GPx in PE.

Objectives: To measure activity and expression of 3 placental GPx-s in Caucasian women with identified PE and matched normotensive controls (NC).

Design: Cross sectional study, approved by Ethical Committee; informed, written consent was obtained from all subjects. PE was defined as resting blood pressure of ≥140/90mmHg in previously normotensive woman, with proteinuria of ≥300mg/L or >2+ on a dipstick, on 2 separate occasions, 6 hours apart after 20 weeks gestation. Placentae were taken at delivery from 27 NC and 23 PE women from 3 standardised locations between cord insertion and placental border to assess potential variations in GPx activity and/or expression. A spectrophotometric assay was used to measure GPx activities. Expression of GPx1, 3, 4 was also examined immunohistochemically in paraffin embedded placental samples from 9 NC and 8 PE, and quantified.

Results: GPx activity and expression were significantly lower in the PE; no differences were found between sampling sites.

Conclusions: Oxidative stress associated with PE may be a consequence of reduced antioxidant defense pathways, specifically involving GPx-s. These results suggest GPx may play a significant role in the pathophysiology of PE, possibly through abnormally increased lipid peroxides resulting in endothelial dysfunction.

GPx activity nmol/L (mean±sd)	Outer Edge	Middle	Near Cord
NC GPx Activity	0.4±0.3	0.4±0.3	0.4±0.3
PE GPx Activity	0.2±0.1*	0.1±0.1*	0.2±0.1*

GPx staining numerical density, arbitrary units (mean±sd)	Outer Edge	Middle	Near Cord
NC GPx1	97.5±40.7	83.8±30.1	82.4±50.3
PE GPx1	59.0±21.1*	47.5±21.4*	41.9±14.2*
NC GPx3	155.2±76.5	200.0±91.1	232.9±98.4
PE GPx3	91.5±24.9*	111.8±43.2*	126.0±42.2*
NC GPx4	144.0±51.5	141.4±67.6	135.4±60.3
PE GPx4	54.4±18.6**	40.1±12.9**	46.0±14.2**

* P<0.05; ** P≤0.001 between NC and PE

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Estrogen Enhances NO Production and Decreases ROS Production in Intact Endothelium of Uterine Arteries from Pregnant Ewes. Fu-Xian Yi,[1] Terrance M Phernetton,[1] Jason L Austin,[1] Ronald R Magness.[1,2,3] *[1]Ob/Gyn, University of Wisconsin-Madison, Madison, WI, USA; [2]Peds, University of Wisconsin-Madison, Madison, WI, USA; [3]Anim Sci, University of Wisconsin-Madison, Madison, WI, USA.*

During pregnancy uterine arteries (UA) show increased vasodilation in response to a number of agonists, which contributes to increases in uterine blood flow (UBF) necessary to meet the needs of the growing fetus. The follicular (Fol) phase of the cycle is also characterized by elevation in UBF. Both pregnancy and Fol phase are associated with increased estrogen levels and eNOS expression in UA endothelium (UAE). Estrogens may act as vasoprotective agents partly by enhancing eNOS activation, and also by reducing oxidative stress so enhancing the bioavailability of NO. Previously, we used fluorescent microscopic imaging to directly and simultaneously monitor NO production and $[Ca^{2+}]_i$ in freshly isolated UAE, our study have shown further enhanced ability of NO production in Preg>>Fol>Lut UAE. **Methods**: We recently developed methodologies to directly monitor in real time reactive oxygen species (ROS) and NO production simultaneously with $[Ca^{2+}]_i$ in intact endothelium *ex vivo* on the vessel surface. We used fluorescent microscopy to directly monitor NO production (DAF-FM DA probe), $[Ca^{2+}]_i$ (fura 2 indicator) and ROS (H2DCFDA indicator) in individual endothelial cells of intact UAs. **Results**: Direct imaging showed that ATP (100 uM), Bradykinin (1 uM) and Ionomycin (2 uM) each stimulated NO in Preg >> Fol > Lut UAE. We also evaluated effect of prolonged I/M injections of Letrozole (3 and 6 mg/day for 11 and 5 days, respectively) on *ex vivo* ATP- and Ionomycin-induced [Ca2+]i and NO production and TNF-a-induced ROS production of intact UAE from late ovine pregnancy (d127-135). These *ex vivo* UAE studies showed that the TNF-a-induced ROS productions were greatly enhanced and ATP- and Ionomycin-induced NO production was substantially depressed in late pregnant sheep given long term treatment with Aromatase inhibitor Letrozole at a level which blocked ovine feto-placental estrogen production. **Conclusion**: The follicular phase and pregnancy are associated with elevations in uterine artery NO production which are consistent with increased estrogen production, UBF, UA shear stresses, and UA eNOS protein expression. Endogenous estrogen during ovine pregnancy maintains NO production and decreases ROS production in UA endothelium. *NIH HL49210, HL87144, HD50578, HD38843.*

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Differential Effects of Hypertension vs. NO Inhibition on Gestational Uterine Artery Remodeling. George J Osol,[1] Maurizio Mandala,[2] Carolyn J Barron.[1] *[1]Ob/Gyn, Univ Vt Coll Med, Burlington, VT, USA; [2]Cell Biology, Univ Calabria, Cosenza, Italy.*

The purpose of this study was to examine the effects of hypertension and/or inhibition of nitric oxide on uterine vascular remodeling during pregnancy. This process consists of arterial axial and circumferential growth (elongation and widening) and is essential for normal pregnancy outcome. Using an established model of hypertension (NO inhibition with L-NAME, 0.5 g/L in drinking water) in nonpregnant (n=8) and pregnant (n=16) rats, comparisons were made to age-matched nonpregnant (n=4) and pregnant controls (n=16). A fifth group of animals (n=8) received hydralazine (25 mg/kg/day, also in drinking water) along with L-NAME during pregnancy to prevent blood pressure elevation while maintaining NO inhibition. Measurements of lumen diameter and wall thickness were made late in pregnancy (day 20/22) in the main uterine (MU) artery, and in the smaller radial pre-placental (RPP) and pre-myometrial (RPM) arteries. Although pregnancy resulted in doubling of MU artery length, and a tripling of the distance between the MU artery and the uterine wall, neither parameter was affected by hypertension/NO inhibition. Conversely, MU artery diameter in control pregnant animals was twice that of vessels from the pregnant hypertensive group (154 ± 8 vs. 82 ± 6 um; $p<0.05$). Wall thickness was unchanged during pregnancy, resulting in a significant increase in the wall: lumen ratio in vessels from the hypertensive vs. normotensive group (1.6 ± 0.12 vs. 0.8 ± 0.08; $p<0.05$). During pregnancy, smaller radial arteries also underwent outward expansive remodeling (widening), with greater changes noted in RPP vs. RPM vessels (lumen diameter increases averaged 52 vs. 11%, respectively). Remodeling of both vessel types was attenuated in hypertensive animals, but not in those made normotensive by co-treatment with hydralazine. Together, these data underscore the complexity of gestational vascular remodeling in terms of vessel size (large vs. small), type (pre-placental vs. pre-myometrial), and pattern (axial vs. circumferential). Some of the adaptive changes observed in L-NAME-treated animals may stem from pressure elevation *per se*, rather than inhibition of endothelial NO production. Interestingly, neither hypertension nor NO inhibition had any measurable effect on arterial elongation, a process that occurs through mechanisms that have yet to be identified.

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Differential Role of Mule in Regulating Cell Death in Preeclamptic and IUGR Placentae. Alessandro Rolfo,[1] Antonella Racano,[1,2] Tullia Todros,[3] Martin Post,[2,4] Andrea Jurisicova,[1] Isabella Caniggia.[1,2] *[1]Ob/Gyn, Mount Sinai Hospital, Toronto, ON, Canada; [2]Physiol., University of Toronto, Toronto, ON, Canada; [3]Ob/Gyn, University of Turin, Turin, Italy; [4]Pediatrics, Hospital for Sick Children, Toronto, ON, Canada.*

Objective: Mcl-1 (Myeloid cell leukemia factor 1) is a Bcl-2 family member with three isoforms: Mcl-1L (pro-survival) and pro-apoptotic Mcl-1S and Mcl-1c. Preeclampsia (PE) and Intra Uterine Growth Restriction (IUGR) are severe human pregnancy disorders characterized by increased placental apoptosis. We recently reported increased expression of Mcl-1 pro-apoptotic isoforms and decreased expression of the pro-survival Mcl-1L in PE placentae (Am.J.Pathol. 2007). Mule, a Mcl-1 Ubiquitin E3 Ligase that targets Mcl-1 and p53 for proteasomal degradation, tightly regulates Mcl-1 protein stability. The objective of this study was to investigate the mechanism of Mcl-1 and p53 degradation mediated by Mule in PE and IUGR placentae.

Methods: Placental tissues from PE (n=24) and IUGR pregnancies with documented AEDF (n=16) and from pre-term controls (PTC, n=14) were used. Mule and Mcl-1 protein were assessed by Western Blot (WB) and immunohistochemistry. Mcl-1/Mule association was examined by Mule immunoprecipitation (IP) followed by Mcl-1 WB. Mcl-1 ubiquitination was assessed by Mcl-1 IP followed by ubiquitin WB.
Results: In contrast to PE, all three Mcl-1 isoforms were decreased in IUGR placentae. Mule protein levels were unchanged in PE and decreased in IUGR placentae vs PTC. Both Mule and Mcl-1 predominantly localized to trophoblast cells. Mcl-1 immunoreactivity decreased in IUGR but was unchanged in PE placentae vs PTC, whereas Mule increased in both PE and IUGR samples. Mcl-1/Mule association and Mcl-1 ubiquitination were increased in IUGR and unchanged in PE placentae. Since pro-apoptotic p53 is another target of Mule, we also investigated its expression and degradation. p53 protein levels decreased in PE and increased in IUGR placentae. Mule/p53 association and p53 ubiquitination were increased in PE while no changes were observed in IUGR placentae vs PTC.
Conclusions: Our findings suggest a different role of Mule in IUGR and PE placentae. In PE, Mule preferentially targets p53 for degradation thereby allowing accumulation of the pro-apoptotic Mcl-1 isoforms (Mcl-1c, Mcl-1S). In IUGR, Mule targets Mcl-1 for degradation, leading to the elevated p53 levels and increased trophoblast apoptosis. (Supported by CIHR and OWH/IGH).

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Identification of Myoferlin Binding Partners in Endothelial Cells through the Use of Glutathione-S-Transferase (GST) Fusion Proteins. Pinar H Kodaman,[1] Pascal N Bernatchez,[2] William C Sessa.[2] [1]*Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT, USA;* [2]*Vascular Biology and Therapeutics, Yale University School of Medicine, New Haven, CT, USA.*
Introduction: The female reproductive system is one of few adult tissues that undergoes physiologic angiogenesis. Myoferlin, a 230 kD transmembrane protein, is highly expressed in endothelial cells, where it is involved in vascular endothelial growth factor (VEGF) receptor-2 stability and function. Deficiency of myoferlin results in impaired endothelial cell proliferation, migration, and nitric oxide release in response to VEGF. Myoferlin has six C2 domains, which mediate binding to calcium and phospholipids, and endothelial cells lacking myoferlin have impaired ability to repair damaged membranes . **Objective:** To identify myoferlin binding partners in endothelial cells through the use of a GST fusion protein system. **Methods**: Three myoferlin constructs, incorporating varying lengths of the amino terminus of myoferlin and up to three C2 domains, were cloned into the PGEX-4T vector, and GST fusion proteins were generated. These proteins were purified and then utilized for pulldown assays with lysates from EA.hy.926 cells, an immortalized line of human endothelial cells. The bound proteins were eluted, electrophoresed, and stained with Coomassie Blue. Differential bands were excised and subjected to proteomic analysis with mass spectrometry. **Results:** Identified myoferlin binding partners included actin and tubulin, components of the cytoskeleton, whose binding to the myoferlin-GST fusion proteins was calcium-dependent. Western analysis confirmed the specific binding of these proteins to myoferlin and also identified additional binding partners, including dynamin-2, caveolin-1, and annexins 1 and 2. Dynamin-2 is a GTPase that mediates vesicle fission and fusion and interacts with several actin-binding proteins, while the annexins facilitate membrane trafficking and cell-matrix interactions. Caveolin-1 is a major structural protein of endothelial caveolar membranes. **Conclusion:** The association of myoferlin with cytoskeletal proteins and proteins involved in vesicle and membrane fusion further suggests a role for myoferlin in endothelial cell membrane trafficking and repair. These findings may have potential implications for angiogenesis during the cyclic remodeling of female reproductive system tissues.

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Induction of Endothelial Dysfunction in Uteroplacental Arteries from Pregnant Rats during Experimental Diabetes. Natalia I Gokina, Olga Y Kuzina, Lindsey L Pryor. *Department of Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA.*
Introduction: Diabetes mellitus is associated with an increased risk for the development of hypertension and preeclampsia in pregnancy that might be due to the development of uteroplacental endothelial dysfunction. The purpose of this study was: (1) To characterize the effect of experimental diabetes during pregnancy on endothelium-dependent vasodilation of uteroplacental arteries; (2) To study the role of endothelium-derived hyperpolarizing factor (EDHF) in the altered arterial reactivity to ACh.
Methods: Diabetes was induced in rats by intraperitoneal injection of 50 mg/kg streptozotocin on day 2 of pregnancy. Four rats were injected with a citrate buffer only (CB). Uteroplacental arteries obtained on day 20 of pregnancy were pressurized, and phenylephrine was added to produce a 50-60 % reduction in the diameter; ACh was then applied in a cumulative fashion (0.03 – 10 μM). The responses to ACh were also studied after inhibition of NO and prostacyclin production with L-NNA and indomethacin. Differences between groups of data were considered significant at $P<0.05$.
Results: Blood glucose was elevated after STZ administration from 86 ± 4 to 420 ± 14 mg/dL in 14 rats (hyperglycemic, HG). 11 rats remained normoglycemic (NH) throughout pregnancy. There were no differences in maternal body weight, placental weight or litter size in HG vs. NG and CB rats. Fetuses from HG mothers were significantly smaller (1.982 ± 0.05g) compared to those from NG (2.432 ± 0.04 g) and CB (2.541 ± 0.14 g) rats. The maximal ACh-induced vasodilation was decreased in arteries of HG (51.9 ± 11.9 %, n = 9) vs. NG (92.2 ± 2.4 %, n = 5) and CB (98.7 ± 1.2 %, n = 4) rats. The concentrations of ACh required to produce half-maximal dilation (EC_{50}) were: 0.288 ± 0.048 vs. 0.080 ± 0.024 and 0.065 ± 0.041 μM for HG vs. NG and CB arteries. ACh-induced EDHF-mediated responses were markedly inhibited in arteries from HG rats; maximal dilatation to ACh was reduced from 65.8 ± 10.6 (NG, n = 5) to 13.4 ± 5.1 (HG, n = 7).
Conclusion: Uncontrolled experimental diabetes during rat pregnancy results in a significant attenuation of endothelium-mediated uteroplacental vasodilation that is most likely responsible for the associated intrauterine growth restriction. Specific impairment of EDHF-mediated vasodilation may be an important contribution to diabetes-induced uterine endothelial dysfunction.
Supported by NIH HL67250.

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Chronic Fetal Hypoxemia Activates a Neuronal iNOS Inflammatory Cascade Causing Fetal Brain Damage That Is Pharmacologically Preventable Despite Continued Fetal Hypoxemia. CP Weiner, W Hou, J Stites, Y Dong. *Obstetrics and Gynecology, University of Kansas School of Medicine, Kansas City, KS, USA.*
OBJECTIVES: We previously demonstrated chronic fetal hypoxemia (HPX)induces gliosis and neuronal apoptosis in the guinea pig (GP) hippocampus associated with induction of iNOS, TNFα and IL-1β in proportion to the HPX. These HPX responses were blocked by maternal therapy with a selective iNOS inhibitor. Herein, we test 2 hypotheses: 1) HPX induces iNOS within the neuron; and 2) oxygen free radical generation is the proximate cause of fetal brain damage associated with chronic HPX.
METHODS: Time-mated GPs were housed in a chamber breathing 10.5% O_2 for 14d (HPX) beginning day 50. HPX GPs were randomly assigned to a 14d therapy with either a potent antioxidant, N-acetylcysteine (NAC) (1g/Kg/day, p.o., HPX-NAC) or placebo (HPX). Control GPs were housed in room air (NMX). Neurons were identified by Nissel staining, reactive astrocytes by GFAP and iNOS by immunohistochemistry. Fetal brain injury (Bax, Bcl2) and inflammation (iNOS, TNF1α and IL-6) were quantified by RT PCR. Reduced (GSH) and oxidized (GSSH) glutathione were measured to determine the adequacy of NAC dosing.
RESULTS: HPX reduced neuronal density, and increased GFAP and apoptosis. iNOS was confined to hippocampal neurons. HPX reduced the GSH/GSSG ratio in fetal liver and brain ($p<0.05$), while HPX-NAC increased it to supraphysiologic levels in both mother and fetus. HPX increased the ratio of Bax / Bcl2 ($p<0.05$), indicating increased apoptosis in parallel to the expected increases in iNOS, TNFα and IL-6 (each $p<0.05$). NAC dramatically reduced the Bax / Bcl2 ratio from >2:1 to 1:1 without altering iNOS expression. It also blunted but did not eliminate the expression of IL-6 and TNF-α (each $p<0.05$).
CONCLUSIONS: Coupled to our prior work, the findings shows HPX produces fetal brain damage by triggering an inflammatory response initiated by the induction of neuronal iNOS (unaffected by NAC) and completed by the generation of ROS. The observation NAC partially blunted IL-6 and TNF-α suggests either inadequate antioxidant capacity or that one or more ROS participate in a positive feedback loop for the transcription of these inflammatory mediators. These studies demonstrate the impact of chronic fetal HPX can be ameliorated despite its continuation, and offers several pharmacological approaches to prospectively treat fetuses in a hostile environment but too premature for delivery.

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Expression of Thyroid Hormone Transporters in Human Fetal Brain. Shiao Y Chan,[1] Azucena Martin-Santos,[1] Laurence S Loubiere,[1] Elisavet Vasilopoulou,[1] Sally R James,[1] Christopher J McCabe,[2] Jayne A Franklyn,[2] Mark D Kilby.[1] *[1]Division of Reproductive and Child Health, University of Birmingham, Birmingham, West Midlands, United Kingdom; [2]Division of Medical Sciences, University of Birmingham, Birmingham, West Midlands, United Kingdom.*

The importance of thyroid hormone (TH) transporters to central nervous system (CNS) development has been highlighted by reports of severe global neurological impairment associated with mutations in the potent TH transporter, MCT8. Even subclinical maternal hypothyroidism in early pregnancy has been associated with long-term neurodevelopmental delay in the offspring suggesting that TH is crucial for fetal brain development from early pregnancy. The cellular supply of TH requires the function of specific plasma membrane transporters. **OBJECTIVE:** To describe the ontogeny of different TH transporters in the human fetal CNS. **METHODS:** Biopsies of cerebral cortex from the human fetus were obtained from normal pregnancies ending between 7-20 weeks gestation (n=65) and compared with adult cerebral cortex (n=10). Nine TH transporters reported to be expressed in either human or mammalian CNS were investigated. Taqman quantitative RT-PCR was used to determine relative mRNA expression. **RESULTS:** Messenger RNA encoding OATP1C1, OATP1A2 and OATP3A1 variant 2 were significantly lower at 7-16 weeks gestation compared with adult, with reductions of 98%, 95% and 91% respectively (ANOVA $p<0.001$). Messenger RNA encoding LAT2 was 92% lower at 17-20 weeks compared with adult (ANOVA $p<0.01$), whilst LAT1 and its obligate heterodimer, 4F2hc, showed no significant changes. MCT8, MCT10, OATP4A1 and OATP3A1 variant 1 mRNA expressions were similar to adult. **CONCLUSIONS:** A range of TH transporters are present in the human fetal cerebral cortex from very early gestation. These include OATPs previously localised to the blood brain barrier (BBB) of adult brains, MCT8 localised in CNS neurons and the choroid plexus (CP), and the LATs expressed in glial cell lines. The concerted effects of these transporters may play differential roles in the physiological regulation of TH entry into the fetal CNS. This includes TH transport across the BBB and CP into the CNS as well as into CNS cells themselves. TH transporters may thus play a part in ensuring that the appropriate amounts of TH for each stage of CNS development are present to facilitate TH action. (Funded by the Health Foundation, Action Medical Research and MRC, UK).

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Regulation of Multidrug Resistance P-Glycoprotein in the Developing Fetal Blood-Brain Barrier. Sophie Petropoulos,[1] Kim Bocking,[1] William Gibb,[4] Stephen G Matthews.[1,2] *[1]Physiology, University of Toronto, Toronto, ON, Canada; [2]Obs-Gyn & Medicine, University of Toronto, Toronto, ON, Canada; [3]Obs-Gyn & Cellular & Molecular Medicine, University of Ottawa, Ottawa, ON, Canada.*

Background: Multidrug resistance P-glycoprotein (Mdr1/P-gp) excludes a range of xenobiotics including therapeutic agents, environmental toxins and hormones from cells. The fetal brain represents a primary target for Mdr1/P-gp substrates, however, to date, no studies have investigated function or regulation of Mdr1/P-gp in the fetal blood-brain barrier (BBB). We have recently shown that *mdr1a* is expressed in the fetal brain capillary endothelial cells and significantly increases near term, coinciding with the rise in both maternal and fetal circulating glucocorticoids (GCs). Synthetic GCs (sGCs) have been shown to regulate Mdr1/P-gp expression in the liver and adrenal gland. In this study, we hypothesize that; 1) GCs upregulate the expression of Mdr1/P-gp in the fetal brain, and 2) this increased expression decreases penetration of xenobiotics into the developing brain. **Methods:** Pregnant FVB mice were treated with dexamethasone (DEX; 1mg/kg) from either embryonic day (E)9.5-15.5 or E12.5-18.5. On E15.5 or E18.5, dams were injected (i.v.) with Mdr1/P-gp substrate, [3H]digoxin, and euthanized 1h later. Fetal brains were collected and either radioactivity accumulation was determined or *mdr1a* mRNA and Mdr1/P-gp protein were measured. **Results:** DEX treatment from E9.5-15.5 prematurely upregulated *mdr1a* mRNA on E15.5. This level of *mdr1a* expression resembled that normally seen near term (E18.5). Further, the sGC induced upregulation of Mdr1/P-gp significantly ($P<0.05$) decreased penetration of [3H]digoxin in the fetal brain. In contrast, DEX treatment during the last week of gestation (E12.5-18.5) did not effect expression or function of Mdr1/P-gp compared to vehicle. **Conclusion:** Mdr1/P-gp mediated protection of the fetal brain increases with advancing gestation and is positively regulated by sGCs. This is of particular importance considering 1 in 10 pregnant women are treated with antenatal sGC for threatened preterm labour. Understanding the regulation of Mdr1/P-gp in the fetal BBB will aid not only in the development of novel treatment modalities but enable improved strategies for protection of the human fetal brain against entry of potentially harmful xenobioitics.

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Medication Use, Nicotinamide Intake, and the Nicotinamide N-Methyltransferase Polymorphism Are New Risk Factors for Congenital Heart Defects. Lydi MJW van Driel,[1,2] Huberdina PM Smedts,[1] Willem A Helbing,[2] Aaron Isaacs,[3] Jan Lindemans,[4] Andre G Uitterlinden,[4,5,6] Cornelia M van Duijn,[6] Jeanne HM de Vries,[7] Eric AP Steegers,[1] Regine PM Steegers-Theunissen.[1,2,3,6] *[1]Obstetrics and Gynecology/Division of Obstetrics and Prenatal Medicine, Erasmus Medical Centre, Rotterdam, Netherlands; [2]Pediatric Cardiology, Erasmus Medical Centre, Rotterdam, Netherlands; [3]Clinical Genetics, Erasmus Medical Centre, Rotterdam, Netherlands; [4]Clinical Chemistry, Erasmus Medical Centre, Rotterdam, Netherlands; [5]Internal Medicine, Erasmus Medical Centre, Rotterdam, Netherlands; [6]Epidemiology, Erasmus Medical Centre, Rotterdam, Netherlands; [7]Human Nutrition, Wageningen University, Wageningen, Netherlands.*

Background: Genetic and nutritional factors in the homocysteine and detoxification pathways play a role in the etiology of congenital heart defects (CHDs). The recently identified nicotinamide N-methyl transferase (NNMT) gene and its substrate nicotinamide are implicated in both pathways.

Objectives: To determine CHD risk in association with the NNMT G/A genotype, maternal periconceptional drug use and/or dietary nicotinamide intake.

Methods: A case-control study of 292 children with CHD and 316 unaffected children and both parents was conducted. Approximately 16 months after the index pregnancy, mothers filled out standardized questionnaires on periconceptional drug use and a validated food frequency questionnaire on current dietary intake. Mothers, fathers and children were genotyped for the NNMT G/A polymorphism (rs694539). The data were analyzed by univariate and multivariate logistic regression analysis using the dominant model.

Results: Maternal periconceptional drug use and a low dietary intake of nicotinamide (≤ 13.8 mg) was associated with CHD risk, (OR(95%CI) 1.5(1.0-2.2) and 1.6(1.1-2.3), respectively. No significant association was found between the NNMT AG/AA genotypes and CHD risk in mothers (0.9(0.7-1.3)), fathers (1.1(0.8-1.6)), and children (1.1(0.8-1.6)). However, periconceptional drug use and dietary nicotinamide intake and the NNMT AG/AA genotype in mothers or children showed risk estimates up to 2.2(0.8-5.7) and 4.0(1.3-12.0), respectively.

Conclusions: The NNMT AG/AA genotype of the mother or child additionally contributes to CHD risk in combination with periconceptional exposure to drugs and/or low dietary nicotinamide intake. These findings provide new insights into the complex etiology of CHDs, and may be important for future preconceptional care.

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Fetal Heart Enlargement Caused by Chronically Elevated Maternal Cortisol Is Mediated by Mineralocorticoid Receptors in the Fetal Sheep Heart. Seth A Reini,[1] Charles E Wood,[1] Maureen Keller-Wood.[2] *[1]Phys and Func Genomics, Univ of Florida, Gainesville, FL, USA; [2]Pharmacodynamics, Univ of Florida, Gainesville, FL, USA.*

Objective: This study was designed to investigate if the enlargement of the fetal heart in response to chronically elevated maternal cortisol levels is mediated by mineralocorticoid receptors (MR) or glucocorticoid receptors (GR) within the fetal heart.

Methods: Pregnant ewes (~120d gestation) were treated with intravenous infusion of saline (S) or cortisol (F, 1mg/kg/day) for ~10 days. Cortisol-treated ewes were further divided into 3 groups at the time of surgery: 2 groups infused with MR antagonist (potassium canrenoate, 600 µg/day; F+ MRa) or GR antagonist (mifepristone, 50 µg/day; F+GRa) directly into the pericardial space from an Alzet minipump, and a group with a sham pericardial catheter. Fetal heart weight, LV, RV, and septal thickness, along with arterial pressure (MAP), and heart rate (HR) data from the 4 groups of ewes were collected and analyzed using ANOVA. Plasma cortisol data was compared between cortisol-treated groups and the saline group using t-test.

Results: Maternal and fetal cortisol levels were significantly and equivalently elevated in the 3 groups of F-treated ewes at day 5 and at sacrifice (9.3 ±1 vs 5.3±1, and 4.2±0.9 vs 2.4±0.9 ng/ml). MAP and HR were not different among groups in either ewes or their fetuses at 5 or 10 days of infusion. Fetal hearts

from F-treated group weighed significantly more (6.8±0.3 g/kg BW) and had significantly thicker LV (5.5±0.3 mm) than hearts from the saline group (5.9±0.2 g/kg and 4.7±0.2 mm) or from F+MRa (5.9±0.2 g/kg and 4.7±0.2 mm), but not F+GRa (6.4±0.2 g/kg and 5.0±0.2 mm) group. The F group also had significantly thicker RV than all other groups (F: 5.0±0.2, MRa: 3.7±0.2, GRa: 4.1±0.1, S: 4.1±0.1 mm). Septal thickness was greater in fetuses in the F group than the F+MRa group (7.1±0.5 vs 5.7±0.2 mm).

Conclusions: Blockade of MR within the fetal heart eliminates the enlargement observed with modest elevations in maternal cortisol. Furthermore, blockade of GR under the same conditions reduces RV wall thickness and tends to decrease the other heart measurements as compared to elevated cortisol alone. These results are consistent with the expected relative occupancies of MR and GR by fetal cortisol concentrations produced secondary to elevations of maternal cortisol, and indicate a role of MR, and to a lesser extent GR, in the fetal heart.

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Assigning Carnegie Stages Using a Novel Virtual Reality System. Christine M Verwoerd-Dikkeboom,[1] Anton HJ Koning,[2] Peter J van der Spek,[2] Niek Exalto,[1] Eric AP Steegers.[1] *[1]Obstetrics and Gynecology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Bioinformatics, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*

OBJECTIVE: The aim of this study was to demonstrate the use of a novel 3D virtual reality system in the assignment of Carnegie Stages to embryos visualized with 3D ultrasound.

METHODS: 3D datasets are usually presented on flat 2D screens or paper, which implies that the third dimension is not used optimally. To benefit from all three dimensions we used a 3D projection system called I-Space. This VR system immerses viewers in a 3D virtual environment and allows depth perception and interaction in an intuitive manner. We analyzed 48 3D ultrasound scans of 19 IVF/ICSI pregnancies at 7-10 weeks' gestation. The 3D datasets were transferred to the I-Space and visualized as 3D "holograms". Embryos were staged according to external morphological features (mainly limb development). After staging, the crown rump length (CRL) was measured. Stage and CRL were compared to gestational age based on the date of oocyte retrieval and with classical data on embryology from the Carnegie Collection.

RESULTS: In all 48 ultrasound scans the Carnegie Stage of the embryo was determined easily. The I-Space allows depth perception, which helps in estimating size and position. Curvature of the elbow for instance, which distinguishes stage 19 from stage 20, was quite obvious, as was the position of the limb buds or hands and feet. The presumed stages corresponded well with the measured CRL. However, in 28 out of 48 cases stages seemed to have been reached earlier than previously described for the Carnegie Collection.

CONCLUSIONS: We successfully demonstrated that embryonic growth and development can be classified into Carnegie Stages using this innovative imaging technique we tentatively named "Virtual Embryoscopy". Being able to accurately determine embryonic stages in the first trimester provides a promising non-invasive tool for early pregnancy evaluation of embryogenesis. It also opens a new area to study the relationship between embryonic growth, development and morphology and 2nd-3rd trimester pregnancy complications.

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Fetal Oxygen Uptake in Normal and GDM Pregnancies. Emanuela Taricco, Tatjana Radaelli, Veronica Cozzi, Gabriele Rossi, Danila Puglia, Giorgio Pardi, Irene Cetin. *Department of Obstetrics & Gynecology "L.Mangiagalli", IRCCS Policlinico, Mangiagalli e Regina Elena, Milan, Italy.*

Background. Diabetes in pregnancy has been associated with alterations of fetal growth probably due to increased nutrient availability and placental transport. Fetal hypoxia and acidemia have been reported in pregestational diabetic pregnancies with poor glicemic control but this is still uncertain in well controlled patients. The role of placental function and the relationship between maternal and fetal circulation is crucial for efficient exchanges of oxygen and nutrients. Since umbilical blood flow can be obtained by US in utero, we studied normal and GDM pregnancies in order to evaluate fetal oxygen uptake.

Methods. 21 normal (N) and 21 GDM pregnancies were studied at term, at the time of elective caesarean section. Umbilical vein volume flow (Qumb) was measured by US before caesarean section and blood samples from umbilical vein (uv) and artery (ua) were obtained. Blood gases and acid-base balance were evaluated.

Results. Average fetal weights were similar in both groups (N=3274±62; GDM=3381±111 g) while placental weights were significantly different (N=474±16; GDM=571±35 g). N and GDM pregnancies showed similar values of Qumb and Qumb/Kg of fetal weight. (Qumb: 245.3±19.0 in N and 242.2±18.5 mL/min in GDM; Qumb/Kg: 74.6±5.0 in N and 71.4±4.6 mL/min/Kg in GDM). In fetuses from GDM pregnancies a significant reduction in O_2 Sat, O_2 Cont and pO_2 and a significant increased Lactate conc was found in both uv and ua compared to N (table 1). O_2 umb uptake (N=0.78±0.08; GDM=0.56±0.08 mmo/l/min) and O_2 umb uptake/kg (N=0.24±0.02; GDM=0.16±0.02 mmo/l/min/Kg) were significantly lower in GDM compared to N fetuses.

Conclusions. Our data indicate that fetuses from GDM pregnancies show a significant reduction in oxygen supply despite a normal blood flow/kg of fetal weight. These data may suggest that a good maternal metabolic control is not sufficient to ensure normal placental oxygen supply and/or utilization by the GDM fetus.

Fetal Oxygenation Data

	pH	O2 Saturation (%)	O2 Content (mmol/L)	pO2 (mmHg)	Lac (mmol/L)
ua Normal	7.323±0.006	15.8±1.5	1.3±0.1	14.3±0.8	1.41±0.06
ua GDM	7.301±0.008	11.1±1.7	0.9±0.1	11.2±1.1	1.76±0.09
uv Normal	7.386±0.007	52.8±3.3	4.5±0.3	28.8±1.3	1.36±0.04
uv GDM	7.01±0.35	40.3±3.2	3.5±0.3	24.0±1.3	1.61±0.10

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Intrauterine Growth Restriction Increases Myocellular Expression of PGC-1α in Male Lambs in Early Postnatal Life. BS Muhlhausler,[1] JA Duffield,[2] JL Morrison,[1] IC McMillen.[1] *[1]Sansom Institute, University of South Australia, Adelaide, Australia; [2]School of Molecular and Biomedical Science, The University of Adelaide, Australia.*

AIMS: Intrauterine growth restriction (IUGR) is often associated with a period of accelerated (catch-up) growth in early postnatal life, and the combination of IUGR and postnatal catch-up growth results in an increased risk of insulin resistance and type 2 diabetes in later life. The molecular mechanisms which underlie catch-up growth are poorly understood. In the present study, we have tested the hypothesis that IUGR results in increased expression of Peroxisome Proliferator Activated Receptorγ Co-activator-1α(PGC-1α), a key regulator of lipid oxidation and insulin sensitivity, the insulin receptor (IR) and GLUT1, a regulator of insulin-independent glucose uptake, in skeletal muscle in male and female lambs in early postnatal life.

STUDY DESIGN: Placental restriction was induced in 9 non-pregnant ewes by the removal of the majority of the endometrial caruncles prior to mating to induce restriction of placental and fetal growth (IUGR). Age-matched ewes who did not undergo caruncletomy were used as controls. Lambs (IUGR, n=9, 5 male, 4 female; Control, n=14, 8 male, 6 female) were born at term and quadriceps muscle samples collected from lambs at 21d of postnatal age. Real Time PCR was used to determine the mRNA expression of PGC-1α, IR and GLUT1 relative to the reference gene RpP0 in all samples.

RESULTS: Birth weight (3.76 ± 0.2 kg vs 5.86 ± 0.1 kg, $P<0.01$) was lower in IUGR compared to Control lambs in both males and females. GLUT1 mRNA tended ($P=0.06$) to be higher in IUGR lambs independent of gender, and the level of GLUT1 expression was inversely related to birth weight ($r^2=0.67$, $P<0.02$). In males, but not in females, the expression of PGC-1α mRNA was higher in the IUGR group (0.053 ± 0.009 vs 0.036 ± 0.003, $P<0.05$). PGC-1α expression was directly related to the expression of the insulin receptor in both males and females ($r^2=0.41$, $P<0.01$).

CONCLUSIONS: Myocellular expression of GLUT1 is related to birthweight such that smaller lambs have higher GLUT1 mRNA expression and this may result in increased glucose uptake into skeletal muscle cells. Furthermore, IUGR in males is also associated with an increase PGC-1α expression in the skeletal muscle. These changes in myocellular gene expression may contribute to the accelerated rate of muscle accretion and postnatal weight gain in IUGR lambs in early postnatal life.

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Hypermethylation of the HOXA10 Gene by *In Utero* DES Exposure: An Epigenetic Mechanism for Altered Developmental Programming. Jason G Bromer, Jie Wu, Hugh S Taylor. *Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Introduction: The HOXA10 gene controls uterine organogenesis during embryonic development. We have shown previously that *in utero* Diethylstilbestrol (DES) exposure induces female reproductive tract anomalies through altered expression of HOX genes. There is increasing evidence that epigenetic changes in gene expression may occur through aberrant DNA methylation. We hypothesized that DES-induced changes in HOXA10 expression and associated developmental abnormalities are due to alterations in HOXA10 DNA methylation.

Methods: 30 pregnant CD-1 mice were treated with intraperitoneal injections of DES (10 mg/kg) or vehicle on days 9-16 of gestation. 2 weeks after birth, the uteri of female offspring were either formalin-fixed for immunohistochemical analysis or homogenized for DNA and mRNA extraction. Genomic DNA was bisulfite-modified and analyzed by Methylation Specific PCR. cDNA was generated, and quantitative real time RT-PCR was performed and normalized to β-actin. All assays were repeated in triplicate. HOXA10 methylation was compared using the Mann-Whitney U test. HOXA10 and DNA methytransferase (DNMT) expression were compared using the Student's T-test.

Results: In the offspring of DES-treated animals, HOXA10 protein expression was shifted to the caudal portion of the uterus relative to its normal pattern in controls. CpG island methylation frequency in the first intronic region of the HOXA10 gene was significantly higher in the offspring of DES-treated animals than controls (100% vs. 60%, $p<0.05$). Methylation levels of the HOXA10 gene were also significantly higher than in controls at the 5' promoter and intron-1 regions (0.42±0.12 vs. 0.31±0.12, $p<0.05$ and 0.59±0.31 vs. 0.25±0.21, $p<0.001$, respectively). Relative fold change mRNA expression in 2 of 3 DNMTs were significantly elevated compared with controls (DNMT1: 4.20± 1.2, $p<0.05$), DNMT3a: 0.98±0.03, $p>0.05$, DNMT3b: 2.95±0.54, $p<0.05$).

Conclusions: *In utero* exposure to DES produces a shift in the normal pattern of uterine HOXA10 expression. This change is accompanied both by alterations in methylation at the promoter and intron regions of HOXA10 and by increased expression of DNMT1 and 3b. Alterations in gene methylation, due to alterations in DNMT expression, mediate the developmental anomalies seen with DES exposure. Epigenetic modifications are one mechanism of endocrine disruptor action.

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CCAAT/Enhancer Binding Protein beta (C/EBPβ) Regulates cAMP-Dependent Aromatase Expression Via Multiple and Novel Sequences in Uterine Leiomyoma. Hiroshi Ishikawa, Veysel Fencki, Erica E Marsh, Ping Yin, Zhihong Lin, You-Hong Chen, Serdar E Bulun. *Reproductive Biology Research, Northwestern University, Chicago, IL, USA.*

Objective: Regulation of aromatase expression giving rise to local estrogen biosynthesis in uterine leiomyoma tissue is clinically important, since aromatase inhibitors reduce the size of leiomyomata in affected women. We previously demonstrated that the cAMP-responsive proximal promoters I.3 and II regulate aromatase expression in leiomyoma smooth muscle cells (LSMCs). We further investigated the mechanism responsible for promoter I.3/II usage.

Methods: We isolated and cultured primary LSMCs from 68 surgical specimens. We used radiolabeled [^{3}H]-release assay and real-time RT-PCR to measure aromatase activity and mRNA levels. We used deletion and site-directed mutants of the promoter I.3/II fused to the luciferase reporter gene for analyzing crucial *cis*-regulatory elements. We used electromobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP)-PCR to analyze binding of transcription factors to these regions and checked protein levels using immunoblotting. We used siRNA to knock down a transcription factor.

Results: A cAMP analog dibutyryl cAMP (Bt_2cAMP) significantly induced promoter I.3/II-specific aromatase mRNA and enzyme activity in LSMCs. Serial deletion and site-directed mutants of promoter I.3/II revealed that the -517/-214 bp region conferred Bt_2cAMP responsiveness, and 5 out of 7 elements, including 3 CCAAT/enhancer binding protein (C/EBP) motifs and 2 cAMP response elements (CREs), were essential for the cAMP-induced promoter activity. EMSA demonstrated that nuclear extracts from LSMCs contain complexes assembled on 4 of the 5 *cis*-elements. C/EBPβ bound to two of the C/EBP motifs including a novel -245/-231 bp sequence. Additionally, ChIP-PCR verified that C/EBPβ is recruited to the aromatase promoter I.3/II region in intact LSMCs. Nuclear C/EBPβ protein levels in LSMCs treated with Bt_2cAMP significantly increased in a time-dependent manner. Conversely, knocking down of C/EBPβ protein dramatically suppressed cAMP-induced aromatase mRNA and enzyme activity.

Conclusion: C/EBPβ regulates cAMP-dependent aromatase expression in LSMCs via binding to multiple and novel *cis*-regulatory elements in the promoter I.3/II region. C/EBPβ activation of promoter I.3/II might be the most prominent pathway controlling aromatase expression in uterine leiomyoma cells.

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Regulation and Signaling of the Membrane Estrogen Receptor, GPR30, in Human Endometrial Cells. Beth J Plante,[1] Lingwen Yuan,[1] Bruce A Lessey,[2] Steven L Young.[1] *[1]Obstetrics & Gynecology, U North Carolina, Chapel Hill, NC, USA; [2]Greenville Hospital, Greenville, SC.*

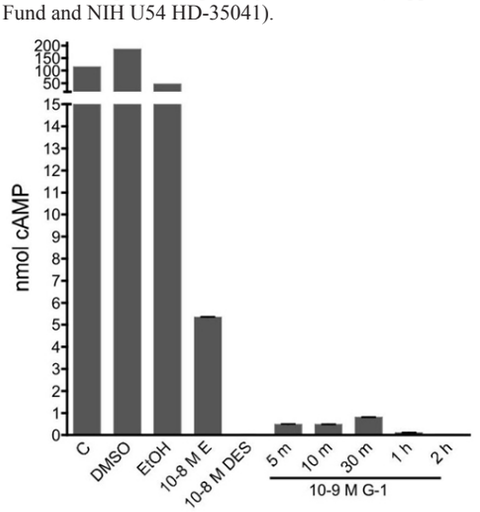
Previous work in our laboratory has demonstrated expression of the transmembrane, G-protein coupled estrogen receptor, GPR30, in normal human endometrial epithelium, with peak expression in the proliferative phase. The endometrial function of GPR30 remains unknown.

Objectives: 1. Determine effects of estrogen on endometrial epithelial GPR30 expression. 2. Determine signaling mechanisms utilized by GPR30 in endometrial epithelium.

Methods: Western blot analysis of GPR30 and p42/44 MAPK utilized rabbit polyclonal antibodies (Abcam ab12563; Cell Signaling 9102). Real-time qRT-PCR utilized predesigned Taqman primer probe sets for GPR30 and constitutive control, PPIA (Applied Biosystems). cAMP was measured by ELISA after cell lysis.

Results: Estradiol (10^{-8} M) or DES (10^{-8} M) treatment of ECC-1 cells resulted in a time dependent increase in GPR30 mRNA, with initial increase detected at 2 hours (1.7 fold) and further increase to 24 hours (3.5 fold). Treatment of ECC-1 cells by DES or E led to increased p42/44 MAPK phosphorylation, by 5 minutes of exposure. Of note, treatment of ECC-1 cells with the GPR30-specific agonist, G-1 at 10-9M resulted in increased p42/44 MAPK phosphorylation by 5 minutes of exposure and persisting to 20 hours of exposure. G-1 shows no detectable binding to ERα or ERβ at 10^{-6} M. Treatment with 10^{-8} M or 10^{-9} M DES or E or 10^{-9} M G1 led to a rapid and profound (50-100 fold) reduction in cAMP levels (see figure).

Conclusions: We have demonstrated for the first time that GPR30 expression can be stimulated by estradiol, consistent with our previous findings of cyclic changes in endometrial GPR30. We also report that estradiol and a GPR30-specific agonist can reduce cAMP levels and increase phosphorylation of p42/44 MAPK in ECC-1 cells. To our knowledge, estrogen treatment has never before been shown to reduce cAMP levels in any mammalian tissue. Further work is focused on the mechanisms conferring GPR30 responses as well as confirming described responses in primary cell culture. (Supported by UNC Nova Carta Fund and NIH U54 HD-35041).



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Membrane Estradiol Receptors Interact with Metabotropic Glutamate Receptors in Hypothalamic Astrocytes. John Kuo,[1,2] Omid R Hariri,[1] Galyna Bondar,[1] Julie Ogi,[1] May S Shung,[1] Paul E Micevych.[1] *[1]Department of Neurobiology, Laboratory of Neuroendocrinology, Brain Research Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; [2]Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.*

Estradiol of ovarian origin induces the synthesis of progesterone in hypothalamic astrocytes that may regulate the estrogen positive feedback response and LH surge. Initial progesterone synthesis in astrocytes is mediated by estradiol acting on membrane estrogen receptors (mER) to increase free cytoplasmic calcium concentrations ($[Ca^{2+}]_i$). We demonstrate that this $[Ca^{2+}]_i$ flux requires involvement of the group Ia metabotropic glutamate receptor (mGluR1a). Co-immunoprecipitation using membrane fractions of hypothalamic astrocytes cultured from postpubertal female rats confirmed the potential for ERa and mGluR1 interaction. $[Ca^{2+}]_i$ flux in astrocytes was monitored with the calcium indicator, Fluo-4, and imaging performed on a Zeiss-510 Meta laser scanning confocal microscope. 17β-estradiol at 1 nM, 10 nM, and 100 nM induced a similarly robust $[Ca^{2+}]_i$ flux of 612 ± 42 relative fluorescent units (RFU) (n=15), 641 ± 47 RFU (n=18), 629 ± 34 RFU (n=16), respectively. These responses were all significantly greater than estradiol at 100 pM with $[Ca^{2+}]_i$ flux of 282 ± 18 RFU (n=25, $p<0.001$). However, even estradiol concentration as low as 100 pM induced a greater $[Ca^{2+}]_i$ flux than controls at 134 ± 16 RFU (n=14, $p<0.001$). Blockade of mGluR1a with the selective antagonist, LY367385 (20 nM), attenuated the estradiol (1 nM)-induced $[Ca^{2+}]_i$ flux from 639 ± 63 RFU to 186 ± 56 RFU (n=5, $p<0.002$). Conversely, high doses of mGluR1a agonist, (*S*)-3,5-dihydroxyphenylglycine hydrate (DHPG), at 100 nM was required to obtain a modest increase in $[Ca^{2+}]_i$ of 346 ± 25 RFU (n=19), which was significantly greater than 146 ± 13 RFU (n=17; $p<0.001$) for DHPG at 10 nM and 188 ± 20 RFU (n=17; $p<0.001$) for DHPG at 1 nM. DHPG did not substitute for estradiol, in fact, DHPG effects appear to be additive, where 1 nM of estradiol in combination with 100 nM of DHPG induced a significantly greater $[Ca^{2+}]_i$ of 790 ± 42 RFU (n=19) than 1 nM of estradiol ($p=0.006$) or 100 nM of DHPG alone ($p<0.001$). In conclusion, mER interacts with mGluR1a to mediate cytoplasmic calcium signaling in hypothalamic astrocytes that is necessary for estradiol induced progesterone synthesis. Supported by HD 42635 and HD 0001281.

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Early Leptin Treatment Decreases GnRH Expression in Later Stages of Hypothalamic and Pituitary Development of the Chicken Embryo. Colleen L Casey,[1] Ed Zelazny,[2] Cynthia J Forehand.[2] *[1]Department of Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA; [2]Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT, USA.*

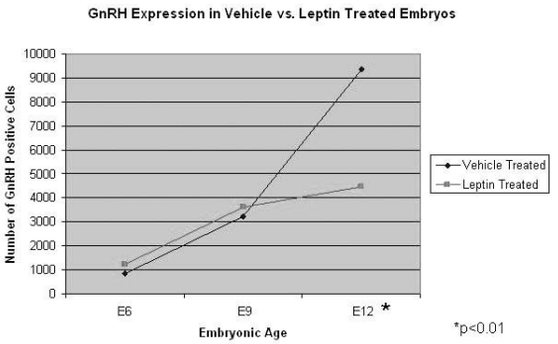
Background: Leptin plays a critical role in energy balance and reproduction and is critical to initiate puberty. We have previously shown that leptin receptors in the brain appear as early as embryonic day 3 (E3) in chicken embryos and that leptin treatment decreases cFos expression in the hypothalamus on E12.

Objective: To determine whether leptin treatment in the chicken embryo alters GnRH expression and, if so, at what stage of development this occurs.

Methods: Leptin (1 μg) in phosphate-buffered saline (PBS) or PBS alone was administered onto the chorioallantoic membrane of chicken embryos at 12 hour intervals from E3 to E5. Embryos were decapitated, fixed, equilibrated in sucrose, frozen and cryosectioned coronally at 30 μm at different ages (E6, E9 and E12). Sections were treated with primary antibody (Rb α LHRH). Control sections were obtained. Sections were then treated with secondary antibody. The total number of GnRH immunoreactive cells in the hypothalamus and pituitary were counted stereologically by a blinded investigator using MBF Bioscience Stereo Investigator Software. Differences between groups were compared by using Student's *t*-test (unpaired). A p value of <0.05 was considered statistically significant.

Results: GnRH was localized in the hypothalamus and pituitary of E6, E9 and E12 embryos in each treatment group (n=5 for all groups). There was a significant decrease in the number of GnRH positive cells compared to controls in E12 embryos that received leptin applications (4442 SEM ±678.1645 vs. 9932 SEM±1282.76, $p<0.01$). There were no significant differences in GnRH expression between groups at E6 or E9.

Conclusions: These results suggest that leptin treatment early in development inhibits GnRH expression at later stages of hypothalamic development. This decrease occurred between E9 and E12. To our knowledge, this is the first study investigating the impact of leptin treatment on the development of reproductive pathways during embryologic development.



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Elevated TNFα and Reduced Adiponectin in Blastocysts Exposed to Diabetic Conditions Correlates with Decreased AMPK Activity. Erica Louden, Maggie Chi, Tiffany Chi, Kelle H. Moley. *Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO, USA.*

Objective: Elevated TNFα levels are associated with lipid accumulation leading to insulin resistance and obesity. TNFα is believed to inhibit the hormone adiponectin, a known activator of AMPK, which modulates glucose uptake in muscle and blastocysts as recently demonstrated by our lab. We sought to determine if activation of AMPK rescues the diabetic blastocyst by reducing the effects of elevated TNFα on apoptosis, glucose uptake, decreased proliferation and lipid accumulation. **Methods:** The new inbred, polygenic Type 2 diabetic TallyHo mice (Jackson Laboratories) were obtained and bred in our animal facility. Age matched C57BL/6 mice were used as the control group. 2-cell embryos and blastocysts were collected from superovulated mice and cultured in either 1) control human tubal fluid (HTF) or 2) HTF with 25ug/ml metformin added. In parallel experiments, trophoblast stem cells (TS) were treated with IGF-1R siRNA as a hyperinsulinemic model. Deoxyglucose uptake was performed on insulin (500nM)-stimulated individual blastocysts. Apoptosis was detected by confocal microscopy using TUNEL assay and Topro-3 nuclear dye, and proliferation was detected using a BRDU assay. Embryos were scored for %TUNEL positive/total nuclei. AMPK activation, TNFα, adiponectin was analyzed by western immunoblot. Lipid accumulation was assayed by Oil Red O. **Results:** We found that diabetic blastocysts and TS cells with knockdown IGF-1R both demonstrated a 60% increase in lipid accumulation that correlated with decreased activation of AMPK. Furthermore, the lipid accumulation corresponded with an elevated level of TNFα and 45% increased apoptosis but reduced adiponectin. Treatment of either diabetic blastocysts with the AMPK activator metformin or TS cells with AICAR reduced apoptosis, and increased glucose uptake by 60% and proliferation. Similarly, knocking down TNFα with siRNA in the TS insulin-resistant cells also reversed apoptosis and the changes in glucose uptake. In addition we found that activation of AMPK reduced resorption rate in diabetic blastocyst. **Conclusions:** Women with obesity and insulin resistance experience poor pregnancy outcomes. Previously we have shown in mouse models of insulin resistance that AMPK activity is decreased and that activators of AMPK reverse the poor embryo outcomes. Here, we demonstrate that elevated TNFα, lipid accumulation and decreased adiponectin may explain these differences in AMPK activity. Targeted therapies at TNFα and adiponectin actions may be more effective at treating the reproductive failures in these women.

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Genetic Variation in Multiple Candidate Genes for Polycystic Ovary Syndrome Affect the Phenotype but Not Risk of PCOS. Olivier Valkenburg,[1] Sharon Lie Fong,[1] Andre G Uitterlinden,[2] Frank H de Jong,[2] Axel P Themmen,[2] Joop SE Laven.[1] *[1]Department of Gynecology and Obstetrics, ErasmusMC, Rotterdam, Zuid-Holland, Netherlands; [2]Department of Internal Medicine, ErasmusMC, Rotterdam, Zuid-Holland, Netherlands.*

CONTEXT: Polycystic ovary syndrome is a complex genetic disorder. Recent literature has identified a number of functional single nucleotide polymorphisms (SNPs) in genes that may have a role in the etiology of PCOS.

OBJECTIVE: In order to study the biological relevance of multiple functional SNPs with regard to PCOS, a two-way approach was applied. First, we studied the influence of multiple SNPs on clinical features. Subsequently, in order to identify risk alleles for PCOS, genotypes were compared in cases and controls.

SUBJECTS: 399 caucasian females with PCOS (2003 Rotterdam consensus criteria) and 3632 unselected controls (the Rotterdam study).
MATERIALS AND METHODS: Patients underwent clinical, ultrasound and endocrine evaluation and were genotyped by PCR (Taqman allelic discrimination assay). SNPs were selected in genes that regulate the synthesis and metabolism of sex steroids and glucocorticoids i.e. estrogen receptor alpha (PvuII and Xba1) and beta (3'UTR+38), aromatase (CYP19: A1531G), glucocorticoid receptor (GR: ER22/23EK, N363S, BclI and exon 9 beta), hexose-6-phosphate dehydrogenase (H6PD: R453Q) and 11beta-hydroxysteroid dehydrogenase type 1 (HSD11B1: 83557insA).
RESULTS: All SNPs were in Hardy Weinberg equilibrium. Homozygous carriers of the CYP19 1531G allele had higher levels of LH (7.8 vs. 6.9 U/l, P=0.01), 17 hydroxyprogesterone (17-OH-Pg) (92.7 vs 79.4 ng/dL, P=0.02) and androstenedione (369 vs 335 ng/dL, p≤0.01), but not testosterone. Haplotype analysis revealed that the polymorphic variant of exon 9 beta of the glucocorticoid receptor (GR) was associated with higher levels of adrenal androgens (androstenedione P=0.05, DHEA P=0.05, DHEAS P=0.02), whereas GR 363S was associated with significantly lower body mass index, 17-OH-Pg and lower frequency of insulin resistance. All polymorphisms except ER22/23EK, which was significantly less common among PCOS cases, were equally distributed among PCOS cases and controls.
CONCLUSION: Polymorphic variations in CYP 17, CYP19 and the cortisol receptor were associated with the severity of clinical features of PCOS, including obesity and hyperandrogenism. However, in a subsequent comparison with unselected controls, these polymorphic variants did not constitute risk alleles for PCOS.

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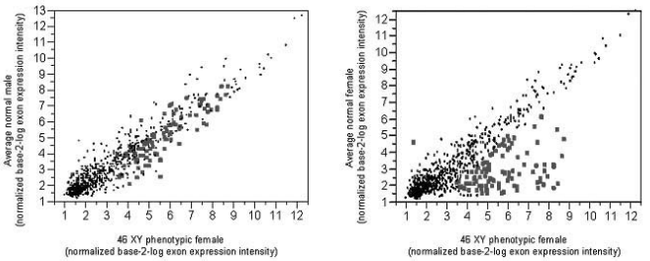
Novel Autosomal Target Genes May Be Responsible for SRY Positive Sex-Reversal: The Results of Exon Expression Analysis Eric D Levens,[1] Xiuli Xu,[2] Peter J Munson,[3] Lawrence M Nelson,[1] Alan H DeCherney,[1] Lynnette K Nieman.[1] *[1]Reproductive Biology and Medicine Branch (RBMB), NICHD, NIH, Bethesda, MD, USA; [2]Vascular Medicine Branch, NHLBI, NIH, Bethesda, MD, USA; [3]Mathematical & Statistical Computing Laboratory, CIT, NIH, Bethesda, MD, USA.*
Objective: Sexual development depends largely upon two sex-determination factors: ancestral Y chromosome sex-determining genes and autosomal genes responsible for masculinizing effects by incompletely understood mechanisms. Examination of 46, XY phenotypic females led to the discovery of the sex-determining region of the Y chromosome, SRY. To identify and characterize other genes responsible for male sexual differentiation, we examined a SRY-positive 46, XY phenotypic female.
Design: G-band karyotype, florescence in situ hybridization (FISH), and exon-level expression profiling experiments.
Materials and Methods: After informed consent was obtained, G-band karyotype analysis followed by FISH using probes to X, Y, and SRY was performed on an XY-phenotypic female patient. Total RNA isolated from PBMCs of 2 normal XX females, 2 normal 46, XY males, and the patient was subjected to Affymetrix GeneChip® Human Exon 1.0 ST Array Gene expression array. Exons demonstrating 8-fold differential expression in the patient compared to control males were analyzed. Additionally, relative expression of Y chromosome targets was compared between subjects. Comparative analyses were performed using JMP (SAS; Cary, NC).
Results: G-band analysis and FISH showed 46, XY karyotype in all cells tested. FISH identified SRY in all cells tested. As assessed by exon gene profiling, expression of SRY, ASMTL, USP9X, and RPS4Y1 (genes present on the Y chromosome) were similar in the patient and normal males. Interestingly, DAZ1 and EIF1AY, other Y-chomosome genes, showed more than 2-fold higher expression compared to normal males. When compared to normal females, a number of Y chromosome targets were over-expressed in the patient (Figure). For non-Y exons, compared to normal males, 189 were 8-fold over-expressed and 104 were 8-fold under-expressed in the patient.
Conclusions: Gene profiling studies in a 46, XY phenotypic female revealed normal or increased male-like transcription levels of many Y-chromosome genes. These data are consistent with the hypothesis that novel autosomal target genes may be responsible for SRY positive sex-reversed males.
Figure: The normalized base-2-log exon expression intensities of selected Y-chromosome targets in a 46, XY phenotypic female (red squares) compared to all Y-chromosome targets in normal males (left) and normal females (right). Red squares indicate probes showing 2-fold over- or under-expression compared to normal females, and fall close to line of identity when comparing to normal males.



Support: This research was supported, in part, by the RBMB, NICHD, NIH, Bethesda, MD.

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Is the Side Population Phenotype an Essential Characteristic for the Somatic Stem Cells in the Human Endometrium? Irene Cervello,[1] Alicia Martinez-Romero,[2] Jose A Martinez-Conejero,[1] Sebastian Martinez-Escribano,[1] Jose A Horcajadas,[1] Antonio Pellicer,[1] Jose E OConnor,[2] Carlos Simon.[1,3] *[1]Fundacion IVI-IUIVI, Univer Valencia, Valencia, Spain; [2]Lab of Cytomics, CIPF-UVEG, Valencia, Spain; [3]Valencia Stem Cell Bank, CIPF-UVEG, Valencia, Spain.*
Objectives: The endometrial somatic stem cell (SSC) population has been described by different groups; however, their characterization and isolation has not been yet achieved. As demonstrated in other tissues, we have have worked with the Side Population (SP) phenotype using the Hoechst extrusion dye as a tool to isolate the SSC from the human endometrium.
The aim of the present study was to determine if the SP technique is a reliable method to obtain the SSC population in the epithelium and stroma of the human endometrium.
Materials and Methods: Human endometrium from healthy donors was obtained. The stromal (n=42) and epithelial (n=7) fractions of the human endometrium, were isolated using an established protocol. Freshly isolated stromal and epithelial cells were incubated with Hoechst/ Ho+Verapamil during 90-120 min at 37ºC. Stromal and Epithelial SP and Non-SP were separated using a cell sorter and resuspended in serum medium. Both SP and Non-SP plus total epithelial or stromal fractions were compared and assessed for clonogenic activity, telomerase activity, undifferentiation markers, and markers for the hematopoietic and mesenchymal lineage.
Results: Stromal and Epithelial SP effluxed the vital dye and treatment with verapamil inhibited dye extrusion. The SP cells represented 0.93 ± 0.46% of the epithelial compartment and 0.25 ± 0.24% of the stromal compartment. Clonogenic activity was analyzed and the results of the cloning efficiency represent 1.28% of the stromal SP versus 1% of the Non-SP and 0.31% of the epithelial SP versus 0.05% of the Non-SP. The telomerase activity of both SP has an intermediate profile between the hESC line VAL4 and differentiated endometrial cells.
Moreover, SP and non-SP cells expressed typical undifferentiated markers although more enriched in the SP population. Both SP fractions were CD90+ indicating the putative mesenchymal origin.
Conclusions: We have isolated and characterized the stromal and epithelial SP of human endometrium. These results strongly suggest that the SP obtained by flow cytometry could represent an enrichment of the SSC population from the human endometrium.
Supported by SAF2004-0024 grant from Ministerio de Educación y Ciencia, Spanish Government.

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Protein Tyrosine Kinases in Leiomyoma Uteri: Inhibition of *Ex Vitro* Myometrial Growth with PDGFC-Antagonist. Yong Jiang,[1] Guangli Suo,[1] Xin Lu,[1] Rana Walley,[2] Linda Wasserman,[1] Bryan D Cowan,[2] Jean Wang.[1] *[1]Moores UCSD Cancer Center, University of California, La Jolla, CA, USA; [2]Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS, USA.*
Background and Objective: Receptor tyrosine kinases (TK) have been implicated in the proliferation of soft tissue tumors, and we hypothesize that specific TK activities are required for the growth of fibroids. The goal of this research is to determine potential molecular candidates to treat fibroid tissues.
Experimental Design, Materials and Methods: We constructed a custom microarray consisting of oligonucleotides derived from the known 90 human receptor TK genes, 45 TK-ligands, and several cyclins and Bcl2-family proteins.

We collected fibroid and adjacent normal tissues following hysterectomy with patient consent and institutional IRBs. To date, 840 data points for each gene from 420 arrays have been collected from 42 patients. The fluorescence readings collected from GenePix 4000B scanner were log-transformed, normalized with the Robust Quartile normalization method, and a Linear Mixture-Effect Model was used to calculate the mean log ratio (fibroid/normal), *p* value, and the false discovery rate (FDR) of signals.

In addition, we established primary cultures of uterine smooth muscle cells (USMC). After cells had grown to confluence, we treated the media with available anti-TK drugs.

Results: The custom microarray profiling identified 38 genes that were differentially expressed between normal and fibroid tissues ($p < 0.01$; FDR<0.13). Among them, 12 genes encode receptor TKs, 15 genes encode TK ligands, and 11 genes encode cell cycle and apoptosis proteins.

Among the ligand genes, we found that PDGFC mRNA was significantly upregulated in ~30% in the fibroid tissues. To determine the role of PDGFC in the proliferation USMC, we treated primary USMC culture established from fresh tissues with imatinib (inhibits PDGFR and Abl tyrosine kinase activities). We found that treatment with 5 μM imatinib significantly reduced the expansion of USMC in *ex vivo* cultures over a seven-day period.

Conclusion: Our results have demonstrated that PDGFC ligand is over expressed in more than 30% of fibroids, and the anti-TK imatinib inhibits *ex vivo* expansion of USMC. These results support the hypothesis that tyrosine kinase ligands are involved in fibroid growth, and the selection of anti-TK medications may offer molecular targets for treatment of fibroids.

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Epigenetic Control of Collagen Regulating Genes in Vascular Smooth Muscle. Renato Cappello,[1] Guadalupe Estrada,[1,2] Phillip M Gerk,[3] Jerome F Strauss,[1] Scott W Walsh.[1] *[1]OB/GYN, Virginia Commonwealth University, Richmond, VA, USA; [2]Direccion de Investigacion, Instituto Nacional de Perinatologia, Mexico City, DF, Mexico; [3]Pharmaceutics, Virginia Commonwealth University, Richmond, VA, USA.*

Epigenetics is the control of gene expression independent of changes in the DNA sequence. DNA methylation is a major epigenetic mechanism to silence gene expression and may serve as a protective mechanism to maintain cell integrity under non-inflammatory conditions. Collagen is an important protein that maintains the structural integrity of tissues. Disruption of smooth muscle collagen could result in vascular dysfunction in women with preeclampsia. 5-Aza-2'-deoxycytidine (5-Aza) inhibits DNA methylation, and so, can be used to study regulation of gene expression by DNA methylation. We hypothesized that epigenetic regulation of collagen regulating genes would be present in vascular smooth muscle cells (VSMC). **METHODS:** Primary cultures of human VSMC were seeded into T-25 flasks (40,000 cells/flask) and grown for 3 days to 70% confluence. The cells were treated for 24 hours with medium control or 5-Aza (10 μM). RNA was extracted from cell homogenates and analyzed for gene expression with an RT2 Profiler PCR Array System for Human Extracellular Matrix Genes (SuperArray). To determine the fold-change of gene expression, the results were first normalized to a housekeeping gene and then ΔΔCt was calculated across two RT-PCR arrays where group 1 was the control and group 2 was the experimental treatment. **RESULTS:** Table 1. **CONCLUSIONS:** 5-Aza increased MMP1 expression 3-fold which is consistent with a role for methylation in silencing MMP1 expression. Interestingly, genes involved in collagen synthesis (COL1A1) or inhibition of MMP1 activity (TIMP-1) were not affected. This suggests that collagen breakdown is epigenetically regulated, but collagen synthesis is not. Epigenetic regulation of MMP1 may be a protective mechanism to silence MMP1 expression and prevent collagen breakdown under non-inflammatory conditions. This regulation may be lost in women with preeclampsia. HL069851, Fogarty 5D43TW007692, P60MD002256.

Table 1. Fold Change in Gene Expression After Treatment with 5-Aza

MMP1	COL1A1	TIMP1
2.93	0.76	0.79

MMP1, matrix metalloproteinase-1; COL1A1, collagen type1, alpha 1; TIMP1, tissue inhibitor of matrix metalloproteinase-1

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Connexin31.1-Null Mutation in Mice Leads to Impaired Placental Development and Intrauterine Growth Restriction. Mark Kibschull,[1] Qingyi Zheng-Fischhoefer,[2] Klaus Willecke,[2] Elke Winterhager,[3] Stephen J Lye.[1] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [2]Institute of Genetics, University of Bonn, Bonn, Germany; [3]Institute of Anatomy, University of Duisburg-Essen, Essen, Germany.*

Differentiation in the mouse trophoblast cell lineage is characterized by a specific spatiotemporal regulation of connexin expression. Connexins form the intercellular gap junction channels which allow the exchange of molecules up to 1 kDa between adjacent cells. Connexins reveal a tissue specific distribution and have shared as well as unique functions.

Expression of the gap junction protein connxin31.1 is induced in the diploid cells of the trophoblast cell lineage after implantation. After chorio-allantoic fusion, connexin31.1 expression becomes restricted to the placental glycogen cells in the mature placenta.

In order to study the role of connexin31.1 we generated a knockout mouse for this gene and analyzed the impact of Cx31.1-deficiency on trophoblast differentiation and placental development.

Inactivation of the connexin31.1 gene in mice results in 30% loss of the embryos *in utero* after ED11.5. Cx31.1-deficient placentas are significantly reduced in weight after ED12.5 until birth and showed 85% of the weight of the wild-type placentas. This reduction in placental weight led to a significant reduction in embryonic weight at the end of pregnancy (Cx31.1 embryos had only 88% of the weight of wild type embryos). After birth the Cx31.1-deficient animals maintained a reduced weight compared to wild type controls.

The Cx31.1-deficient placenta shows impaired differentiation of the labyrinth and spongiotrophoblast layers at ED10.5. The spongiotrophoblast layer revealed an increased thickness and appears more compact, with enlarged cell sizes compared to wild type controls. Real-time mRNA analysis of marker genes for spongiotrophoblast differentiation like *Tpbpa, Pl-1* and *Cx31* indicate a reduction of diploid cell numbers in this layer. In the labyrinth layer the inter-labyrinth spaces tended towards reduced sizes and conversely the diameter of the fetal vessels tended towards increased areas, compared to wild type placentas. These placental malformations seem to be a critical parameter for embryonic nutrition and survival of the conceptuses.

In conclusion the connexin31.1 gap junction protein is critical for placental development and embryonic growth in mice.

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Autophagy Is a Physiologic Process Regulated by Glucose Availability in the Murine Preimplantation Blastocyst. Amber Cooper, Rachael Sheridan, Katie Boehle, Joan Riley, Kelle H Moley. *Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO, USA.*

Autophagy is a programmed process of protein degradation and recycling of the necessary cellular building blocks of glucose, amino acids and fatty acids. This process is critical to cell survival during periods of nutrient and specifically glucose deprivation, and during development and differentiation. We hypothesize that under normal physiologic conditions, the blastocyst stage embryo undergoes basal autophagy, but that autophagic activity is modulated by starvation, regulates selective changes in cell homeostasis and metabolism, and results in selective degradation of proteins, which may direct development and morphogenesis. In this study, we employed blastocyst stage embryos and trophoblast stem cells to test these hypotheses. We determined that autophagy is present in the murine blastocyst by electron microscopic identification of autophagosomes. In addition, we detected autophagic protein beclin 1 at all stages of embryonic preimplantation by confocal immunofluorescent microscopy as well as western immunoblotting. Beclin 1 protein also increased in expression in response to both physiologic (starvation of 0.2 mM glucose-3-fold increase) and pharmacologic (Rapamycin at 5 uM-5-fold) activation of autophagy, in a dose response fashion in blastocyst stage embryos (n=15; 0.2mM vs. n=17 in 2.8mM glucose; n=20 in 5uM Rapamycin vs. n=21 in control media). Similar results were found using trophoblast stem (TS) cells. Furthermore, the lysosomal membrane degradation product LC3 was detected in blastocysts and TS cell, both in a basal state as well as under physiologic and pharmacologic activation. Autophagic inhibition, with the compound 3-methyladenosine (3-MA), reversed these effects. Finally in individual blastocysts, by inhibiting autophagy with 3-MA, we detected a significant increase in the enzymatic activity of GAPDH, one of the long-lived enzyme proteins known to be degraded by autophagy (2.54+0.12 vs. 2.04+0.11pmol/embryo/hr). In conclusion, this is the first study to identify autophagic proteins in the murine blastocyst and to demonstrate physiologic and pharmacologic conditions that alter this process. These alterations correspond to metabolic

changes in the activity of key glycolytic enzymes. Poor pregnancy outcomes related to fluctuations in glucose availability may be avoidable and/or reversible by changing the pattern of autophagy.

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Increased Efficiency of Meiotic Silencing of Unsynapsed Chromatin in the Presence of Irradiation-Induced Extra DNA Double Strand Breaks.
Sam Schoenmakers,[1] Evelyne Wassenaar,[2] Peter de Boer,[3] Joop SE Laven,[1] J Anton Grootegoed,[2] Willy M Baarends.[2] *[1]Division of Reproductive Medicine, Obstetrics and Gynaecology, Erasmus MC University Medical Center Rotterdam, Rotterdam, Zuid Holland, Netherlands; [2]Reproduction and Development, Erasmus MC University Medical Center Rotterdam, Rotterdam, Zuid Holland, Netherlands; [3]Obstetrics and Gynaecology, University Medical Center St. Radboud, Nijmegen, Gelderland, Netherlands.*
CONTEXT: In meiotic prophase of male placental mammals, the heterologous X and Y chromosomes remain largely unsynapsed. This activates a mechanism named meiotic silencing of unsynapsed chromatin (MSUC), leading to formation of the transcriptionally silenced XY body. MSUC can also silence autosomal unsynapsed chromatin lacking a homologous pairing partner, but heterologous synapsis and escape from silencing can occur, when such non-homologous chromatin is not detected. In yeast and mammalian species, enzymatic formation of DSBs by SPO11 during leptotene precedes meiotic chromosome pairing. These DSBs are essential to achieve full synapsis of homologous chromosomes, and are subsequently repaired.
OBJECTIVE: To evaluate if the detection of non-homologous chromatin, and subsequent initiation of MSUC, might be linked to the presence of persisting, unrepaired meiotic DSBs in such non-homologous regions.
DESIGN: We exposed mice carrying 1^{13} and 13^{1} translocation chromosomes that display synaptic problems during meiotic prophase to 4 Gray of whole body irradiation with the intention to generate more (meiotic) DSBs.
RESULTS: We generated 25% extra meiotic DSBs by whole body irradiation of the mice. This led to a small significant increase in meiotic recombination frequency. We observed a 1.7-fold increase in the efficiency of MSUC of the non-homologous region of the small 1^{13} bivalent, following irradiation. However, the non-homologous region of the large 13^{1} translocation bivalent, escapes from MSUC in almost 100% of the nuclei, irrespective of exposure to irradiation.
CONCLUSION: We propose that activation of MSUC is functionally linked to the presence of unrepaired meiotic double strand breaks in the non-homologous region. In addition, spreading of synaptonemal complex formation from regions of homology may act as an opposing force against MSUC, and drive heterologous synapsis. Furthermore, we have shown that irradiation can be used to introduce extra meiotic DSBs in the male mouse.

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***In Utero* and Lactational Exposure to Nicotine: Alterations to the Intra-Ovarian IGF System.**
Carolyn E Cesta,[1] Jim J Petrik,[2] Alison C Holloway.[1] *[1]Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada; [2]Biomedical Sciences, University of Guelph, Guelph, ON, Canada.*
Introduction: It is well documented that cigarette smoking is associated with a number of adverse obstetrical outcomes yet 15-20% of all pregnant women smoke. In human populations there is evidence that *in utero* exposure to cigarette smoke results in decreased fertility in female offspring. We have demonstrated that fetal and neonatal exposure to nicotine, the major addictive component of cigarette smoke, results in impaired fertility and increased follicular atresia in the adult female offspring. We propose that this effect is due to disruption of the intra-ovarian IGF system. The IGF family includes the IGF ligands (IGF-I and -II), IGF binding proteins (IGFBP1-6) and IGF receptors (IGFR-I and -II). In most mammalian species IGFs and IGFBPs are considered, respectively as stimulators and inhibitors of follicular growth and maturation and the loss of IGF-I *in vivo* is associated with impaired ovulation and sterility. Therefore dysregulation of the intra-ovarian IGF system could have significant implications for ovarian function and subsequent fertility.
Objective: To determine if reduced fertility in adult rats exposed to nicotine during fetal and neonatal development can be attributed to alterations in the intra-ovarian IGF system.
Methods: Maternal rats were exposed to nicotine (1mg/kg/d) for 2 weeks prior to mating until weaning. Ovaries were collected on the morning of estrous from sexually mature saline and nicotine exposed offspring. IGF-I expression was quantified by in formalin-fixed paraffin embedded tissues. The expression of IGF-I, IGF-II; IGFR-I, IGFR-II; and IGFBP1-6 in the whole ovary was determined by semi-quantitative reverse transcriptase-PCR.
Results: Nicotine exposure significantly reduced IGF-I expression ($p<0.01$) relative to saline controls. Furthermore, nicotine-exposed offspring had significantly reduced IGF-II mRNA expression ($p<0.01$) in the ovary. There was no effect of nicotine exposure on the mRNA expression of any other component of the intra-ovarian IGF system.
Conclusion: Results from this study suggest that the decreased fertility and increased follicular atresia in nicotine-exposed animals may be due, in part, to disruption of the IGF regulation in the ovary.

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ChIPping at the Human Genome To Characterize Functional Estrogen Response Elements.
Christopher E Mason,[1] Roland G Kallen,[2] Sujun Hua,[3] Kevin P White,[3] Tianwei Yu,[4] Cheng Wang,[5] Caleb B Kallen.[5] *[1]Human Genetics, Yale University School of Medicine, New Haven, CT, USA; [2]Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; [3]Joint Institute for Genomics and Systems Biology, University of Chicago and Argonne National Laboratory, Chicago, IL, USA; [4]Department of Biostatistics, Emory University School of Medicine, Atlanta, GA, USA; [5]Gynecology and Obstetrics, Emory University School of Medicine, Atlanta, GA, USA.*
Until recently, few (<40) bona fide Estrogen Response Elements (EREs) were described and tested within the Human Genome. We employed whole genome ChIP-on-chip to identify the genomic locations of Estrogen Receptorα- (ERα)-bound cis-regulatory elements (EREs) in MCF7 breast cancer cells. ERE-like DNA sequences were identified at 1017 of the highest confidence ER-bound loci. The specificity of our approach was confirmed at selected ChIP loci using ChIP-PCR, gel shift, and luciferase reporter assays. At low stringency (up to 20% mismatch from the consensus ERE sequence), ~500 genomic loci demonstrated ~650 putative ERE sequences: roughly 75% of these loci contained a single ERE and ~25% demonstrated two or more predicted EREs detected within 2000 bp of the center of the respective ChIP site. Most ERα-bound EREs are not perfect 15 bp consensus EREs, the latter representing under 2.5% of all EREs identified. Our set of ERE-like sequences was therefore used to derive an improved position-weighted matrix that can be used to identify novel ERα-regulated elements. In addition, half of the ERα-bound loci in MCF7 cells do not possess an ERE. These likely represent sites of ERα "tethering" to non-ERE sequences via protein-protein interactions. Our data demonstrate that ERα binds to variant EREs in vivo with less sequence specificity than had previously been suspected. We have codified new sequence determinants that should accurately predict ERα binding to variant ERE sequences in vivo. Finally, our results indicate that many genes without EREs are subject to regulation by ERα, most likely via indirect binding.

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Effect of Betamethasone Treatment on Brain Cell Death and Neurosteroidogenic Pathways in a Guinea Pig Model of Growth Restriction.
Amy A McKendry,[1] Hannah K Palliser,[1] Della M Yates,[1] Robert KP Sullivan,[3] David W Walker,[2] Jonathan J Hirst.[3] *[1]Mothers & Babies Research Centre, University of Newcastle, Newcastle, NSW, Australia; [2]Physiology, Monash University, Clayton, VIC, Australia; [3]School of Biomedical Sciences, University of Newcastle, Newcastle, NSW, Australia.*
Objectives: Neurosteroids have potent neuroprotective actions in the fetal and adult brain. We have shown that key neurosteroidogenic enzyme, 5α reductase (5αR), is upregulated in response to acute hypoxia in the late gestation fetus and the resultant increase in 5α-reduced steroids reduces hypoxia-induced apoptotic cell death in the hippocampus (Yawno et al, 2007 Neurosci 146: 1726). Repeated treatment with betamethasone, a synthetic corticosteroid, may interfere with these processes and adversely affect the fetal brain. The aim of this study was to determine the effect of repeated betamethasone treatment on the expression of key enzymes in the neurosteroid synthetic pathway and on the level of apoptotic brain cell death in normal and growth restricted fetuses.
Methods: Placental insufficiency in guinea pigs was produced by the ablation of uterine artery branches at mid gestation (term 68d) in order to induce fetal growth restriction (GR). Sham and GR fetuses were treated with vehicle or betamethasone (1mg/kg/day) for 4 days prior to sacrifice (65d). Western blot and real time PCR were used to quantify the effects on key neurosteroidogenic enzymes (5αR and P450scc) in fetal brain, adrenal glands and placenta. Apoptotic cell death was assessed by the measurement of activated caspase-3 by immunohistochemistry in the fetal brain.
Results: Betamethasone treatment resulted in decreased placental weight as a proportion to body weight in normal pregnancies and reduced brain weight (inhibiting brain sparing) when administered during compromised

pregnancies. This treatment also reduced 5αR expression in the placenta by 51% (P=0.029), but not in the brain. Activated caspase-3 staining was not affected by betamethasone alone however, a marked increase in staining was seen in the cortex and hippocampus of GR fetuses receiving betamethasone.
Conclusions: These findings indicate betamethasone treatment while not affecting apoptotic cell death in normal fetuses, causes a potentially detrimental increase in cell death in fetuses already compromised. The placental deficit, due to betamethasone treatment, in the capacity to synthesize protective neurosteroids may contribute to these effects.

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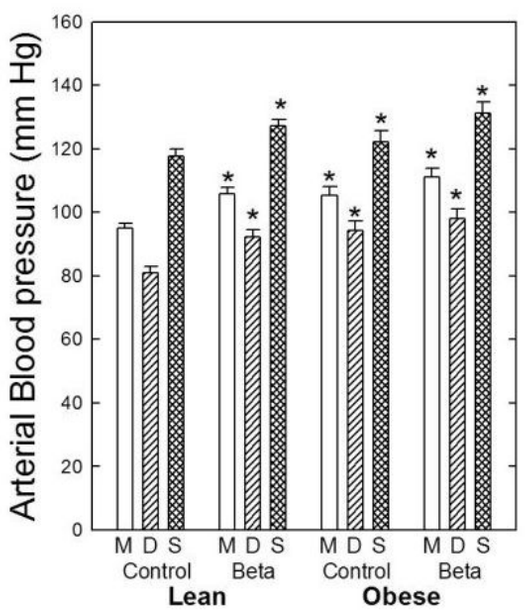
Effects of Diet-Induced Obesity on Arterial Blood Pressure in Adult Sheep Exposed Antenataly to Glucocorticoids. Jie Zhang, Victor M Pulgar, Angela G Massmann, Jorge P Figueroa. *Department of Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*
Obesity is considered a central component of the Metabolic Syndrome as increased adiposity is associated with insulin resistance and hypertension. In sheep, exposure to glucocorticoids (GC) in the perinatal period is associated with an elevation in blood pressure in adult life. Furthermore, antenatal exposure to GC has been shown to alter glucose tolerance in animals and in people. The aim of the present study was to determine the impact of diet induced obesity on blood pressure regulation in adult sheep exposed antenatally to GC.
METHODS: Pregnant sheep were treated with two IM doses of betamethasone (BM, 0.17 mg/kg) or vehicle (CTR) 24-hs apart at 80 days gestational age and allowed to deliver at term. At 9 mo of age, female sheep were randomly allocated to be fed at either 100% of recommended nutritional allowance or ad libitum for three months. Sheep were chronically instrumented under general anesthesia to place intravascular catheters. Arterial blood pressure was measured continuously for a minimum of 2 days. Data Mean±SEM were analyzed by ANOVA and/or two sample t test.
RESULTS: After 12 weeks sheep fed ad libitum had gained > 50% of the original weight, whereas sheep receiving 100% dietary allowance had stable weight. As shown on the figure, diet-induced obesity significantly increased arterial blood pressure in control and BM-exposed sheep. The highest blood pressure values were observed in the obese BM-exposed group.
CONCLUSION: Our data show that prenatal exposure to a single course of GC at 0.55 gestation has long-term effects on blood pressure control mechanisms. BM-exposed sheep exhibit elevated blood pressure that is further enhanced by obesity. HL 68728.



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Transgenerational Memory of Antenatal Synthetic Glucocorticoid Exposure: Modification of Stress Responsiveness. Alice Kostaki,[1] Sophie Petropoulos,[1] Dawn Li,[1] Stephen G Matthews.[1,2] *[1]Physiology, University of Toronto, Toronto, ON, Canada; [2]Ob-Gyn & Medicine, University of Toronto, Toronto, ON, Canada.*
Background: Synthetic glucocorticoids (sGC) are given to mothers at risk of preterm delivery to promote fetal lung maturation. Evidence is emerging indicating long-term effects of such treatment on endocrine function and behavior in offspring. However, virtually nothing is known concerning potential transgenerational influences on growth, endocrine function and behaviour. We hypothesize that repeated treatment of grandmothers (F0) with sGC will alter hypothalamic-pituitary-adrenal (HPA) function in F2 offspring with no manipulation of the F1 pregnancy.
Methods: Pregnant guinea pigs (F0) were subcutaneously injected with betamethasone (Beta; 1mg/kg) or vehicle (Veh) on gestational days 40/41, 50/51 & 60/61. Adult F1 female offspring from each group were mated with control males. HPA function was assessed in adult F2 offspring by non-invasive measurement of salivary cortisol concentrations: 1) under basal conditions, 2) during and following exposure to psychological stress (high frequency strobe light) or psychological/physical stress (forced swim) and, 3) following dexamethasone suppression.
Results: There was no effect of Beta (F0) on bodyweight from birth to adulthood in F2 offspring. Basal salivary cortisol in the BetaF2 females was lower than in the VehF2 group in the morning but not the afternoon; there were no differences in male F2 offspring. In contrast, both male and female BetaF2 failed to mount adrenocortical responses to psychological stress compared to VehF2 offspring that mounted robust responses ($P<0.02$). Swim stress induced robust adrenocortical responses in all groups ($P<0.0001$), however, the response was consistently lower in BetaF2 offspring. Beta exposure also led to a significant difference ($P<0.003$) in the cortisol response to dexamethasone suppression in female (F2) offspring, with a similar trend in males.
Conclusion: Prenatal exposure to sGC (F0) causes transgenerational programming of adrenocortical function in adult F2 offspring. Grand maternal exposure to Beta results reduced basal adrenocortical activity in BetaF2 female offspring, and caused stress hypo-responsiveness in both males and females. Dexamethasone suppression tests indicate altered central glucocorticoid feedback. These findings have important ramifications for the management of human preterm labor.
Support: Canadian Institutes of Health Research.

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Nitric Oxide Inhibits ACTH Induced Cortisol Production in the Long Term Hypoxic Sheep Fetus. Tshepo R Monau, Vladimir E Vargas, Kanchan M Kaushal, Steven M Yellon, Lubo Zhang, Charles A Ducsay. *Center for Perinatal Biology, Loma Linda Univ., Loma Linda, CA.*
BACKGROUND: We previously reported that in the sheep fetus, long term hypoxia (LTH) resulted in elevated basal $ACTH_{1\text{-}39}$ while basal cortisol levels were not different from normoxic controls. We also observed that LTH enhanced eNOS expression in sheep fetal adrenal. Since nitric oxide (NO) inhibits steroidogenesis in different tissue types, including adult adrenal cortex, we designed the present study to test the hypothesis that in the LTH fetus, endogenously expressed eNOS regulates cortisol to maintain basal cortisol secretion within a normal range.
METHODS: Pregnant ewes were maintained at high altitude (3,820 m) from ~40 days' gestation (dG) to near term (term = 146 days). Between 138-141 dG, fetal adrenal glands were collected from LTH and age-matched normoxic control fetuses (n=7-8 per group). Dual-staining immunofluorescence for eNOS and CYP17 was used to determine if cortisol producing cells express eNOS. Dispersed adrenal cortical cells were pre-treated with sodium nitroprusside (SNP;1.0 mM), L-NAME (1.0 mM), or L-arginine (2.0 mM) then challenged with 10nM ACTH. Cortisol responses were compared after 1 h. NOS activity was also measured in the adrenocortical cells.
RESULTS: eNOS co-localized with CYP17 in the zona fasciculata/reticularis cells of the adrenal cortex. ACTH induced cortisol secretion (ng/ml) was significantly higher in LTH vs. control (2.6 ± 0.3 vs. 1.1±0.3, respectively, $p<0.01$). In the presence of SNP, this response was reduced only 3% in the control group but in the LTH group, a significant 28% reduction in ACTH-mediated cortisol production was observed ($p<0.05$ compared to ACTH alone). L-arginine induced a similar effect with a significant (58%, $p<0.05$) reduction in cortisol in the LTH group. Inhibition of NOS with L-NAME significantly increased cortisol production in the LTH group (57%, $p<0.05$ compared to ACTH) while the effect on the control group was not significant. Following ACTH treatment, NOS activity was significantly higher in the LTH group compared to control (9.39 ± 1.19 vs. 3.86 ± 0.33; uM nitrite produced, $p<0.05$).
CONCLUSIONS: Together, these data indicate that LTH enhances sensitivity to the inhibitory effects of NO on cortisol production. NO may therefore play an important role in regulating ACTH-induced cortisol production in the LTH fetal adrenal. (NIH grant HD 31226).

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Growth Restriction Is Transmitted to the Next Generation Fetus with Compensation in Early Postnatal Life. Mary E Wlodek, Kerryn T Westcott. *Physiology, The University of Melbourne, Melbourne, Victoria, Australia.*

Objectives: In the rat, uteroplacental insufficiency restricts fetal growth and impairs mammary development further compromising postnatal growth. Both male and female small offspring have a reduced nephron endowment but only males develop hypertension with glomerular hypertrophy all of which can be reversed by improving the lactational environment. Female offspring have impaired uterine vascular function and increased vessel wall stiffness, which may restrict uterine blood flow during pregnancy, thereby altering fetal development, providing a mechanistic pathway for intergenerational programming. Our aim was to explore whether growth restriction and hypertension can be passed onto the next (F2) generation.

Methods: Uteroplacental insufficiency and fetal growth restriction of F1 offspring was achieved by bilateral uterine vessel ligation (Restricted, R) or Sham (Control, C) surgery on WKY rats on day 18 of pregnancy. F1R and F1C females (F1 generation) were mated at 10-15 weeks with normal males generating F2 offspring. Blood pressure (9 week tail-cuff; F2 day 18 of pregnancy) and body weight and dimensions (day 20 of pregnancy; postnatal days 6, 14 and 35; 9 weeks) were measured in F1 (F1C and F1R) and F2 (F2C and F2R) offspring.

Results: F1R and F2R litter size was reduced after birth ($p<0.05$), but not during pregnancy. The blood pressure of F2 females prior to or during pregnancy was not different between groups. F1R and F2R fetuses were smaller (F1R by 10%; F2R by 5%) and shorter than controls ($p<0.05$). F1R, but not F2R, offspring were smaller and shorter from birth to 9 weeks with a smaller head size early in life ($p<0.05$). Although body weight was not different between F2C and F2R offspring after birth, male pups had shorter crown rump length and smaller head length in early life ($p<0.05$). Male, but not female, F1R offspring were hypertensive by 9 weeks ($p<0.05$), with no blood pressure differences in F2 offspring at 9 weeks.

Conclusions: Our study demonstrates that uteroplacental insufficiency reduces litter size after birth and that this is transmitted to the subsequent generation. Adverse pregnancy adaptations may have contributed to the F2R fetal growth restriction during pregnancy. The F2R fetal growth restriction is compensated for in early postnatal life possibly through effects on lactation. Intergenerational transmission of growth restriction suggests that programming of hypertension and other diseases may emerge later in life.

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Failure To Suppress Adipogenic Transcription Factor (PPARγ) Activity Leads to Programmed Obesity in IUGR Offspring. Mina Desai,[1] Robert H Lane,[2] Guang Han,[1] Michael G Ross.[1] *[1]Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Dept. of Pediatrics, Univ. of Utah, SLC, UT, USA.*

Objective: Enhanced adipogenesis is one of key features of programmed obesity. Adipogenesis occurs via induction of adipogenic transcription factors, in particular PPARγ2, which promote adipocyte differentiation and lipid storage by adipocytes. We have established a rat model of maternal food restriction that results in IUGR newborns which paradoxically develop adult obesity. Specifically, IUGR offspring exhibit upregulation of the adipogenesis signaling cascade prior to the development of obesity. Sirtuin (SIRT1) is a stress-response and chromatin-silencing factor that represses the activity of PPARγ2 by recruiting corepressors (nuclear receptor corepressor, NcoR and silencing mediator for retinoid and thyroid hormone receptor, SMRT). This complex binds to specific DNA sequences in the promoter region of PPARγ target genes, and inhibits their transcription. We hypothesized that downregulation of SIRT1 activates PPARγ2 and enhances lipid accumulation in IUGR adipocytes.

Methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21. At birth, litter size was culled to 4 males and 4 females. All pups were nursed by Control dams and weaned at 3 weeks to ad libitum feed. At 1d and 9 months of age, adipose tissue was analyzed for SIRT1, NcoR and SMRT protein expression (Western Blot) in IUGR and Control male offspring. Data is normalized to β-actin (similar levels seen in IUGR and Control offspring) and presented as fold change.

Results: At 1 day of age, IUGR pups showed changes consistent with starvation with significantly increased protein expression of SIRT1 (1.5-fold, $P<0.01$) and SMRT (2.6-fold, $P<0.001$) as compared to Controls. However, the expression of NcoR was significantly reduced (0.5-fold, $P<0.01$) in IUGR pups. In contrast, at 9 months of age IUGR adults, now obese, had decreased expression of SIRT1 (0.5-fold, $P<0.01$) and SMRT (0.2-fold, $P<0.001$) with unchanged NcoR levels.

Conclusion: This suggests that in IUGR newborns, SIRT1 mediated repression of PPARγ2 transactivation may prevent lipid accumulation in IUGR adipocytes. However following obesity, downregulation of SIRT1 enhances activation of PPARγ2, leading to increased lipid accumulation and programmed obesity.

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Persistent Pulmonary Hypertension of the Newborn Even Following Transient Exposure of the Pregnancy to High Altitude. EA Herrera,[1,3,5] G Ebensperger,[1] RA Riquelme,[2] RV Reyes,[1] BJ Krause,[1] DA Giussani,[5] AJ Llanos.[1,3,4] *[1]Facultad de Medicina; [2]Facultad Cs Quimicas y Farmaceuticas; [3]INCAS, Universidad de Chile; [4]Universidad de Tarapaca, Chile; [5]University of Cambridge, United Kingdom.*

High altitude promotes pulmonary hypertension in man and animals. In addition, pregnancy at high altitude in women and animals yields neonates with persistent pulmonary hypertension (Keyes *et al*, 2003; Herrera *et al*, 2007), despite enhanced pulmonary NO function (Herrera *et al*, 2007). It remains unknown whether pulmonary hypertension of the newborn persists following transient, rather than sustained, exposure to high altitude during pregnancy. We investigated at sea level the effects on pulmonary function of newborn sheep whose mothers were transiently exposed to high altitude during pregnancy. **Methods:** Pregnant ewes were divided into two groups: conception, pregnancy and delivery at lowland (Santiago, 500m, LLL; n=10) and conception at lowland, taken to high altitude (Putre, 3,600m) from 30% of gestation until delivery, and return to lowland (LHL; n=4). Under general anaesthesia, lambs were instrumented with pulmonary catheters and studied at 7-8 d of age. Pulmonary arterial pressure(PAP) was measured *in vivo* during basal and acute hypoxic conditions before and after treatment with L-NAME. *Ex vivo* wire myography was performed on isolated small pulmonary arteries.

Results: LHL lambs had a higher basal PAP and a greater increment in basal PAP following NO blockade than LLL lambs(Fig. 1A). During acute hypoxia PAP in LHL relative to LLL reached higher values both during vehicle and following LNAME treatment(Fig. 1B). Isolated pulmonary arteries from LHL compared to LLL had a greater maximal contraction to K^+(1.34 ± 0.05 *vs.* 1.16 ± 0.05 N/m, Fig. 1C) and a higher sensitivity to SNP(7.31 ± 0.07 *vs.* 5.77 ± 0.12, Fig. 1D).

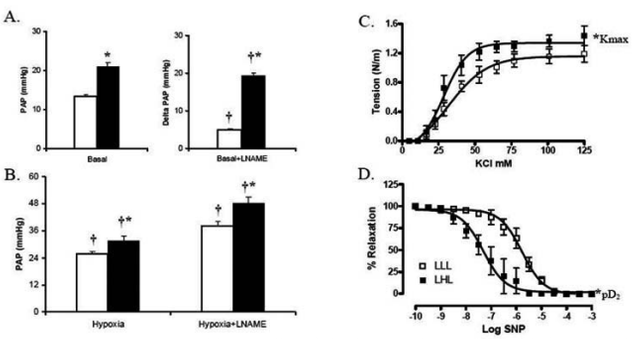

Figure 1: Pulmonary arterial pressure (PAP) in LLL (white) and LHL (black) lambs during basal and acute hypoxic conditions with and without treatment with L-NAME (A, B). Reactivity of small pulmonary artery isolated from LLL (white) and LHL (black) lambs (C,D). Significant differences (P≤0.05): *, LHL *vs.* LLL; †, *vs.* Basal period.

Conclusion: Transient exposure to high altitude during pregnancy still yields newborn offspring with persistent pulmonary hypertension, despite enhanced pulmonary NO function. These findings are relevant to pregnant women considering trips to high altitude.

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Adaptive Changes in Gene Expression in White Adipose Tissue Following Long Term Hypoxia in the Ovine Fetus. DA Myers,[1] K Hanson,[1] M Mlynarczyk,[2] KM Kaushal,[2] CA Ducsay.[2] *[1]Ob/Gyn, Univ. Oklahoma HSC, Oklahoma City, OK; [2]Ctr. for Perinatal Biol., Loma Linda Univ., Loma Linda, CA.*

Background: In the late gestation fetus/neonate, white adipose (WA) tissue expresses uncoupling protein (UCP) 1 , 2 and 3. UCP1 catalyzes adaptive thermogenesis normally associated with brown adipose tissue but is also expressed in fetal/neonatal WA. Adipose is also innervated by sympathetics and catecholamines play a key role in regulating both thermogenesis and lipolysis. Recently, adipocyte expression of neuropeptide Y (NPY) has been linked to stress-induced adipogenesis. We previously reported that long term hypoxia (LTH) in fetal sheep alters catecholamine biosynthesis in the adrenal medulla and function of the fetal hypothalamo-pituitary-adrenocortical axis.

We also found increased fetal plasma leptin levels with enhanced WA leptin expression. The present study was designed to elucidate the effects of LTH on expression of UCP1, UCP2, beta 3 adrenoreceptor (B3AR) and neuropeptide Y (NPY) in WA tissue in the late gestation sheep fetus.
Methods: Pregnant ewes were maintained at high altitude (3,820 m) from day ~ 40 to 137 of gestation when they were returned to the lab for placement of a maternal tracheal catheter. Reduced maternal PO_2 was maintained at a level comparable to that at altitude (~60 mmHg) by nitrogen infusion. Periadrenal WA was collected from LTH (n=6) and age-matched normoxic control fetuses (n=6) at 137 to 140 dG. Quantitative real time reverse transcription PCR was used to quantify mRNA. Cyclophilin was used as a housekeeping mRNA. All data are presented as fg mRNA/50 ng total RNA.
Results: LTH significantly increase UCP1 mRNA (133.4 ± 60.4 control vs. 450.3 ± 119.3 LTH; p=0.04); UCP2 mRNA was not different between groups (12.4 ± 3.1 control vs. 17.6 ± 1.8 LTH). B3AR mRNA was also significantly elevated in WA tissue from LTH fetal sheep (1.2 ± 0.4 control vs. 3.9 ± 0.7 LTH; p=0.01). Similar to UCP2, NPY mRNA was not altered in response to LTH (117.6 ± 62.3 control vs. 210.9 ± 98.9 LTH).
Conclusions: LTH significantly increases thermogenic UCP1 but not the lipogenic UCP2. In addition, an increase in B3AR mRNA suggests an increased sensitivity to sympathetic activation of adipose tissue at birth in the LTH fetus. We found no evidence of increased stress sensitive adipose NPY expression. Thus, in addition to leptin, LTH alters expression of key genes regulating adipose function in fetal sheep. (Supported my NIH grants HD33147 and HD31226).

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Specification of the Trophectoderm Lineage and Production of Trophoblast Giant Cells. Matthew J Kohn, Rieko Yagi, Zakir Ullah, Melvin L DePamphilis. *LMGR, NICHD, Bethesda, MD, USA.*
In mammals, the trophectoderm (the outer layer of cells in a blastocyst) is the first lineage to arise. It is required for implantation of the embryo and produces the placenta. Mammals have four, highly conserved, TEAD transcription factors, two of which, TEAD2 and TEAD4, are expressed during zygotic gene activation. We discovered that only TEAD4 is required for preimplantation development, and that it is the earliest transcription factor expressed during mammalian development that is known to be required for specification of the trophectoderm. In the absence of TEAD4, the POU transcription factor OCT3/4, which is required for formation and pluripotency of the inner cell mass, is expressed in all blastomeres. Moreover, the homeobox transcription factor CDX2, previously identified as a key determinant of trophectoderm cell fate, is not expressed in the absence of TEAD4. By cotransfection and microinjection of Cdx2 promoter reporter constructs, we found that TEAD4 is a site-specific activator of Cdx2 transcription, but only in the presence of the YAP1 coactivator protein. Thus, Cdx2 is a direct transcriptional target of TEAD4.
Trophoblast giant (TG) cells are a direct descendent of the trophectoderm that is required for implantation. TG cells are one of only two cell types in mammals that are programmed for endoreduplication, multiple rounds of genome duplication without mitosis. We have now identified CDK1/CDC2 as the key regulator for the transition from mitotic genome duplication to endoreduplication. CDK1, in cooperation with a Cyclin, is the protein kinase required for entry into mitosis. Specific inactivation of CDK1 using either a chemical inhibitor or siRNA rapidly triggered both endoreduplication and differentiation of trophoblast stem cells into TG cells. Normally this transition is triggered by withdrawal of FGF4, which results in a rapid increase of the CDK-specific inhibitors p21 and p57 with concomitant suppression of CDK1 activity and initiation of endoreduplication. The ability to induce both endoreduplication and differentiation of trophoblast stem cells simply by inactivating CDK1 implicates CDK1 as the ultimate target of FGF-signaling. TG cells survive this event whereas embryonic stem cells do not, because TG cells inactivate the DNA damage checkpoint, thereby enabling them to escape apoptosis. These results mark defining stages in the molecular events that specify the trophectoderm lineage and production of trophoblast giant cells.

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eNOS Promotes Utero-Placental Remodeling and Blood Flow, and Feto-Placental Oxygen Delivery during Pregnancy in Mice. Shathiyah Kulandavelu,[1,2] J Mu,[2] K Whiteley,[2] S Lee Adamson.[1,2] *[1]Physiology, Obstetrics & Gynecology, U of T; [2]Samuel Lunenfeld Research Institute, ON, Canada.*
Introduction: In mice lacking the eNOS gene, the normal increases in maternal cardiac output and enlargement of the heart and aorta during pregnancy are blunted, and fetal body weight is reduced (-17%). Given that eNOS is expressed in the uterine and umbilical vasculatures, we hypothesized that their remodeling would also be blunted in pregnant eNOS -/- mice, leading to an elevated vascular resistance and decreased blood flow to the placenta contributing to fetal growth restriction.
Methods: Utero- and umbilical-placental blood velocity waveforms and umbilical arterial diameters were measured using 40 MHz ultrasound biomicroscopy in control (C57Bl/6J) and eNOS -/- mice at 17.5 days of pregnancy (N=6 mothers). Spiral artery and fetal capillary morphologies, and uterine arterial diameters were evaluated from vascular corrosion casts. Tissues were collected for Hydroxyprobe-1 and actin immunohistochemistry to identify hypoxic and smooth muscle regions. We calculated Resistance Index ((S-D)/S) from systolic (S) and diastolic (D) velocities, and blood flow from mean velocity and vessel area.
Results: Calculated uterine blood flow normalized to the weight of the uterus and its contents was 55% lower ($P<0.05$) in pregnant eNOS -/- mothers due to large reductions in uterine artery diameter (-33%, $P<0.001$) and mean velocity (-17%, $P<0.05$). Uterine arterial Resistance Index was 52% higher ($P<0.001$), and the spiral arteries were less coiled and contained more smooth muscle actin in eNOS -/- mice than controls. More intense hypoxic immunoreactivity was detected in the spongiotrophoblast and trophoblast giant cell layers of the junctional zone of eNOS -/- placentas, whereas fainter staining was only detected in the spongiotrophoblast cell layer in controls. In the umbilical circulation, flow normalized to fetal weight was not significantly changed although the Resistance Index was slightly elevated (4%, $P<0.05$) and capillary lobule length was reduced by (-28%, $P<0.05$). Fetal organs showed increased hypoxic immunoreactivity suggesting reduced organ oxygen delivery in eNOS -/- fetuses.
Conclusions: Results suggest that eNOS plays an important role in uterine and spiral artery remodeling and in augmenting utero-placental blood flow during pregnancy. It also appears to enhance oxygen delivery to the placenta and fetus. eNOS may contribute to normal fetal growth by these mechanisms.

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Integrin $\alpha5\beta1$ Involves the Angiogenesis Formed by Placenta-Derived Multipotent Mesenchymal Stromal Cells. Chie-Pein Chen,[1,2] Jian-Pei Huang,[1] Yi-Hsin Wu,[2] Chia-Yu Chen,[2] Pei-Chun Chen,[2] Chun-Chuan Ko,[2] Ming-Yi Lee.[2] *[1]Division of High Risk Pregnancy, Mackay Memorial Hospital, Taipei, Taiwan; [2]Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan.*
Objective: The aims of this study are to investigate if human placental multipotent mesenchymal stromal cells (hPMCs) are capable of differentiation into endothelial cell lineage and if the integrin $\alpha_5\beta_1$ molecule involved in the process of angiogenesis formed by the differentiated hPMCs in vitro and in vivo.
Methods: hPMCs isolated from term placentas were assessed for their phenotype markers, mutilineage capacity, and the expression of integrin molecules. The hPMCs were induced to endothelial cell differentiation in the presence of Endothelial Cell Growth Medium 2 with 2% of FCS and 50 ng/ml VEGF for 14 to 21 days. The angiogenesis ability of these cells was demonstrated by using an in vitro angiogenesis kit and in vivo chick chorioallantoic membrane assay from ten-day-old embryos. Blocking antibodies specific to integrin α_4, α_5, and β_1 subunits were used to inhibit its capillary-like tube formation and angiogenesis ability in vitro and in vivo.
Results: hPMCs were demonstrated to be multipotent. The cells expressed high levels of integrin subunits α_2, α_4, α_5, and β_1, very low level of integrin β_2, but no β_4. Under differentiation condition, hPMCs showed several characteristics of endothelial cells, including the expression of CD31, CD34, CD105, von Willebrand factor, VE-cadherin and VEGF receptors 2. The differentiated cells significantly increased the expression of integrins α_5 and β_1, but not α_4. These differentiated endothelial cells could form capillary-like tube structure in vitro and increase the neovascularization in the chick chorioallantoic membrane assay. The cells that involved in capillary-like structure in vitro or neovaecularization in vivo also expressed von Willebrand factor, CD31 and CD105. However, the capillary-like tube structures in vitro or neovascularization in vivo were inhibited when the differentiated cells were pretreated with blocking antibodies specific to integrin α_5 and β_1 subunits, but not integrin α_4.
Conclusions: These observations provide evidences that hPMCs have the potential for endothelial cell differentiation and the ability of neovascularization formed by differentiated hPMCs is mediated by integrin $\alpha_5\beta_1$. Differentiated hPMCs may participate in the placenta angiogenesis and vasculogenesis.

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Urocortin 2 and Urocortin 3 mRNA and Protein Expression Are Increased in Preeclamptic Placentae Specimens. Alberto Imperatore,[1,2] Alessandro Rolfo,[3] Felice Petraglia,[2] John RG Challis,[1] Isabella Caniggia.[3] *[1]Departments of Physiology, Obstetrics and Gynecology and Medicine, University of Siena, toronto, ON, Canada; [2]Department of Pediatrics, Obstetrics, and Reproductive Medicine, University of Siena, Siena, Italy; [3]Department of Obstetrics and Gynecology, Samuel Lunenfeld Research Institute, Toronto, ON, Canada.*

Objective: Urocortin 2 (Ucn2) and urocortin 3 (Ucn3) are new members of the CRH family of peptides binding selectively the CRF-R2. Their expression and localization in human placenta has been described but their regulation remains unknown. Preeclampsia (PE) is a pregnancy-related disorder in which oxidative stress plays a central role in disrupting maternal endothelial functions and decreased placental perfusion. The aims herein were to investigate: 1) effects of low-oxygenation on placental explants and trophoblast cell culture Ucn2-Ucn3 expression; 2) first trimester explants Ucn2-Ucn3 expression after hypoxia-reoxygenation (HR); 3) Ucn2-Ucn3 mRNA and protein expression in preeclamptic specimens. **Materials and Methods:** first trimester placental explants were obtained from voluntary pregnancy interruption and: 1) incubated at 20%O_2, 8%O_2 or 3%O_2; 2) exposed to HR. Trophoblast cell culture were obtained from healthy women undergoing elective cesarean section and treated with different oxygen tensions. Specimens from preeclamptic placentae were collected from women undergoing elective cesarean section. Total mRNA and proteins were extracted for real-time qPCR and western blotting analysis. **Results:** an increase in Ucn2-Ucn3 transcript occurs when oxygen tension is low. Significantly higher Ucn2-Ucn3 mRNA expression was found when first trimester explants were kept in hypoxic environment. Moreover, Ucn2-Ucn3 transcript levels in first trimester explants exposed to HR were higher than in controls. Also in placental cell culture, Ucn2-Ucn3 transcript levels were ten to thirtyfold higher when exposed to 3%O_2. PE samples expressed significantly higher Ucn2-Ucn3 mRNA and protein levels than controls. **Discussion:** Placental Ucn2-Ucn3 mRNA expression is sensitive to O_2 tensions suggesting that these peptides may be involved in trophoblast differentiation and proliferation at low oxygen conditions. Moreover, their increase in PE samples suggests their possible involvement in the pathogenesis of preeclampsia, an oxidative stress-related condition.

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A Role for MicroRNAs in Human Parturition. Daniel Montenegro,[1] Roberto Romero,[1,2] Sung-Su Kim,[1] Adi Tarca,[1,3] Sorin Draghici,[3] Juan Pedro Kusanovic,[1] Jung-Sun Kim,[1,4] Offer Erez,[1] Francesca Gotsch,[1] Sonia S Hassan,[1,5] Chong Jai Kim.[1,4] *[1]Perinatology Research Branch, NICHD, NIH, DHHS, Detroit, MI, USA; [2]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA; [3]Department of Computer Science, Wayne State University, Detroit, MI, USA; [4]Department of Pathology, Wayne State University, Detroit, MI, USA; [5]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*

Objective: microRNAs (miRNAs) are critical regulators of gene expression that promote mRNA degradation or translational repression. miRNA expression profiling of the preterm and term chorioamniotic membranes was performed by microarray analysis. The purpose was to determine gestational age-dependent changes in miRNA expression and to assess the functional significance of miRNA in human parturition.

Methods: The expression profile of 455 miRNAs in the chorioamniotic membranes from term (n=10) and preterm gestations (n=10) was determined using miRCURY™ locked nucleic acid microarrays. Samples were labeled using the miRCURY™ Hy3™/Hy5™ labeling kit and hybridized on the miRCURY™ LNA Array (v.8.1). Endogenous miR-338 in primary decidual cells was inhibited by transfection with anti-miR-338. miR-338 and cytosolic phospholipase A2 (cPLA2) mRNA expression was determined using TaqMan assays.

Results: 1) The expression of ten microRNAs (miR-449, miR-136, miR-199a*, miR-142-3p, miR-25, miR-202*, miR-101, miR-135a, miR-154, miR-338) was lower in term chorioamniotic membranes than in those of preterm gestations ($p<0.05$); 2) The change in miR-338 expression detected by microarray was confirmed by real-time qRT-PCR (fold change = 2.12; $p<0.05$); 3) Computational analysis identified cPLA2 as a putative target of miR-338; 4) The inhibition of endogenous miR-338 with anti-miR-338 resulted in a significant increase in the mRNA expression of cPLA2 (fold change = 3.7; $p<0.05$), a phospholipase implicated in human parturition.

Conclusions: 1) Microarray studies demonstrated that all the differentially expressed miRNAs in the chorioamniotic membranes decreased with advancing gestational age; 2) Cytosolic phospholipase A2 was identified as a target for miR-338; 3) Collectively, these observations suggest that miRNAs play a role in the control of human parturition.

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Adenosine 3',5' Cyclic Monophosphate (cAMP)-Protein Kinase A (PKA) Pathway Stimulates Human Trophoblast Placental Growth Factor (PlGF) Gene Expression. Christophe Depoix, Robert N Taylor. *Gynecol/Obstet, Emory University, Atlanta, GA, USA.*

Objective: Cyclic AMP regulates genes controlling trophoblast invasiveness and differentiation. A cDNA microarray study of forskolin-treated BeWo cells revealed a 10-fold upregulation of PlGF. We confirmed and extended these results by characterizing the pathway regulating this important angiogenic protein.

Methods: PlGF was analyzed by real-time quantitative RT-PCR and ELISA in JEG3 choriocarcinoma cells and placental explants treated with activators of the cAMP signaling pathway. JEG3 cells also were transfected with a PlGF promoter-luciferase vector to assess transcriptional activation.

Results: Treatment of JEG3 cells with 1 mM dibutyryl cAMP for 8^h induced a 7-fold increase in steady-state PlGF mRNA, accompanied by a 4-fold increase in protein secretion. Treatment of placental villi with 1 mM cAMP increased PlGF mRNA 3-fold. Treatment with 10 μM forskolin, which directly activates adenylate cyclase, increased PlGF mRNA in JEG3 cells and placental villi by 9- and 3-fold, respectively. To determine if $G\alpha_s$ regulated the PlGF gene, we treated the samples with cholera toxin. PlGF mRNA increased in JEG3 cells and villi by 9- and 2-fold using this toxin. PlGF protein secretion from JEG3 cells treated with cholera toxin also increased 9-fold. To analyze transcriptional regulation, JEG3 cells were transiently transfected with a vector containing 3.5 kb of the human PlGF promoter. Treatment with the same compounds showed similar stimulation. The primary protein kinase involved in cAMP signaling is PKA. To confirm that PKA was responsible for increased PlGF expression, we treated JEG3 cells with a combination of forskolin or cholera toxin and the specific inhibitor of PKA, H89. 10 μM H89 blunted forskolin and cholera toxin-induced PlGF mRNA accumulation by 56% and 65%. The effect of H89 was even stronger in transient transfection experiments, inhibiting PlGF promoter activation by 85% and 90%, respectively.

Conclusions: Our experiments demonstrate that PlGF mRNA and protein production in JEG3 cells and placental explants are regulated robustly by cAMP via PKA. Current studies focus on cytokines that activate this pathway upstream and transcription factors that mediate downstream effects on the PlGF gene promoter. We postulate that these approaches will lead to therapeutic strategies to modulate PlGF production in clinical conditions such as preeclampsia and fetal growth restriction.

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Palmitic Acid Exposure Alters Trophoblast Apoptotosis and Proliferation. Emily S Jungheim, Joan K Riley, Kelle H Moley. *Obstetrics & Gynecology, Washington University, St. Louis, MO, USA.*

Background and objective: Maternal obesity is associated with complications in pregnancy including an increased risk of developing preeclampsia. Elevated circulating levels of long chain saturated fatty acids like palmitic acid (PA) have been implicated in some of the morbidity associated with obesity as they have been found to induce apoptosis and alter cellular proliferation in several different cell types. It has been suggested that endoplasmic reticulum stress plays a role these lipotoxic events. Given that altered proliferation and apoptosis of the trophoblast have been implicated in the development of preeclampsia, we sought to determine the effect of PA on TS cells.

Methods: TS cells generated from murine C57BL/6 embryos were cultured in varying doses of palmitic acid (0mM-0.6 mM) for 30 hours and subjected to terminal uridine deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Apoptosis was quantified by BD FACSCalibur flow cytometer. An ELISA for bromodeoxyuridine (BrdU) uptake was also performed as a measure of cellular proliferation, and resultant optic density was quantified by spectrophotometer. Treated TS cells were also subjected to SDS-PAGE and Western blot analysis for expression of GRP78, a marker of endoplasmic reticulum stress.

Results: Cellular proliferation as measured by BrdU uptake was decreased in TS cells in response to palmitic acid (OD reading: 0mM PA=2.98, 0.4mM PA=2.64, 0.6mM PA 2.69), and apoptosis as determined by TUNEL assay increased with increasing doses of palmitic acid (geometric mean: 0mM

PA=38.24, 0.4mM PA=55.07, 0.6mM PA=155.32). GRP78 expression also increased in response to increasing doses of PA (fold change: 0mM PA=1, 0.4mM PA=1.39, 0.6mM PA=1.30).
Conclusion: This is the first study to demonstrate the apoptotic and anti-proliferative effect of PA on TS cells. Endoplasmic reticulum stress may play a role in the liptoxicity of PA on TS cells. Elevated circulating levels of long chain saturated fatty acids like those seen in obesity may prove to be a contributing factor in the development of preeclampsia.

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Leukemia Inhibitory Factor Promotes Human Extravillous Trophoblast Adhesion to Extracellular Matrix and Secretion of Tissue Inhibitors of Metalloproteinases-1 and -2. Evdokia Dimitriadis,[1] Alejandro Tapia,[1] Ursula Manuelpillai,[2] Lois A Salamonsen.[1] *[1]Prince Henry's Institute of Medical Research, Melbourne, Victoria, Australia; [2]Obstetrics and Gynaecology, Monash University, Melbourne, Victoria, Australia.*
BACKGROUND Leukemia inhibitory factor (LIF) is essential for blastocyst implantation in mice. In humans, implantation involves the apposition, adhesion and migration of trophoblast across the endometrial luminal epithelium. This is followed by the differentiation of trophoblast into the invasive phenotype, the extravillous trophoblast (EVT), which can transverse the uterine stroma and engraft the maternal blood vessels. LIF is produced by human EVT cells and it has been suggested may play a role in EVT invasion, however very little is known about its mechanisms of action. The aim of this study was to examine whether LIF regulates EVT adhesion. **METHODS** Primary first trimester human EVT cells were isolated, characterised and treated with/without LIF (N=4 per group) for 24 hours. The effects on EVT cell adhesion to fibronectin (FN), vitronectin (VN), laminin (LN) were assessed. EVT integrin and extracellular matrix (ECM) mRNA expression was determined by PCR arrays containing 96 genes. EVT integrin (IT) molecule mRNA expression was examined by quantitative real-time RT-PCR. Matrix metalloproteinase (MMP) 2 and MMP9 secretion was assessed by gelatine zymography and tissue inhibitors matrix metalloproteinase (TIMP) -1 and TIMP-2 secretion by ELISA. Results were expressed as mean±SEM and % change from control. Data was analysed by the Mann Whitney test with appropriate post-hoc analysis. **RESULTS** EVT cells showed maximal binding to FN followed by VN and LN. EVT cell adhesion increased to all three ECM elements in response to LIF compared to controls (20±3%, FN; 20±4%,VN and 29±3%, LN; $p<0.05$ respectively). EVT cell adhesion molecule mRNA expression was consistent with an in vivo EVT phenotype. ITα1, α5, α6, αv, β1 and β3 mRNA did not change, but ITβ4 mRNA levels decreased by 50±4% following LIF treatment compared to controls ($p<0.05$). MMP2 and MMP9 secretion was not affected by LIF but there was a significant increase in TIMP-1 and -2 secretion following LIF treatment compared to controls (12±2%, 18±3% $p<0.05$ for TIMP-1 and -2 respectively). **CONCLUSION** This is the first study to demonstrate LIF regulation of EVT adhesion and ITb4 mRNA expression. This supports an important role for LIF in trophoblast invasion and early placental development.

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Maternal Systemic Inflammation Reduces Neural Progenitor Cells: Involvement in Cerebral Palsy Etiology. Natalia S Mattan,[1] Julie Boles,[2] Cristina A Ghiani,[1] Michael G Ross,[2] Jean de Vellis.[1] *[1]Mental Retardation Res. Ctr., Semel Inst. for Neurosci. and Human Behav., David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; [2]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*
Objective: A hallmark of cerebral palsy is underdeveloped white matter and poor myelination, likely due to impaired oligodendrocyte development. *Fetal* infection appears to contribute to central inflammation and potentially cerebral palsy, yet there is little understanding of the potential adverse effects of systemic *maternal* infection/inflammation on fetal brain development. In the present study, we sought to determine the effect of maternal lipopolysaccharide (LPS) injection on fetal brain cytokine levels and on neural progenitor development, with particular focus on oligodendrocyte development.
Methods: Pregnant Sprague Dawley rats were randomized to receive two consecutive injections of LPS (200 µg/ kg) or saline on gestational day 15 and 16. The dams were sacrificed on day 18, and fetuses rapidly extracted of which some were fixed in 4% paraformaldehyde to be processed for immunohistochemistry. The forebrains of littermates were dissected out to prepare whole tissue extract for western blot or to extract total RNA. RNA levels of cytokines were determined by real time RT-PCR. Expression of neural progenitor markers was determined by western blot and immunohistochemistry.
Results: Following maternal LPS injection, IL-6 mRNA levels were significantly increased in fetal forebrains as compared to saline-injected dams. LPS-treated fetuses displayed lower expression of neural cell markers, including markers for both committed and uncommitted progenitors.
Conclusions: Maternal systemic inflammation during early stages of fetal brain development induces a brain inflammatory (i.e., cytokine) response and affects the expression of oligodendrocyte cell markers. These findings suggest that maternal infection/inflammation may interfere with fetal neural progenitor development and contribute to the development of cerebral palsy.

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Retinol Binding Protein Polymorphisms and Gestational Diabetes. Mark K Hiraoka, Johann Urschitz, Omar Sultan, Kenneth Ward. *Pacific Research Center for Early Human Development, University of Hawaii, John A. Burns School of Medicine, Honolulu, HI, USA.*
Objective: Native Hawaiians have a higher prevalence of type 2 diabetes (T2DM), a higher prevalence of gestational diabetes (GDM) and higher complication rates associated with GDM. This increased incidence is multi-factorial and is likely to include genetic factors. Increased plasma levels of retinol binding protein 4 (RBP4), a transport protein for retinol (vitamin A), have been associated with T2DM and GDM. Recently, it was discovered that RBP4 is produced in adipose tissue and plays a role in insulin resistance. Single nucleotide polymorphisms (SNPs) in the RBP4 gene have been linked to serum RBP4 levels and to occurrence of T2DM. Hypothesizing a link between RBP4 and GDM, we performed a candidate gene study to look for an association between an important RBP4 gene polymorphism (rs3758539) and GDM.
Methods: Blood was collected from Caucasian, Asian and Pacific Islander women who met the Coustan-Carpenter criteria for GDM and from ethnically matched non-diabetic controls. DNA was extracted and real time PCR technology (*TaqMan, Applied Biosystems)* used to screen for the rs3758539 SNP located 5' of exon 1 of the RBP4 gene.
Results: Genotype and allele frequencies in the controls and GDM cases were tested using chi-square contingency tests. The findings are displayed in the table below. Genotype frequencies were in Hardy-Weinberg equilibrium. There was no association between the rs3758539 RBP4 SNP and GDM in the Caucasian, Asian or Pacific Islander groups.
Conclusion: Curiously the rs3758539 RBP4 SNP was not found to be associated with GDM. This study was adequately powered to detect association in the Caucasian and Asian group. The absence of association suggests that GDM and T2DM may have more divergent molecular pathophysiology than previously suspected.

rs3758359 SNP and Allele Frequenices

	CC Genotype	TT Genotype	CT Genotype	T Allele
Caucasian GDM (n=88)	64%	1%	35%	17%
Controls (n=315)	72%	3%	24%	15%
Asian GDM (n=71)	78%	1%	21%	12%
Controls (n=99)	82%	1%	17%	10%
Pacific Islander GDM (n=19)	95%	0%	5%*	3%
Controls (n=32)	72%	0%	28%	14%

*p=0.047 with 95% CI

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Maternal Obesity Combined with a High Plane of Nutrition during Pregnancy Alters Glucose (GL) Tolerance of the Ewe and Her Offspring. Stephen P Ford,[1] Lindsey A George,[1] Alison N Iroz,[1] Bret W Hess,[1] Gary E Moss,[1] Peter W Nathanielsz.[1,2] *[1]Center for the Study of Fetal Programming, Department of Animal Science, University of Wyoming, Laramie, WY, USA; [2]Department of Obstetrics and Gynecology, University of Texas Health Sciences Center, San Antonio, TX, USA.*
Introduction: Last year we reported that ewes fed an obesogenic (OB; 150% of NRC recommendations) diet from -60 days to 75 days of gestation (dG) became obese compared to control (C; 100% of NRC) ewes. At midgestation, fetuses of OB ewes were heavier and had an enlarged pancreas with increased numbers of β-cells ($P<0.05$) compared to fetuses of C ewes. Further, both OB ewes and their fetuses were hyperinsulinemic and hyperglycemic compared to C ewes and fetuses.
Hypothesis: From these data we hypothesized that OB ewes and their offspring would exhibit altered glucose (GL) tolerance compared to C ewes and offspring.
Methods: Using the experimental paradigm above, C (n=5) and OB (n=6) ewes were fasted for 12 h on 75 dG and subjected to a GL tolerance test (GTT). Blood samples were taken for GL and insulin (INS) at 15 and 5 min before,

and 2, 5, 10, 15, 30, 60, and 120 min after, intravenous GL infusion(0.25g/kg body wt.). Both groups of ewes continued on their respective diets, allowed to lamb, and returned to a normal diet after delivery. At 2 months of age, their lambs were given an identical GTT.

Results: Gestation was shorter in OB than C ewes (145±1 vs.151±1 days; P<0.01), while birth weights were similar (6.0±0.3 vs.5.3±0.5 kg). OB ewes had greater baseline plasma GL and INS, and greater area under the curves (AUC) for both GL and INS during GTT than C ewes (P<0.05). Further, INS: GL ratios before GL infusion and at INS maximum (+10 min) were greater for OB than C ewes (P<0.05). First phase INS response to GTT was greater (P<0.05) in OB ewes than C Ewes (1.37±0.25 vs.0.21±0.03). Baseline INS was lower in lambs from OB than lambs from C ewes. AUC for GL during GTT was less for lambs from OB ewes than from C ewes (P<0.05). AUC for INS was greater (P<0.06) for lambs from OB ewes during GTT than for lambs from C ewes.

Conclusions: Increased INS:GL ratios in OB versus C ewes at midgestation prior to GL infusion, and at INS maximum during GTT suggests INS resistance. The reduced AUC for GL in offspring of OB versus C ewes suggests either increased pancreatic sensitivity to GL and/or increased peripheral INS sensitivity. NIH INBRE 1P20RR16474.

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High Predictive Value of Amniotic Fluid RT-PCR for Prenatal Diagnosis of Congenital CMV Infection. Simcha Yagel,[1] Orit Kaplan,[2] Nurith Mador,[2] Lorinne Levit,[2] Bracha Shainberg,[3] Zvi Roash,[4] Nili Yanai,[1] Dana Wolf.[2] *[1]Obstetrics and Gynecology; [2]Clinical Virology, Hadassah Hebrew University Medical Centers; [3]Macabbi Health Services; [4]Meuchedet Health Services, Jerusalem, Israel.*

Background: The predictive value of PCR for fetal infection, its sensitivity, specificity, PPV, NPV range between 80-100%. Real time PCR (RT-PCR) amniotic fluid (AF) viral load has been examined in limited studies.

Aims: To determine the predictive value of AF real-time PCR for the presence of congenital CMV infection and to examine the possible correlation of AF viral load with disease outcome.

Patients and Methods: AF specimens were obtained prospectively over 4 years (2003-2007) from women with suspected infection, after 21 wks gestation and at least 6 wks from documented maternal infection. gB RT-PCR was performed with a sensitivity of 50 cop/ml and a linear quantitation over a 6-log range. IE RT-PCR was employed to detect viral strains not detected by the gB assay. To evaluate infection and disease outcomes, ultrasound, histopathologic findings, newborn urine cultures, hearing and developmental evaluation were performed.

Results: A total of 539 AF specimens were examined for CMV, 462 of these had known serology data. CMV was detected in 28/462 (6.1%). All of these were from women with primary CMV infection by seroconversion (149 patients). The transmission rate in primary infection was 18.8% (28/149). 18/18 (100%) showed congenital infection. 121 patients with primary CMV infection returned negative AF RT-PCR results, congenital infection was found in 1/65 (1.5%). In cases of primary CMV infection the sensitivity of RT-PCR was 94.7%, specificity 100%, PPV 100%, and NPV 98.5%, compared with culture and PCR, which showed sensitivity of 89.5%, specificity 100%, PPV 100%, and NPV 96.9%. Only 5/28 (17.9%) cases with positive AF RT-PCR showed findings on ultrasound. There was an observed trend for higher proportion of viral load$\geq 10^5$ cop/ml in those with disease (*P=0.09*); a threshold value of $\geq 10^5$ cop/ml had a sensitivity of 100% but low specificity as a prenatal marker for disease (specificity 40%; PPV 57.1%). No disease developed with viral load $< 10^5$ cop/ml (NPV 100%).

Conclusions: AF RT-PCR is a highly reliable prenatal diagnostic assay for congenital infection. Higher median AF viral load is found in pregnancies with congenital disease, however the low PPV and specificity of viral load threshold preclude reliable prenatal disease prediction.

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Gene Expression Signature in Leukocytes Accurately Predicts Preterm Delivery in Women with Threatened Preterm Labour. Craig E Pennell,[1,2] Karen J Bosel,[2] John E Perkins,[1] Alan D Bocking,[1] Stephen J Lye.[1] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; [2]School of Women's and Infants' Health, The University of Western Australia, Perth, Western Australia, Australia.*

INTRODUCTION: The ability to predict preterm birth (PTB) in women with threatened preterm labour (T-PTL) is poor. Preliminary data from our group has demonstrated that leukocyte gene expression profiles may accurately predict PTB in women with T-PTL.

OBJECTIVE: To define a gene expression signature from peripheral leukocytes in women with T-PTL that accurately predicts PTB in <48hours of clinical presentation.

METHODS: Maternal blood samples and cervical fetal fibronectin (FFN) were collected from 90 women admitted with T-PTL. RNA was extracted from leukocytes and Affymetrix U133Plus2.0 Arrays were performed on each patient after globin reduction. Gene expression data was analysed to predict timing of delivery. Class prediction was performed using permutation testing of cross validated misclassification rate. Biological significance of the genetic signature was assessed using pathway analysis and gene-ontology analysis.

RESULTS: Of the 90 women with T-PTL, 27 delivered within 48hours of admission, 36 delivered preterm and 51 delivered at term. Three patients were excluded for iatrogenic PTB. FFN testing was positive in 44% of those without contraindications for testing (52%). The expression of 66 genes was significantly different between outcome groups. Delivery within 48 hours of admission was accurately predicted from a subset of 12 genes (subsequently confirmed by RT-PCR) with 82% accuracy (sensitivity 97%, specificity 48%, PPV 81%, NPV 87%). For delivery within 48 hours of presentation FFN had an accuracy of 73% (sensitivity 81%, specificity 75%, PPV 50%, NPV 91%). Genes that discriminated between outcome groups had functions including: extracellular matrix receptor interactions; cytokine signalling; MAPK signalling; TGF beta signalling, anti-apoptosis and toll-like receptor pathways.

CONCLUSION: We have identified a gene expression signature in peripheral leukocytes that is capable of predicting the pre-term delivery within 48 hours in women presenting with T-PTL. Unlike some other tests this signature can be applied to all women with T-PTL. Development of a diagnostic test based on this signature could reduce hospital admissions and enable therapies to be targeted to those who would be most likely to benefit from intervention.

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Weight Reduction (WR) in Obesity Is Associated with Changes in Metabolic, Vascular and Inflammatory Pathways in Peripheral Blood Mononuclear Cells (PBMCs). Natalia E Schlabritz-Loutsevitch,[1] Matthew P Johnson,[2] Katherine A Freed,[2] Antony G Comuzzie,[2] Susan L Jenkins,[1] Peter W Nathanielsz,[1] Eric K Moses.[2] *[1]Obstetrics and Gynecology, University of Texas Health Sciences Center, San Antonio, TX, USA; [2]Department of Genetics, Southwest National Primate Research Center, Southwest Foundation for Biomedical Research, San Antonio, TX, USA.*

Introduction: One third of women in the USA are obese (OB). Maternal pre-pregnancy (pre-P) OB is associated with a high incidence of P complications and dysregulation of metabolic, vascular and inflammatory pathways. PBMCs play a critical role in fat metabolism and placentation. WR is a major measure for the prevention of OB-related P complications. The rate and degree of WR prior to P remains controversial. **Aim:** To determine whether WR as a results of three months dietary restriction is associated with corrections of pathways altered by OB. **Methods:** We evaluated serum cytokine [Adiponectin (AD), Leptin (LEP), MCP-1, insulin (I), IL-8, IL-6 and IL1β, (Luminex, Millipore, USA)] and PBMC whole genome expression (Illumina human-6 (v2) beadchip) profiles in the follicular (FP) and luteal phases (LP) of the menstrual cycle in two group of baboons: OB (n=5) and lean (n=4). Animals were placed on *ad lib* and then on nutrient restricted (NR, during three months fed 70% of *ad lib* feed) diet. Results were assessed using t-tests with Bonferroni correction for multiple comparisons. Pathways were analyses using impact analyses (Genome Research, 17:1537-1545, 2007). **Results:** OB animals lost 4.8 ± 2% of their weight. LEP, AD, MCP-1, IL-6 and IL1β concentrations did not differ between groups. Serum IL-8 was higher in OB vs. lean animals ($p<0.05$). LEP was lower in OB animals after NR ($p<0.05$). Forty eight PBMC genes were up-regulated (with PLAC8 at $p<0.00001$) and 20 genes down-regulated in LP vs. 89 up-regulated and 343 down-regulated genes in FP in the OB group compared to lean. NR was associated with 265 up-regulated and 22 down-regulated transcripts in FP in OB animals. Pathways altered in OB and corrected by WR were cell adhesion molecules, Type I diabetes mellitus, leukocytes transendothelial migration, antigen processing and presentation and JAK-STAT ($p<0.05$).

Conclusion: 4.8 ± 2% of weight reduction in obesity as a result of NR decreased serum LEP concentration and changed PBMC global genome expression in a hormonal phase sensitive manner with involvement of key pathways associated with maternal obesity.

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A Physiological Approach to Stimulating Labour: PULSE a Randomised Controlled Trial of Pulsatile *Versus* Continuous Oxytocin Administration.
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Introduction: In the UK, induction and augmentation of labour are associated with high rates of medical intervention. Continuous oxytocin infusion protocols are routinely used to stimulate uterine contractions, but can cause uterine hyperstimulation and fetal distress. As continuous exposure to oxytocin is associated with oxytocin receptor down regulation *in vivo and in vitro*, pulsatile oxytocin infusion protocols may provide an effective alternative approach to stimulation of uterine contractions (Dawood, 1995). Previous small studies have been inadequately powered to demonstrate benefit in relation to mode of delivery.
Aim and Objectives: The aim was to improve the current method of oxytocin induction and augmentation of labour, in order to reduce associated intervention rates and maternal and fetal morbidity. We hypothesised that pulsatile infusion of oxytocin is associated with lower caesarean section and intervention rates compared to a continuous oxytocin infusion protocol.
Methods: A randomised controlled trial (n =1031 women) was conducted in two large UK maternity units with local ethics committee approval. Pregnant women requiring oxytocin for induction or augmentation were recruited, with written informed consent, and randomised to either a continuous or pulsatile (administered as discrete 10 second boluses every six minutes) oxyotcin infusion protocols (infusion dose was increased every 30 minutes according to the National Institute of Clinical Excellence guidelines). Primary outcome measures were caesarean section rate, instrumental delivery rate and time to delivery.
Results and Conclusion: Recruitment has finished with 98 % of outcome data collected. Overall, 382 women (37.8%) had caesarean section; 265 (26.2%) had instrumental deliveries which include 105 (10.4%) by forceps, 160 (15.8%) by ventouse, and 363 (35.9%) were vertex vaginal deliveries. Full analysis will be completed by January 2008.
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Pathophysiologic Pathways in Racial Disparity of Preterm Birth.
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OBJECTIVE: Etiologic and pathophysiologic heterogeneities complicate understanding and assessment of racial disparity in preterm birth (PTB). This study utilizes bioinformatics tools to delineate high throughput genetic analysis data to determine distinct pathways in preterm birth in Caucasians (C) and African-Americans (AA).
METHODS: High-throughput candidate gene association studies were performed on 1442 SNPs in 134 genes of known PTB-associated genes in a case (preterm birth <36 weeks) control study (term birth >37 weeks) using maternal DNA from 370 (172 cases and 198 controls) C and 279 (82 PTB and 197 term) AA. Single locus association analyses followed by hierarchical clustering of data were ordered based on p values for significant associations in both races. Using Ingenuity Pathway Analysis software known pathophysiologic pathways impacted in both races were determined.
RESULTS: Several differences between variants in PTB candidate genes and their association with PTB were observed between races. Forty seven in C and 96 in AA genes had at least 1 marker significant at either allele or genotype level and 23 of them were common between races. The single strongest association in C was for SNP in plasminogen activator tissue gene (C-4443T) at both allelic ($p=2.00x10^{-3}$) and genotypic ($p=2.0x10^{-6}$) level and in AA the strongest association was in Interluekin-15 (C13929T, allele $p=2.91x10^{-4}$, genotype $p=2.0x10^{-3}$). Pathway analysis revealed that hematological pathways were the top-ranked disease functions in C, with 32% (15/47) of the affected genes mapping to hematological disease functions compared to only 17% (16/96) in AA. Affected genes in both C and AA also significantly mapped to disorders in inflammation ($p=3.62x10^{-13}$ and $p=9.40x10^{-16}$, respectively) and collagen metabolism ($p=2.1x10^{-10}$ and $2.04x10^{-15}$, respectively).
CONCLUSION: Pathway analysis verified differences in genetic associations and pointed to discrete operational pathways in PTB between races. In C variations in hematological/coagulation genes dominate pathways leading to PTB. Although the terminal events mediated by uterotonins leading to PTB may be same in two races, initiator and operator pathways could be different.

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Mouse Fetuses Produced by *In Vitro* Fertilization (IVF) Show Delayed Development with Smaller Placentas Than *In Vivo* Conceived Mice.
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Objective: Low birth weight, increase in imprinting disorder and increase in malformation have been associated with IVF. The cause of these findings is not known and abnormal placentation is a potential candidate: placenta development is in fact the first step of the complex maternal-fetal interaction. This study evaluates the effect of the method of conception on placentation in a mouse model system.
Methods: Oocytes were collected from superovulated CF-1 and CD-1 mice and fertilized *in vitro* with cauda epididymal sperm from B6D2F1/J mice. Fertilized eggs were cultured in Whitten medium under 5% CO2 in humidified air at 37 °C for 96 h. Blastocysts were transferred to the uteri of pseudo-pregnant recipients. Control mice were allowed to conceive *in vivo*. At 10 and 14 days post fertilization (DPF), implantation sites were collected, fixed in 4% paraformaldehyde and fetuses checked for developmental stage (DS) according to external features (Gruneberg, 1943). Six µm paraffin embedded, hematoxylin and eosin stained sections were compared and the labyrinth and total fetal area of the placenta were calculated using the Image Processing toolkit in Photoshop (Reindeer Graphics). Means were compared using a t-test.
Results: There is some variation in DS within litters, between maternal strains and types of conception. When the fetus DS was examined, the IVF fetuses displayed a significantly delayed development compared to *in vivo* embryos in all strains at 14 DPF and in CD1 at 9 DPF. Furthermore, at 14 DPF, IVF fetuses weighed significantly less than controls (56.45 mg vs. 113.64 mg; $p<0.05$); IVF placentas were smaller both in weight (53.27mg vs. 75.37mg; $p<0.05$) and cross-sectional areas (4.44mm² vs. 5.26mm²; $p=0.041$).
Conclusion: *In vitro* fertilization affects fetal development and placentation. IVF fetuses manifest a delay in development at each developmental stage checked. After controlling for developmental stage, IVF fetuses are smaller than *in vivo* generated fetuses and have, though absolutely smaller, relatively larger placentas than controls. This finding should be further studied as placentation defects might lead to low birth weight and associated diseases.

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Spastin Confers Specificity to HOXA10 Mediated Transcriptional Regulation in Reproductive Tissues.
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Objective: HOXA10 is a transcription factor required for uterine development and endometrial receptivity. Spastin, a AAA ATPase is necessary for neurotransmitter trafficking. Mammalian spastin unlike invertebrate orthologs demonstrates nuclear localization. Here we characterize the role of spastin as a HOXA10 transcriptional cofactor.
Methods: We identified Spastin as a member of the HOXA10 trancriptional complex by immunoprecipitation of Ishikawa nuclear extract followed by mass spectrophotometry and sequencing. Spastin expression was determined in Ishikawa, primary endometrial epithelial and stromal cells and in a breast adenocarcinoma cell-line (BT-20) by RT-PCR. In Luciferase reporter assays, Ishikawa and BT-20 cells were transfected with either 4.0 µg spastin-myc chimera, 4.0 µg pcDNA/HOXA10 or both and empty pcDNA as a control. Cells were additionally transfected with a HOXA10 responsive EMX2-luciferase vector. Transfections were done in triplicate. Luciferase activity was assayed 48 hours post-transfection. Co-immunoprecipitations were performed on Ishikawa and BT-20 nuclear extracts obtained from cells transfected with the chimeric proteins HOXA10-Flag and GFP-spastin or HOXA10-Flag alone.

Anti-GFP or Anti-spastin were used to immunoprecipitate chimeric or native Spastin respectively. Western blot using Anti-FLAG was performed to confirm co-immunoprecipitation of HOXA10 with Spastin.
Results: Spastin expression was identified in primary endometrial cells as well as in a breast adenocarcinoma cell-line (BT-20), where it co-localized with HOXA10 in the nucleus. Spastin also co-immunoprecipitated with HOXA10 using nuclear extracts from these cells. In EMX2-Luciferase assays, reporter activity decreased with HOXA10 transfection ($p<0.001$) as previously shown, whereas it increased with spastin ($p<0.03$) transfection. In BT-20 cells, spastin reversed HOXA10 mediated repression of EMX2-Luciferase activity ($p<0.05$).
Conclusion: Spastin is mutated in hereditary spastic paraplegia. Here we characterize its novel role, as a cofactor of HOXA10 in uterine and extrauterine tissues. Spastin and HOXA10 are co-localized and co-expressed in endometrial and breast adenocarcinoma cells. Additionally, Spastin abrogates HOXA10 mediated target gene repression in these cells. It is likely that Spastin enhances specificity of HOX gene transcription not only in the uterus but in other HOXA10 responsive tissues as well.

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HBEGF-Induced Downstream Regulators of Trophoblast Functions. PA Jessmon,[1] BA Kilburn,[1] R Romero,[2] RE Leach,[3] DR Armant.[1,4] *[1]Obstetrics & Gynecology, Wayne State University, Detroit, MI, USA; [2]Perinatology Research Branch, National Institute of Child Health & Human Development (NICHD), NIH, Bethesda, MD, USA; [3]Obstetrics & Gynecology, Michigan State University College of Human Medicine, Grand Rapids, MI, USA; [4]Reproductive Biology & Medicine Branch, NICHD, NIH, Bethesda, MD, USA.*
Background: Heparin-binding EGF-like growth factor (HBEGF) is expressed by trophoblast cells throughout gestation, but is absent in placentas from women with preeclampsia. HBEGF protects first trimester trophoblast cells from apoptosis induced by hypoxia, hypoxia/reoxygenation (H/R) injury or ethanol exposure, and mediates their differentiation to an invasive phenotype. Therefore, we have examined the role of downstream signaling pathways induced by HBEGF in mediating these cellular activities, which are deficient in preeclampsia.
Methods: Two first trimester cytotrophoblast cell lines, HTR-8/SVneo and SW71, were used in these studies with similar results. Cytotrophoblast cells were exposed to H/R by culturing 2 h at 2% O_2, then 6 h at 20% O_2. Cell death was assessed by fluorescent in situ TUNEL with a DAPI nuclear counterstain to determine the percent dying cells. Cells migrating through semi-permeable inserts coated with fibronectin were counted after 8 h. Western blotting of cell extracts with antibodies against ERK1/2, p38 or AKT, and with antibodies recognizing their phosphorylated forms, was used to assess pathway activation.
Results: Recombinant HBEGF (1 nM) significantly increased trophoblast migration and prevented apoptosis of cells exposed to H/R. HBEGF induced transient phosphorylation of ERK1/2, p38 and AKT within 15 min. To determine which pathways regulate survival and migration, specific inhibitors of the ERK upstream regulator MEK1/2 (U0126), the AKT upstream regulator PI3 kinase (LY294002) and p38 (SB203580), along with inactive structural analogues, were added to the culture medium. Only SB203580 specifically prevented HBEGF rescue of cells exposed to H/R, while HBEGF-stimulated cell migration was attenuated by SB203580 or LY294002, but not U0126.**Conclusions:** HBEGF advances trophoblast extravillous differentiation through activation of PI3 kinase and p38 MAP kinase, while only p38 is required for anti-apoptotic activity. Signaling through these pathways and ERK1/2 could be disrupted in placentas of preeclamptic women that lack HBEGF. Supported by NIH grant HD040093-06 and the intramural research program of NICHD.

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Circulating Endothelial Progenitor Cell Number and Function during the Menstrual Cycle. Fiona C Denison,[1] Amy O Robb,[1] Imogen B Smith,[1] Nicholas L Mills,[2] Hilary OD Critchley,[1] David E Newby.[2] *[1]Centre for Reproductive Biology; [2]Centre for Cardiovascular Sciences, The University of Edinburgh.*
BACKGROUND: Uterine endometrium has a unique cycle of physiological angiogenesis. In mice, endothelial progenitor cells (EPC) contribute to endometrial angiogenesis being incorporated after oestradiol administration. OBJECTIVE: To determine whether circulating © EPC number and function vary through the menstrual cycle in response to changes in circulating sex steroid concentrations. METHODS: Ten healthy, nulliparous, pre-menopausal, non-smoking women (mean age 31 years) with regular menses (27-29 days) were studied. Venous blood was collected during menstrual, follicular, periovulatory and luteal phases of a single cycle (days 1-3, 6-9, 13-15, 20-22). cEPCs, serum oestradiol (E) and progesterone (P) were measured at each phase. cEPCs were quantified by phenotype using flow cytometry (leukocytes co-expressing CD133, KDR and CD34) and function by the EPC-Colony Forming Unit (CFU) assay. EPC-CFUs were stained for endothelial markers including uptake of acetylated low-density lipoprotein, binding of lectin (*Ulex europaeus)* and endoglin. RESULTS: Luteal P was >30 nmol/L in all women. cEPC numbers increased in the menstrual and follicular phases being 3-fold higher in the follicular compared to periovulatory phase ($p<0.05$). There was no significant variation in cEPC function over the menstrual cycle. There was no correlation between serum E or P levels and cEPC number or function. CONCLUSION: cEPCs number but not function (EPC-CFU assay) vary during the menstrual cycle with numbers increasing during the menstrual and follicular phase and falling in the periovulatory and luteal phase of the menstrual cycle. This may represent mobilisation of cEPCs from the bone marrow and subsequent incorporation into the endometrium. Neither function nor cEPC number correlate with serum P or E.

	Menstrual	**Follicular**	**Periovulatory**	**Luteal**	ANOVA
Serum Oestradiol (pmol/L)	179.5±20.9	317.6±68.4	1342±274.4	826.9±93.4	p<0.0001
Serum Progesterone (nmol/L)	3.5±0.3	3.3±0.2	6.5±2.1	47.3±4.5	p<0.0001
Numbers of cEPCs/ml of peripheral blood)	569±174	993±292*	293±103*	420±163	p=0.04
	*p<0.05 Follicular vs Periovulatory				

Statistical analyses were performed using one-way ANOVA with repeated measures and Bonferroni's post tests for multiple comparisons. Values are reported as mean± SEM

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A Factor VII/IgG1 Fc Immunoconjugate Molecule (Icon) Regresses Established Disease in a Mouse Model of Human Endometriosis. Graciela Krikun,[1] Kevin Osteen,[2] Kaylon Brunner-Tran,[2] Zhiwei Hu,[1] Frederick Schatz,[1] William Konigsberg,[1] Alan Garen,[1] Charles J Lockwood.[1] *[1]Ob/Gyn & Mol Biophys. Biochem., Yale University, New Haven, CT, USA; [2]Ob/Gyn, Vanderbilt University, Nashville, TN, USA.*
Introduction: Our previous studies demonstrated that tissue factor (TF), which binds factor VII to act as a potent pro-coagulant and angiogenic factor, is over-expressed in endometriotic lesions. Thus, we determined whether TF could serve as a target for the elimination of pre-established ectopic human endometrial growth in a mouse model. Icon is composed of a mutated factor VII (fVII) domain targeting TF and an IgG1 Fc (fVII/IgG1 Fc) effector domain that activates antibody-dependent immune responses against TF bearing endothelial cells.
Methods: Athymic, ovariectomized and estrogen-treated mice received intraperitoneal (i.p.) injections of 1.0 mg of proliferative phase human endometrial tissue derived from normal (disease-free) women. Twelve days after inoculation to establish lesions, Icon protein (10 ug) was delivered i.p. once a week for 4 weeks. After sacrifice, animals were subject to gross inspection. Residual endometriotic tissue was formalin fixed, paraffin embedded and immunostained for von Willebrand's factor (vWF) and TF.
Results: Compared to control mice, treatment with 10ug of Icon abolished all lesions in 7 of 10 mice and reduced both size (2.33 to 1.4mm) and number of lesions (1.9 to 1 per diseased mouse) in the remaining mice. Moreover, residual lesions from Icon treated mice were atrophic and displayed significant reductions in vessel areas of 87% +/- 26% ($p=0.002$, mean +/-SEM, $n=3$) as determined by vWF immunostaining. No hemorrhagic or thrombotic sequelae were observed in Icon treated mice.
Conclusions: Unlike other treatments that target developing angiogenesis, Icon can target both developing and established human endometriotic lesions in athymic mice. The gross and microscopic vessel analysis suggests that Icon directly or indirectly destroys endometriotic vessels. Thus, Icon presents a novel, non-toxic therapy for endometriosis.

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ERβ Is a Suppressor of ERα Gene Expression in Endometriosis. Elena Trukhacheva, Zhihong Lin, You-Hong Cheng, Serdar Bulun. *Obstetrics and Gynecology, Northwestern Unuiversity, Feinberg School of Medicine, Chicago, IL, USA.*

Background: ERβ levels are 36 fold higher, whereas ERα levels are 4 fold lower in endometriosis compared to endometrium. We investigated E2 regulation of ERα gene via specific promoters and presented preliminary evidence that ERβ mediates this effect. Here, we provide mechanistic evidence that ERβ in endometriosis acts as a suppressor of ERα gene.

Objective: To investigate ERβ-dependent regulation of ERα via specific cis-regulatory elements in multiple ERα gene promoters.

Methods: Stromal cells from ovarian endometriomas (n=10) and eutopic endometrium (n=10) were used in primary culture. ERβ was selectively knocked down by siRNAs and overexpressed using the plasmid containing human ERß gene. ERα and ERβ protein levels were determined by Westerns. Real-time PCR employing exon-specific primers was used to quantify total and promoter (A, B, or C)-specific ERα mRNA levels in the presence of ERβ knockdown or overexpression. Lucefirase construct Luc-2ERE-pS2(-91/+10) was used to determine ERβ effect on ERα transcriptional activity. BRDU staining was performed to evaluate ERß regulation of cell cycle. ChIP assay was used to determine ERβ binding to specific ERα promoter regions.

Results: In response to ERβ knockdown ERα mRNA levels in endometriotic stromal cells significantly increased. This increase was mediated primarily through ERα promoter C specific mRNAs. ERβ overexpression resulted in decreased ERα mRNA levels in both cell types. Promoter (A, B, or C)-specific ERα mRNAs contributed to observed downregulation. Lucefirase experiments demonstrated a dose-dependent downregulation of ERα transcriptional activity with ERβ overexpression. ERβ knockdown resulted in increased proliferation of endometriotic stromal cells. ChIP assay demonstrated ERβ binding to AP-1 and Sp-1 motifs in the promoters A and C and to classic ERE in promoter B of the ERα gene. ERβ binding activity was increased with E2 treatment.

Conclusions: ERβ regulates ERα transcriptional activity and response to E2 in endometrial and endometriotic stromal cells via binding to classical and non-classical cis-regulatory elements in specific promoters of the ERα gene. High levels ERβ suppress ERα expression in both cell types. Since ERα regulates PR, it is possible that decreased ERα levels might lead to progesterone resistance observed in endometriosis. Based on the above results ERβ may serve as novel therapeutic target for endometriosis.

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Gene Profiling of Uterine Decidua from Women with Tubal and Intra-Uterine Pregnancies To Identify Candidate Genes as Biomarkers for Diagnosis of Ectopic Pregnancy. AW Horne,[1] L Udby,[2] S Burgess,[3] P Lorenco,[1] P Cornes,[1] P Ghazal,[3] HOD Critchley.[1] *[1]Reproductive and Developmental Sciences, University of Edinburgh, Edinburgh, United Kingdom; [2]Granulocyte Research Laboratory, Copenhagen University Hospital, Rigshospitalet, Denmark; [3]Pathway Medicine, University of Edinburgh, Edinburgh, United Kingdom.*

Introduction: One in 80 pregnancies is ectopic and >98% implant in the oviduct. A reliable blood test that can differentiate a tubal from an intra-uterine (IU) pregnancy is not available. Microarray genomic technology was used to determine which genes were up- and/or down-regulated in uterine decidua of women with tubal compared to IU pregnancies to identify a biomarker for ectopic pregnancy.

Methods: Uterine decidua and sera were collected from women undergoing surgical management of tubal pregnancy (n=11; gp 1); surgical management of miscarriage (n=6; grp 2); and surgical termination of pregnancy (TOP) (n=8; gp 3). Total decidual RNA was analyzed by Affymetrix U133 plus 2.0 human whole genome arrays (54,000 genes) according to standardised protocols and differential gene expression, between tubal and IU samples (groups 2+3), was investigated. Decidual samples were further analyzed by quantitative Taqman RT-PCR (Q-RT-PCR) and immunohistochemistry (IHC). Sera was stored for analysis by ELISA.

Results: 669 genes were differentially expressed (FC>2, $p<0.05$) in the tubal compared to the IU pregnancy group. The gene list was further analysed for potential biomarkers using Ingenuity Pathway Analysis. Cysteine-rich secretory protein 3 (CRISP3), was one of the genes up-regulated in the tubal pregnancy group (FC 13.10, $p<0.05$). CRISP3 can be detected in human blood, and as such, has potential as a diagnostic tool for ectopic pregnancy. Q-RT-PCR confirmed increased CRISP3 decidual expression ($p<0.05$) in tubal compared to the miscarriage and TOP groups. IHC for CRISP3 demonstrated increased secretion of CRISP3 in the glandular epithelium and expression in the leucocytes of the tubal uterine decidua.

Conclusions: There are differences in decidual gene expression in tubal compared to IU pregnancies. We believe that potential biomarkers of tubal pregnancy can be discovered by focusing on secreted proteins associated with uterine decidualization. One of these proteins, CRISP3, is significantly increased in decidua of tubal ectopic pregnancies and we are currently investigating its expression pattern in sera from women with tubal compared to IU pregnancies.

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Progesterone and HOXA 10 Regulate GABA-A pi Receptor Expression, Membrane Translocation and Activation. Homayoun Sadeghi, Hugh S Taylor. *Obstetrics, Gynecology and Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA.*

Objective

The expression of the GABA-A pi receptor has been previously described in the human endometrium in both luminal epithelium and stroma. It is upregulated during stromal decidualization in the rat and in the implantation window of human endometrium. The GABA receptor is modulated by progesterone metabolites, with the resultant opening of the receptor ion channels which allow the water flux necessary for trophoblast attachment. Here we identified regulators of pi subunit receptor gene expression and activity.

Study Design

The well-differentiated human endometrial adenocarcinoma cell line (Ishikawa) and human endometrial stromal cells (HESC) were transfected with HOXA10, treated with progesterone, or treated with vehicle or empty plasmid controls. GABA-A pi receptor mRNA upregulation was evaluated by real time RT-PCR. Protein expression was evaluated using immunohistochemistry.

Results

GABA-A pi receptor mRNA expression was increased with either progesterone treatment (31%, $p=0.049$) or HOXA10 transfection (11%, $p=0.027$). Co-administration of progesterone along with increased HOXA10 transfection had no additive effect on the expression of GABA-A pi receptor mRNA ($p=0.98$). GABA-A pi receptor protein expression was similarly increased by each treatment. Either HOXA10 or progesterone independently caused translocation of the GABA receptor from the cytoplasm to the cell membrane in Ishikawa cells.

Conclusion

GABA-A pi receptor expression is increased in the human luminal epithelium and stroma in the window of implantation. Activation of the pi subunit leads to opening of ion channels, likely allowing flux of water into the epithelial cells and out of the uterine lumen. Progesterone and HOXA 10 each increase both pi subunit receptor expression and membrane translocation. The lack of additive effect suggests progesterone induced pi subunit receptor expression is likely mediated indirectly through progesterone's regulation of HOXA 10 expression. Finally, after receptor expression and translocation, progesterone mediated GABA receptor ion channel activation mediates water resorption necessary for implantation.

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HuR Binds to a Novel 99nt Non AU-Rich Element in 3'UTR *c-fms* RNA, Regulating Its Expression, and Is a Poor Prognostic Factor in Breast Cancer. Y Zhou,[1] C David,[1] W Zheng,[1] H Woo,[1] X Yi,[1] M Gilmore,[2] E Ulukus,[2] H Kluger,[2] J Stoffer,[1] SK Chambers.[1] *[1]AZ Cancer Ctr, Tucson, AZ; [2]Yale Univ, New Haven, CT.*

Background: The role of RNA binding proteins in cancer biology is increasingly being recognized. The nucleocytoplasmic shuttling and AU-rich RNA binding protein HuR stabilizes several target mRNAs. Expression of the proto-oncogene *c-fms*, whose 3'untranslated region (3'UTR) is not AU-rich, can be regulated on the basis of mRNA stabilization and its expression is associated with metastasis and poor prognosis in breast cancer patients. Physiologic levels of glucocorticoids (GC) markedly up-regulate *c-fms* expression.

Materials and Methods: HuR bound specifically to 3' UTR *c-fms* RNA, as assessed by RNA gel shift and UV crosslinking assays. *In vivo* binding of HuR to *c-fms* RNA was demonstrated by IP-RT-PCR assays. HuR siRNA demonstrated regulation of *c-fms* mRNA and protein by HuR. A large cohort tissue microarray of 670 human breast cancer specimens was used to investigate the clinical role of HuR.

Results: We define a novel 99nt non AU-rich containing region in the 3' UTR of *c-fms* RNA as a target binding motif for HuR. Deletion of the mid 69nt of

the target motif abolishes binding of HuR. *In vivo* assays of HuR/*c-fms* RNA protein binding in BT20 breast cancer cells confirmed binding of HuR to 3'UTR *c-fms* RNA. Northwestern analyses showed additional proteins binding the 99nt *c-fms* RNA element. HuR silencing significantly interferes with GC stimulation of *c-fms* mRNA and protein. By tissue microarray, increased nuclear HuR expression was associated with lymph node metastasis (p=0.037) and independently with poor survival (p=0.032, RR 1.45). Breast tumors with nuclear HuR, co-expressed *c-fms* in the cytoplasm (p=0.0007).

Conclusions: We are the first to describe that HuR specifically bound to a 99nt non AU-rich element in 3'UTR *c-fms* RNA, and regulated its expression. This 99nt element likely represents a target for protein binding. Furthermore, in the largest breast cancer cohort studied to date, we found that nuclear, but not cytoplasmic, HuR expression was an independent poor prognostic factor. Human breast cancer cells *in vivo* are continuously exposed to circulating GCs and we find GC stimulation of *c-fms* to be largely dependent on HuR's presence. In line with this, we observe that HuR was co-expressed with *c-fms* in the breast tumors. Collectively, our findings suggest that HuR may play a supportive role for *c-fms* in breast cancer progression.

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Estrogen Stimulates the Human Endometrium To Express a Factor(s) That Promotes Vascular Smooth Muscle (VSMC) Migration as an Early Step in Microvessel Remodeling. Jeffery S Babischkin,[1] Laurence C Udoff,[1] Christine O Vergara,[2] Robert O Atlas,[2] Gerald J Pepe,[3] Eugene D Albrecht.[1] *[1]Obstet, Gynecol, Reprod Sci, Univ Maryland School of Medicine, Baltimore, MD; [2]Obstet Gynecol, Mercy Medical Center, Baltimore, MD; [3]Physiol Sci, Eastern Virginia Medical School, Norfolk, VA.*

New blood vessels formed via angiogenesis undergo remodeling via migration of VSMC to and coupling with endothelial cells, during each menstrual cycle to support growth of the endometrium for implantation. Ovarion estrogen has a pivotal role in establishing the endometrial vascular bed, however, relatively little is known about the mechanisms by which estrogen regulates this fundamentally important process. In the present study, we established *ex vivo* explants of human endometrium incubated with estrogen to assess the impact on VSMC migration. Endometrial tissue was obtained from untreated women in the proliferative phase of the menstrual cycle after hysterectomy for benign gynecological conditions, minced (2 mm^3) and incubated on 30 mm Millicell culture inserts in serum-free DMEM/F12 supplemented with 2.5 mM glutamine and antibiotics at 37 C (95% air: 5% CO_2) for 24 h in the presence/absence of 10^{-8}M estradiol and/or 10^{-7}M progesterone. Endometrial explants were comprised of well developed glandular epithelial and stromal components the integrity of which was maintained by estradiol. Conditioned medium (CM) from the endometrial explants (n=4) was then added (20% vol: vol) to human aortic VSMC (25,000 cells, Clonetics, Inc) that were applied to 8µm-pore polyethylene teraphthalate membranes coated with 10µg/ml human fibronectin and incubated 21 h at 37 C in 0.25 ml DMEM/F12 supplemented with 0.1% BSA. CM from endometrial explants incubated with estradiol stimulated VSMC migration (number of VSMC migrated across membrane) to a value (169 ± 18 cells/mm^2 membrane) 3.6-fold greater ($P<0.001$, ANOVA) than with DMEM alone (46 ± 10) and greater ($P<0.05$) than with CM from explants incubated with DMEM (123 ± 11), estradiol/progesterone (118 ± 3) or progesterone (123 ± 8) alone, while estradiol plus progesterone added directly to VSMC had no effect (55 ± 11). We propose that the increasing levels of ovarian estrogen in the proliferative phase stimulate endometrial gland and/or stromal cells to secrete a factor(s) that promotes VSMC migration as an essential step in microvessel remodeling necessary for development of the endometrial vascular network. Supported by NIH U54 HD36207 SCCPRIR.

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Vascular Endothelial Growth Factor Pathway Blockade in Endometrial Cancer. Suzy Davies, Donghai Dai, Kimberly K Leslie. *Obstetrics and Gynecology, University of New Mexico, Albuquerque, NM, USA.*

Objectives: Endometrial cancer is the most frequent gynecologic cancer in women. It affects an estimated 40,000 women in the US every year, and long term outcomes for patients with advanced stage or recurrent disease are poor. Targeted molecular therapy against the vascular endothelial growth factor (VEGF) and its receptors constitute a new therapeutic option for patients that is now under study by the Gynecologic Oncology Group in a phase 2 trial, GOG 229E (now in second stage accrual). The goal of our work was to assess the potential effectiveness of VEGF/VEGFR blockade in preclinical endometrial cancer models.

Methods: These studies employed two agents, bevacizumab (Avastin, a VEGFA blocking antibody) and vandetanib (Zactima, a tyrosine kinase inhibitor of EGFR and VEGFR2). IC_{50} experiments were performed on endometrial cancer cells in culture using four established cell line models. Xenografted athymic mice were also employed to test the ability of compounds to inhibit tumor growth in vivo. Tumors were isolated from controls and treated animals, mRNA was extracted, and Affymetrix gene expression arrays were performed to determine the genes consistently modulated by treatment.

Results: Compared to vandetanib with an IC_{50} of 1.15 µM, bevacizumab showed little activity on cell proliferation in vitro, and cell numbers were not reduced by 50% using concentrations up to 2.5 µM. However, bevacizumab demonstrated robust activity in the athymic mouse model, resulting in a significant decrease in tumor formation and growth compared to vehicle treated animals when dosed bi-weekly in a concentration of 0.2 mg/mouse IP. Tumors from this model demonstrated that eighteen genes were consistently up or down regulated in the presence of bevacizumab. Among the regulated transcripts was microRNA21, which was significantly down-regulated. MicroRNA21 is an anti-apoptotic factor, and its inhibition by bevacizumab predicts for increased expression of the tumor suppressor PTEN, decreased cell proliferation, and a reduced capacity for metastasis.

Conclusions: These studies confirm that the VEGF pathway is a good target for new therapies against endometrial cancer. Blocking this pathway not only inhibits angiogenesis, but also results in changes in gene expression that enhance apoptosis and reduce cellular proliferation and tumor invasion.

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Progesterone Inhibition of Estrogen Signaling during the Menstrual Cycle: A Role for Wnt Signaling and the Wnt-Inhibitor DKK1. Leen J Blok,[1] Yongyi Wang,[1] Elisabeth C Kuhne,[2] Riccardo Fodde,[3] J Anton Grootegoed,[2] Curt W Burger.[1] *[1]Obstetrics and Gynaecology, Erasmus University Medical Center, Rotterdam, Netherlands; [2]Reproduction and Development, Erasmus University Medical Center, Rotterdam, Netherlands; [3]Pathology, Erasmus University Medical Center, Rotterdam, Netherlands.*

Objectives: Wnt/beta-catenin signaling has a central function in the maintenance and control of stem cell compartments where it regulates the fine balance between stemness (Wnt-on) and differentiation (Wnt-off). During the menstrual cycle there is a balance between estrogen-induced build-up and progesterone-induced differentiation of the endometrium. We postulate an important role for Wnt/beta-catenin signaling in the cyclic renewal of the endometrium.

Methods: Genome wide microarrays (Affymetrix) were used to produce endometrial gene expression profiles from healthy postmenopausal patients treated with estradiol or estradiol+progestagens (Hanifi-Moghaddam et al., 2007). Using NCBIs GEO DataSet (in PubMed) raw endometrial gene expression profiles from different stages during the menstrual cycle were extracted (Talbi et al., 2006). Ishikawa cells were used for transfections with PRA/B and DKK1 measuring Wnt activation using the TOP/FOP system.

Results: Using genome wide microarray studies it was observed that 65% and 27% of estrogen regulated genes were inhibited fully or partly back to control levels after progesterone administration. Furthermore, these same data indicated that the Wnt-signal-inhibitor DKK1 is a progesterone regulated gene, highly expressed during the mid-secretory phase of the menstrual cycle. These results led us to hypothesize that progesterone may inhibit Wnt-signaling by the induction of DKK1. Using the well differentiated Ishikawa endometrial cancer cell line we could show that Wnt-signaling was indeed inhibited by progesterone as well as DKK1 and we could also show that progesterone activates transcription from the DKK1 promoter.

Conclusions: Progesterone inhibits Wnt signaling in the human endometrium, putatively by inducing the Wnt inhibitor DKK1.

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Protein Tyrosine Kinases in Leiomyoma Uteri: Expression of Two Unique Ligands. Yong Jiang,[1] Drew Kennedy,[1] Xin Lu,[1] Max Chen,[1] Rana Walley,[2] Linda Wasserman,[1] Bryan D Cowan,[2] Jean YJ Wang.[1] *[1]Moores UCSD Cancer Center, University of California, San Diego, La Jolla, CA, USA; [2]Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS, USA.*

Background and Objective: Receptor tyrosine kinases (TK) have been implicated in the proliferation of soft tissue tumors, and we hypothesize that specific TK activities are required for the growth of fibroids.

Experimental Design, Materials and Methods: We constructed a custom microarray consisting of oligonucleotides derived from the known 90 human receptor TK genes, 45 TK-ligands, and several cyclins and Bcl2-family proteins.

We collected fibroid and adjacent normal tissues following hysterectomy with patient consent and institutional IRBs. To date, 840 data points for each gene from 420 arrays have been collected from 42 patients. The fluorescence readings were log-transformed and normalized with the Robust Quartile normalization method. Quality control of the normalized data was performed to remove 13 arrays that deviated from twice the inter-quartile range calculated from the 420 array signals.

Results: The custom microarray profiling identified 38 genes that were differentially expressed between normal and fibroid tissues ($p < 0.01$; FDR<0.13). Among them, 12 genes encode receptor TKs, 15 genes encode TK ligands, and 11 genes encode cell cycle and apoptosis proteins. Clustering analysis of the mean log2 ratios of these 38 genes has led to the division of the 42 patients into two major groups. Thirty four (81%) belong to a group characterized by the significant downregulation of the Cyr61 (CCN1) gene in fibroid tissues. The Cyr61-down group can be further divided into two sub-groups based on the expression of another TK ligand, EFN4A. Other receptor differentially expressed TK did not segregate with the three defined sub-groups. Finally, there was no sub-group segregation based on age of the patient, menstrual phase, or weight of the fibroid.

Conclusion: Our results have demonstrated that tyrosine kinases and their ligands are uniquely differentially expressed between normal and fibroid tissues. Unique tyrosine kinase ligands in our population were Cyr61 and EFN4A, and these markers created three molecular classification groups based on their differential expression. These results support the hypothesis that tyrosine kinase ligands are involved in fibroid growth, and may offer targets for a strategy to avoid hysterectomy.

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Microvascular Perfusion Sonographic Imaging To Detect Early Stage Ovarian Cancer. Joanie Mayer Hope,[1] Arthur C Fleischer,[2] Brian Day,[1] Stephanie V Blank,[1] Bhavana Pothuri,[1] Robert Wallach,[1] John P Curtin,[1] David A Fishman.[1] *[1]Gynecologic Oncology, New York University School of Medicine, New York, NY, USA; [2]Radiology, Vanderbilt University Medical Center, Nashville, TN, USA.*

Objective: Epithelial ovarian cancer (EOC) is the 4th leading cause of death in US women due to the inability to detect early stage disease. Recent sonographic developments involving harmonics, pulse inversion, and the use of contrast agents justify the hope that depiction of aberrant tumor microvascularity associated with early disease can occur. This study utilizes these new techniques to assess the unique microvascularity associated with early stage EOC.

Methods: We used pulse inversion harmonic microvascular imaging (MVI) technology to depict differences between benign and malignant ovarian lesions. Contrast enhanced harmonic transvaginal (TV) sonography was performed using the Philips iU22 scanner after intravenous injection of 1 µg of Definity (Bristol-Myer-Squibb). Split screen real-time images were acquired displaying conventional sonographic views adjacent to harmonic, low mechanical index images. Morphologic features (thickened wall, papillary excrescence, calcifications) and aberrant vascularity were noted. Q-lab quantification of wash-in, peak enhancement, and wash-out times as well as area-under-the-curve (corresponding to microvessel perfusion) were compared using student's T-tests.

Results: To date, contrast enhancement patterns of 93 ovaries have been analyzed, 78 benign and 15 malignant. Of the malignancies (3 fallopian tube, 12 ovarian: 3 stage I, 12 stage III), 12/15 women were correctly identified using conventional TV imaging and 3 others were identified using MVI 15/15. All benign lesions were correctly identified by MVI 78/78 while TV detected 81 normals (3 false negatives). The lesions detected as malignant by MVI were 2-Stage I fallopian tube and 1-Stage I EOC. Contrast enhancement kinetics of malignant lesions demonstrated similar wash-in (26.1 ± 6.3 vs 24.9 ± 7.6, $p = 0.7$), greater peak enhancement (23.3 ± 2.8 vs 12.3 ± 3.9, $p < 0.01$), longer wash-out (139.9 ± 43.6 vs 46.3 ± 19.7 ($p < 0.01$), and greater perfusion (2012.9 ± 532.9 vs 523.8 ± 318, $p < 0.01$) when compared to benign lesions.

Conclusion: Contrast enhancement patterns are significantly different in benign vs. malignant ovarian masses. This technique has clear potential in differentiating benign from malignant lesions and for detecting occult Stage I disease.

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Identification and Characterization of miR-199a as Regulator of IKKβ Expression and Its Function in Ovarian Cancer Cells. Rui Chen, Ayesha B Alvero, Thomas Rutherford, Gil Mor. *Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT, USA.*

Introduction: The proinflammatory environment associated with tumor growth and chemoresistance is produced by both immune cells and cancer cells. The NF-κB pathway plays a critical role mediating the capacity of cancer cells to produce pro-inflammatory cytokines. Recently, we described a group of epithelial ovarian cancer (EOC) cells characterized by IKKβ expression as the main factor promoting NF-κB activation and cytokine production. In this study we evaluated the regulation of IKKβ in EOC cells. We describe the identification and characterization of miR-199a as a regulator of IKKβ expression, thus indirectly of NF-κB activity and function.

Materials and Methods: 11 human EOC cell lines were established from malignant ovarian cancer ascites. Protein expression were determined by western blotting. IKKβ mRNA was measured by RT-PCR. Cytokines were profiled by the Luminex 200 system. IKKβ transfection was done with Roche FuGENE6 Transfection Reagent. miRNA microarray was done with Invitrogen NCode Multi-Species miRNA Microarray Kit. miR-199a qRT-PCR was performed with Invitrogen NCode SYBR GreenER miRNA qRT-PCR Analysis Kit. miRNA transfection was done with Ambion siPORT NeoFX Agent. The IKKβ 3'-UTR Luciferase reporter plasmid was established based on Ambion pMIR-REPORT miRNA Expression Reporter Vector.

Results: IKKβ expression was associated with NF-κB cyclic activity and the ability of Type I EOC cells (but not Type II) to produce inflammatory cytokines. Transfection of IKKβ into Type II EOC cells reversed their phenotype. miRNA microarray identified 18 miRNAs differentially expressed in Type I versus Type II cells, one of which, miR-199a, had 3 putative binding sites in the 3'-UTR of IKKβ mRNA. miR-199a introduction into Type I cells inhibited IKKβ expression, and direct inhibition through IKKβ's 3'-UTR was confirmed by luciferase assay.

Conclusion: We describe for the first time the identification of IKKβ as a potential key switch between chemo-resistant and chemo-sensitive phenotypes, by regulating NF-κB activity in EOC cells. Furthermore, we identified miR-199a as a direct regulator of IKKβ expression. IKKβ expression may represent an adaptational stage in tumor progression allowing cancer cells to create their own inflammatory environment. These findings may provide novel molecular targets and potential markers for individual therapy selection.

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Regulation of CD55 in the Rat Uterus by Nitric Oxide and the Involvement of P13 Kinase Pathway. Uma Yallampalli, Rebakah Elkins, Pawel Goluszko, Chandra Yallampalli. *Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Objective. CD55 is expressed in many cell types including uterine cells and has been shown to play an important role in protecting against compliment attack. We have previously shown in endometrial cell lines that CD55 levels were down regulated by nitric oxide (NO). In this study we extend our previous observations to determine if NO down regulates CD55 in rat uterine tissues and assess its mechanisms of action.

Methods. Non pregnant rats (200g; B wt) were bilaterally ovariectomized under ketamine anesthesia. Groups of ovariectomized rats were implanted with Alzet mini pumps to deliver nitro-L-arginine melthylester (L-NAME) at 10 or 50/mg/rat/day in saline or saline alone. Uteri were obtained from these rats at 48 or 72 hours after infusion. In another set of experiments uteri were obtained from ovariectomized rats and cut in to small pieces and incubated in vitro with either L-NAME (3mM), diethylenetriamine-NO (DETA-NO; 1mM) or Worthmanin (0.1 µM) in MEM without phenol red for 24 hours. Uterine tissues were homogenized in Trizol and mRNA levels for CD55 were measured using RT-PCR and expressed as a ratio to 18S.

Results. Results show that in vivo treatment with L-NAME to ovariectomized rats caused elevations in uterine CD55 mRNA levels in a time and dose dependent manner with maximal responses seen with 50mg L-NAME and at 72 hours. In vitro studies show that DETA-NO suppressed CD55 mRNA levels in the rat uterus. Both L-NAME and PI3 Kinase inhibitor, Worthmanin caused increases in CD55 levels in these tissues and the effects of Worthmanin are reversed by DETA-NO. These results suggest that CD55 levels in the rat uterus are down regulated by NO and are upregulated when NO synthesis is inhibited by L-NAME. Further, PI3 Kinase appears to be involved in CD55 regulation in the rat uterus and NO donor appears to modulate this response.

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Maternal Progesterone Treatment Retards Labor in Mice by Antagonizing Inflammatory Response Pathways and SP-A Secretion by the Fetal Lung. LaKeitha R Foster,[1] Daniel B Hardy,[2] Carole R Mendelson.[2] *[1]Dept of Pediatrics; [2]Depts of Biochemistry and Ob/Gyn, UT Southwestern Medical Center, Dallas, TX, USA.*

During ~95% of human pregnancy, the maternal uterus is maintained in a state of almost complete quiescence by elevated circulating levels of progesterone (P_4). We previously observed that P_4 acting through the progesterone receptor (PR) serves an anti-inflammatory role and inhibits uterine contractility by antagonizing nuclear factor κB activation and cyclooxygenase-2 (COX-2) expression (Hardy *et al.*, 2006). Our previous findings also suggest that the fetus provides an important signal for the initiation of labor near term through augmented secretion of the major lung surfactant protein, SP-A, into amniotic fluid (Condon *et al.*, 2004). Secreted SP-A, in turn, activates fetal macrophages which migrate to the maternal uterus where they release cytokines and promote an inflammatory response, leading to labor. In the present study, we tested the hypothesis that maternal P_4 also maintains uterine quiescence by inhibiting SP-A production by the fetal lung. Age matched ICR mice were injected *s.c.* once daily either with sesame oil (control) or P_4 (1 mg/ml) from 15 to 19 days post-coitum (dpc). As expected, treatment with P_4 delayed parturition by 24-48 h. This also was associated with a decrease in uterine COX-2 mRNA expression, as compared to the vehicle-injected controls. Interestingly, maternal P_4 treatment caused a marked decrease in the levels of immunoreactive SP-A protein secreted by the fetal lungs into in amniotic fluid at 19 dpc. Furthermore, SP-A protein and mRNA levels were reduced in the fetal lungs of P_4-injected mothers, as compared to controls. This was associated with an inhibitory effect of P_4 treatment on COX-2 protein and mRNA levels in the fetal lungs. These findings were of interest, since COX-2 expression is markedly upregulated during differentiation of human fetal lung (HFL) explants in culture (Hardy *et al.*, 2005) and endogenous and exogenous prostaglandins increase SP-A expression in HFL (Acarregui et al., 1990). Collectively, these findings suggest that maternal P_4 treatment prevents increased uterine contractility, in part, by inhibiting inflammatory response pathways within the fetal lung. This, in turn, blocks the developmental induction of SP-A expression and its secretion into amniotic fluid. In this manner, maternal P_4 inhibits an important fetal signal leading to labor. Supported by NIH P01 HD011149; NIH R37 HL050022.

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Stretch of Primary Rat Myometrial Cells Enhances Monocyte Chemotaxis and Uterine Inflammation Via a Ccl-2-Dependent Mechanism; an Effect Blocked by Progesterone. Oksana Shynlova,[1] Anna Dorogin,[1] Stephen Lye.[1,2,3] *[1]Samuel Lunenfeld Research Institute, Mt Sinai Hosp, Toronto, ON, Canada; [2]Ob/Gyn; [3]Physiology, University of Toronto, ON, Canada.*

Recent evidence suggests that leukocytes infiltrate uterine tissues at the time of parturition implicating inflammation as a key mechanism of human labor. CCL-2 is a pro-inflammatory cytokine that may contribute to the development of inflammatory reaction in the myometrium. Previously we showed up-regulation of rat *Ccl-2* gene expression in myometrium during term and RU486-induced preterm labor. Also *Ccl-2* was elevated specifically in the gravid horn of unilaterally pregnant rats suggesting that mechanical strain imposed by the growing fetus controls its expression in the myometrium. The **objective** of this study was to investigate the role of mechanical stretch as a possible regulator of myometrial leukocyte infiltration and Ccl-2 as a mediator of this stretch response. We also studied the effect of progesterone (P4) on the myometrial secretion of Ccl-2.

Methods. We used primary culture of rat myometrium smooth muscle cells (SMCs) to study *in vitro* Ccl-2 gene and protein induction by static mechanical stretch. Ccl-2 gene expression analysis was performed by real-time RT-PCR and immunoreactive (IR) protein content was measured by ELISA assay. We used primary rat monocytes to access whether stretch-induced Ccl-2 production by myometrial SMCs resulted in enhanced monocyte chemotactic activity.

Results. Myometrial cells were stretched for 2-24 hours and the supernatants collected. Analysis of media conditioned by primary myometrial SMCs revealed that static mechanical stretch (25% elongation for 24 hours) caused a significant accumulation in IR Ccl-2 which was repressed by pretreatment with P4 (1 uM). The rise in Ccl-2 protein levels was preceded by a transient increase on *Ccl-2* mRNA. The migration of primary rat monocytes in response to conditioned medium from stretched myometrial SMCs was much greater than that of conditioned medium from control non-stretched cells. Co-incubation with a neutralizing antibody to Ccl-2 significantly reduced the chemotaxis of monocytes in response to the stretch-conditioned medium. **Conclusion:** Uterine SMCs play an active role in uterine inflammation by producing chemokines and promoting the chemotaxis of immune cells into the myometrium. The blockade of this effect by P4 offers a potential explanation for the therapeutic actions of this hormone in the prevention of preterm birth.

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Changes in Leukocyte Subpopulations in the Choriodecidua and Fetal Membranes during Human Labor. Nardhy Gomez-Lopez,[1,2] Guadalupe Estrada-Gutierrez,[1] Lourdes Vadillo-Perez,[1] Felipe Vadillo-Ortega.[1] *[1]Direction of Research, Instituto Nacional de Perinatologia, Mexico City, DF, Mexico; [2]Escuela Nacional de Ciencias Biologicas, IPN, Mexico City, DF, Mexico.*

Introduction. Leukocytes arriving to the choriodecidua (CHD) during labor are capable to secrete cytokines and matrix metalloproteinases that may play a role in the fetal membranes (FM) extracellular matrix degradation.

Objective. The aim of this work was to identify changes in the leukocyte subpopulations in the CHD and FM during human labor.

Methods: FM were obtained from two groups of women: 1) term without labor (n=4) and 2) term with spontaneous labor (n=4). CHD cells were isolated and analyzed by flow cytometry. Explants of FM were embedded in paraffin and analyzed by confocal microscopy. In both techniques, CD45, CD56, CD14, CD19 and CD3 subpopulations of leukocytes were identified. Intracellular MMP-9 was also identified in these cells.

Results: Major changes in leukocytes subpopulations during labor involved a higher amount of CD3+, CD+14 and CD56+ cells, both in the CHD and inside the FM. MMP-9 was associated to CD 56+ cells. CD3+ exhibited a more widespread localization in the FM and CD56+ cells were localized in the contact with the trophoblast layer during labor.

Conclusions: Leukocyte populations changes both in the CHD and FM during labor and are characterized by arrival and infiltration of specific subpopulation of lymphocytes and monocytes. NK cells are enriched in MMP-9, which may be related to a role in extracellular matrix degradation leading to the rupture of fetal membranes.

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Racial Disparity in Genotype-Phenotype Interactions in Preterm Birth. Stephen J Fortunato,[1,3] Digna R Velez,[2] Errol R Norwitz,[3] Charles J Lockwood,[3] Scott M Williams,[2] Ramkumar Menon.[1] *[1]The Perinatal Research Center, Centennial Women's Hospital, Nashville, TN, USA; [2]Vanderbilt University, Nashville, TN, USA; [3]Obstetrics and Gynecology, Yale University, New Haven, CT, USA.*

OBJECTIVE: To study the association of maternal and fetal single nucleotide polymorphisms (SNPs) in TNFα and its receptor genes (TNFR1 and TNFR2) with amniotic fluid (AF) TNFα and soluble TNFR (R1 and R2) concentrations in preterm birth (PTB-cases) in Caucasian (C) and African Americans (AA) and to understand interaction of this association with microbial invasion of amniotic cavity (MIAC) in preterm birth (PTB).

METHODS: AA and C maternal and fetal DNA samples were genotyped for 34 single nucleotide polymorphisms [SNPs]) in 3 genes (TNFα, TNFR1 and TNFR2) and analyzed for association with AF TNFa and sTNFR1 and sTNFRR2 concentrations. AA maternal-35 cases, 57 controls (term birth), AA fetal-34 cases, 54 controls; C maternal-88 cases, 40 controls, C fetal- 86 cases, 36 controls were studied. Analysis of variance was performed with genotype, status (case/control), and maker-status interaction in the model for cytokine concentrations. Case only analyses including MIAC were also performed. We assessed independent effects as well as interactions. $p<0.01$ was significant.

RESULTS: In C, no significant associations were observed between SNPs in TNFα or TNF receptor genes in both maternal and fetal DNA and AF TNFα and sTNFR1 and R2 concentrations. Similarly, no significant interactions were observed between MIAC and the genotypes with AF analyte concentrations. However, in AA maternal DNA, TNFR2 SNPs (rs#816050; intron14842) and TNF concentration and TNFR2 SNPs (rs#522807;promoter-2910, rs#652625;promoter-1798) with sTNFR2 concentration showed significant association with pregnancy outcome. In AA fetal DNA, TNF SNP (rs#769178;downstream 3995) was associated with sTNFR1 and sTNFR2 concentrations. Presence of MIAC and maternal TNFR2 SNP (rs#235219) was associated with changes in sTNFR2 concentration, similarly fetal TNFR2 SNP (rs#235219) and MIAC resulted in differences in AF TNFα concentrations.

CONCLUSIONS: Racial disparity exists in genotype-phenotype interactions in PTB with several SNPs associating with AF analyte concentrations in AA but not C samples. MIAC and genotype also modifies analyte concentrations in AA but not in C. This study documents underlying differences in genetic regulation of AF analytes in AA that may result in changes that affect PTB.

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Histone 2B in Mid-Trimester Amniotic Fluid Down-Regulates Lipopolysaccharide-Induced Tumor Necrosis Factor Alpha Production by Amniocytes. Shari E Gelber, Devrim Sezen, Ann Marie Bongiovanni, Uma Perni, Daniel Skupski, Steven S Witkin. *Obstetrics and Gynecology, Weill Cornell Medical Center, New York, NY, USA.*

Objective: We hypothesized the existence of compounds in the amniotic cavity that recognize lipopolysaccharide (LPS) and neutralize LPS-induced pro-inflammatory immune system activation. The presence in mid-trimester amniotic fluid (AF) of histone 2B, and its ability to bind to LPS and inhibit LPS-induced TNF-alpha production by ex vivo-cultured whole AF, was determined.

Methods: Supernatant fractions from 68 mid-trimester amniotic fluids (15-19 weeks gestation) were added to wells of a microtiter plate that were coated with LPS from Escherichia coli (3 mg/well). The binding of histone 2B to LPS was quantitated by ELISA. The final values were adjusted to the total protein concentration of each AF. Unfractionated aliquots of 14 of the same amniotic fluids were cultured ex vivo for 24 hours in the presence or absence of 50 ng/ml E. coli LPS. The supernatants were tested for TNF-alpha and interleukin (IL)-10 concentrations by ELISA.

Results: All AFs were positive for histone 2B. The median (range) value was 72.7 (19.1-440.9) ng/mg total AF protein. The intraamniotic histone 2B concentration was inversely proportional to LPS-induced TNF-alpha production by the ex vivo-cultured amniocytes ($r = -.6035$, $P = .022$). There was no association between the histone level and IL-10 production. The mid-trimester intraamniotic histone 2B concentration was proportional to subsequent gestational age at delivery, increasing from 37 to 41 weeks gestation ($r = .5507$, $P = .041$). There was no association between the histone 2B level and maternal age, gravidity, parity or fetal gender. The AF histone 2B concentration was higher in Asian women than in White women ($P = .02$).

Conclusion: Histone 2b in mid-trimester AF binds to E. coli LPS and inhibits cells within the amniotic cavity from initiating LPS-induced production of TNF-alpha. The down-regulation of pro-inflammatory cytokine production at this site by components of amniotic fluid provides a mechanism to prevent the premature expulsion of the developing fetus from the uterus.

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Lactobacilli Supernatant Inhibits LPS-Activated TNF-α and Stimulates IL-10 Production in Human Chorion Trophoblast Cells. Maryam Yeganegi,[1,3] Carole S Watson,[3] Sung Kim,[2] Gregor Reid,[2] John RG Challis,[1] Alan D Bocking.[1,3] *[1]Dept. of Physiology & Ob/Gyn, University of Toronto, Toronto, ON, Canada; [2]Dept. of Microbiology & Immunology, University of Western Ontario, London, ON, Canada; [3]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada.*

Objective: Bacterial Vaginosis (BV) is characterized by the presence of gram-negative bacteria, and the absence of endogenous *Lactobacillus*. BV is associated with a 1.4-fold increased risk of preterm birth. Previously, we have demonstrated that *Lactobacillus rhamnosus* GR-1 supernatant is able to down-regulate LPS-stimulated TNF-α production in human placental trophoblast cells. Since the primary site of activity in an ascending infection, however, is more likely the fetal membranes, we hypothesize that probiotic lactobacilli would also antagonize the actions of LPS in chorion trophoblast cell cultures.

Methods: Term placentae were collected from women undergoing elective Caesarean section. Chorion trophoblasts were isolated using a percoll gradient according to established primary culture protocols. Cells were pretreated with lactobacilli supernatant (1:20 dilution) and subsequently treated with 100 ng/ml of LPS. TNF-α, IL-1β, and IL-10 concentrations were measured in the media using ELISA. **Results:** LPS stimulation caused a marked increase in production of TNF-α in chorion trophoblasts (10.2±6.5 pg/ml to 210.5±88.7 pg/ml, $p<0.05$). Pretreatment with lactobacilli supernatant markedly inhibited this increase (44.3±21.0 pg/ml, $n=7$, $p<0.05$). LPS and lactobacilli supernatant both stimulated IL-10 production (7.9±1.0 pg/ml to 23.6±8.8 pg/ml and to 11.8±1.8 pg/ml respectively, $n=4$, $P<0.05$). There were no changes in IL-1β concentrations with any treatment. **Conclusion:** Probiotic lactobacilli supernatant down-regulates LPS-activated pro-inflammatory cytokine TNF-α and up-regulates anti-inflammatory IL-10 in chorion trophoblast cells as in placental trophoblast cells. We propose that these activities counter the effects of LPS or other pathogenic bacteria in infection driven preterm birth in vivo and support a role for endogenous lactobacilli in decreasing the risk of spontaneous preterm birth.

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Cross-Talk between NF-κB, C/EBP and AP-1 in Regulating Human IL-8 Gene Expression. Shirin Khanjani, Yun S Lee, Sheri Lim, Suren R Sooranna, Mark R Johnson, Phillip R Bennett. *IRDB, Imperial College, London, United Kingdom.*

OBJECTIVES: Interleukin-8 is a chemokine, which recruits and activates neutrophils. It is present in uterine tissue, is upregulated by IL-1β and by NF-κBp65 overexpression, and its concentration increases in the third trimester and with labour. The IL-8 promoter contains a transcriptional enhanceosome (-125 to -73), with binding sites for NF-κB, AP-1 and C/EBP. Here, we report a functional and physical interplay of these Transcription factors in regulating IL-8 in human myocytes.

METHODS: Myometrium was collected from women undergoing elective caesarian section before the onset of labour. Cells were cultured until 90-95% confluent and then stimulated with 1ng/ml IL-1β for 6 hrs. For ChIP analysis, DNA protein complexes were crosslinked and antibodies recognizing p65, C/EBP, AP-1, H4 (+ve control) and IgG (-ve control) were used for immunoprecipitation. Primers were designed to target the enhanceosome. Si RNA was used to knock down the endogenous NF-κBp65 and the effect of IL-1β on IL-8 protein was measured using ELISA. Different combinations of expression constructs for NF-κB, C/EBPβ and AP-1(c-Jun and c-Fos) were transiently cotransfected to examine the effects on an IL-8 promoter reporter construct. Cells were cultured for a total of 48 hrs, followed by harvesting and analysis with a dual firefly/Renilla luciferase assay. To further study interactions between these transcription factors, a 6xNF-κB consensus/LUC reporter construct was cotransfected with each expression vector.

RESULTS: NF-κB, C/EBP and AP-1 separately drove the IL-8 promoter. However, transfections of all three together caused greatest promoter activity. ChIP assay showed no constitutive in vivo binding. However, binding of p65, C/EBP β and δ, AP-1(c-Jun and c-Fos) and H4 was seen in the cells stimulated with IL-1β. SiRNA against p65 abolished the IL-8 response to IL-1β, proving a central role for NF-κBp65. Transfection of the NF-κB reporter construct with expression vectors for either C/EBP or AP-1 caused a significant reduction in the basal promoter activity suggesting that both C/EBPβ and AP-1 can bind to NF-κB. These interactions were confirmed using a TF-TF interaction array.

CONCLUSION: The findings of this study suggest that binding of all three transcription factors to the enhanceosome and their physical interaction is needed for maximal induction of the IL-8 promoter. How they interact, is a question yet to be answered.

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The Requirement for MyD88 in *E. coli* Induced Preterm Labor: Mechanisms. Yana Filipovich,[1] Emmet Hirsch.[1,2] *[1]Department of Ob/Gyn, Evanston Northwestern Healthcare, Evanston, IL; [2]Department of Ob/Gyn, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

Objective: Myeloid differentiation primary-response gene 88 (MyD88) is one of two adaptor proteins that mediate the effects of toll-like receptor 4 (TLR4, the lipopolysaccharide receptor). We have demonstrated previously that *E. coli*-induced preterm delivery in the mouse has an absolute requirement for MyD88 but not for the other adaptor protein, TRIF ('Toll/IL-1 receptor (TIR)-domain-containing adaptor protein inducing IFNβ'). Here we examine the mechanisms underlying this requirement.

Methods: Wild-type (WT) mice or mice deficient in MyD88 (MyD88-KO), TRIF (TRIF-KO), or both MyD88 and TRIF (DKO) were inoculated into the uterus with either saline or 5×10^9 killed *E. coli* (which induces preterm delivery in 100% of WT and TRIF-KO mice and 0% of MyD88-KO and DKO mice). Nuclear translocation of nuclear factor κB (NFκB, a transcription factor controlling the expression of inflammatory mediators) was determined immunohistochemically 3h later. Expression of interferon β (IFNβ), interleukin 1β (IL-1β), tumor necrosis factor α (TNFα) and inducible nitric oxide synthase (iNOS) within the uterus was evaluated by RT-PCR 3 and 5 hours after treatment. Circulating progesterone levels were determined by ELISA 5 hours after treatment.

Results: *E. coli* induced a strong nuclear signal for NFκB in WT and TRIF-KO mice, but not in MyD88-KO and DKO mice. The baseline expression (i.e. after saline treatment) of IL-1β, TNFα and iNOS but not IFNβ was lower in MyD88 and DKO mice than in wild-type and TRIF-deficient mice ($p<0.05$). Treatment with *E. coli* resulted in upregulation of all these transcripts in WT controls ($p<0.05$ for all). TRIF-KO animals resembled WTs except for IFNβ, which, as expected, was not inducible in animals lacking TRIF. In contrast, none of the examined transcripts was inducible in DKO animals. In some bacterially treated MyD88-KO mice IL-1β, TNFα and IFNβ mRNA were

induced, though at levels lower than those of wild-type animals. A decline in serum progesterone occurred in *E. coli*-treated WT and TRIF-KO mice ($p<0.03$), but not in MyD88-KO or DKO animals.

Conclusions: MyD88 is required for *E. coli*-induced preterm delivery, for nuclear translocation of NFκB and for a decline in circulating progesterone in the mouse. In contrast, *E. coli*-induced upregulation of IL-1β and TNFα requires both MyD88 and TRIF.

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A History of Pre-Eclampsia Identifies Women Who Have Underlying Cardiovascular Risk Factors. Graeme N Smith,[1] Mark C Walker,[2] Aizhong Liu,[3] Shi Wu Wen,[2] Michelle Hladunewich.[4] *[1]Obstetrics & Gynecology, Queen's University, Kingston, ON, Canada; [2]Obstetrics & Gynecology, Ottawa Health Research Institute, Ottawa, ON, Canada; [3]School of Public Health, Central South University, China; [4]Nephrology, University of Toronto, Toronto, ON, Canada.*

BACKGROUND

Women who have had a pregnancy complicated by pre-eclampsia (PE) are at increased risk of cardiovascular disease. The primary objective of this study was to prospectively compare physical and biochemical cardiovascular risk markers in women who developed PE compared to those that did not, at one year postpartum.

METHODS

Following an overnight fast, previously PE (n=70) and normotensive women (n=70) had weight and blood pressure (BP) recorded, a.m. blood for insulin, glucose, C reactive protein, lipids and cholesterol and first a.m. urine for microalbumin and creatinine. Body mass index (BMI), HOMA index and incidence of Metabolic Syndrome were determined. Mathematical modelling was used to predict the risk of major cardiac events for both groups.

RESULTS

Baseline pregnancy characteristics were similar between groups. At one year post partum, there were significant differences in weight, BMI, change in BMI from delivery to follow up, systolic and diastolic BP, HOMA index, cholesterol, LDL and microalbumin/creatinine between the groups. There were also significant differences in the number of women classified as having abnormal values. Mathematical modelling of cardiovascular event risk suggests that PE increases the risk by 2-3 fold; the risk was greatest for women with severe or early onset PE.

CONCLUSIONS

By one year postpartum, women who had developed PE had increased BP, total cholesterol, higher LDL, triglycerides, increased BMI, fasting insulin, HOMA index and microalbumin/creatinine ratio. We have identified one of the earliest clinically identifiable markers of a woman's heightened risk of cardiovascular risk.

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Characterization of Maternal Innate and Adaptive Inflammatory Patterns in Women with a History of Early-Onset Preeclampsia. Bas B van Rijn,[1] Arie Franx,[2] Ger T Rijkers,[3] Hieronymus A Voorbij,[4] Mark Roest,[4] Hein W Bruinse.[1] *[1]Perinatology & Gynecology, University Medical Center Utrecht, Utrecht, Netherlands; [2]Obstetrics & Gynecology, St Elisabeth Hospital, Tilburg, Netherlands; [3]Pediatric Immunology, University Medical Center Utrecht, Utrecht, Netherlands; [4]Clinical Chemistry & Hematology, University Medical Center Utrecht, Utrecht, Netherlands.*

Objective: Preeclampsia is characterized by generalized endothelial dysfunction as a result of an inappropriate maternal immune response against the fetus. In this study, we used multiplexed cytokine mapping, to determine maternal constitutional innate and adaptive inflammatory patterns related to early-onset preeclampsia.

Methods: Sixteen primiparous women with a history of early-onset preeclampsia (delivery before 34 weeks gestation) and 16 controls with a history of only uneventful pregnancies were included. Whole blood samples were obtained at least six months after delivery and co-cultured with medium, LPS, IFNγ, CD2/28 and PHA for 18, 48 and 72 hours. Subsequently, simultaneous detection of IL1β, IL4, IL5, IL6, IL8, IL10, IL13, IFNγ, TNFα, MIF, sICAM, sVCAM, MCP-1 and IL18 was performed by a previously validated multiplexed fluorescent microsphere immunoassay. Data were analyzed by conventional parametric and non-parametric statistical tests, as well by as by cluster analyses using color array techniques.

Results: Women with a history of early-onset preeclampsia exhibited characteristically different cytokine profiles in response to inflammatory stimulation, when compared to healthy controls, predominantly involving pro-inflammatory (IFNγ, $P<0.05$; IL1β, $P=.07$), cell migratory (IL8, $P=.004$) and innate immunity regulatory cytokines (IL18, $P<0.05$; MIF).

Conclusion: Cytokine mapping of women with a history of early-onset preeclampsia reveals altered innate and adaptive inflammatory patterns in response to mild inflammation. Further understanding of the complex immunoregulatory processes that constitute the maternal inflammatory response could be of crucial importance to predict adverse pregnancy outcome.

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Body Composition by Dual Energy-X-Ray Absorptiometry in Women with a History of Preeclampsia or Intrauterine Growth Restriction. Anne L Berends,[1] Maria C Zillikens,[2] Christianne J de Groot,[1] Fernando Rivadeneira,[2] Ben A Oostra,[3] Cornelia M van Duijn,[4] Eric A Steegers.[1] *[1]Obstetrics and Gynecology, Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands; [2]Internal Medicine, Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands; [3]Clinical Genetics, Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands; [4]Epidemiology and Biostatistics, Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands.*

Background:

Women with a history of a pregnancy complicated by preeclampsia or intrauterine growth restriction (IUGR) have an increased risk of future cardiovascular disease. Excessive weight, particularly abdominal fat mass, is associated with cardiovascular morbidity and mortality.

Objectives:

The aim of this study was to investigate differences in body composition and fat distribution between women with a history of preeclampsia or IUGR and uncomplicated pregnancies.

Methods:

From a genetically isolated population in the Southwest of the Netherlands, non-pregnant women with a history of preeclampsia (n=45), IUGR (n=53) and uncomplicated pregnancies (n=106) were recruited at a mean follow up time of 10.8 years after pregnancy. Body composition and fat distribution were assessed by dual energy-X-ray absorptiometry (DXA) and anthropometric measurements.

Results:

Women with a history of preeclampsia compared to controls had higher mean total-, fat- and lean mass ($P<0.05$) as well as higher mean indices of body mass, fat mass and lean mass ($P< 0.05$). No significant differences were found for these variables between women with a history of IUGR and controls. Women with a history of preeclampsia had higher waist circumferences and waist-to-hip ratios ($P<0.001$) as well as excess of android fat mass and increased android-to-fat ratios ($P<0.05$). Women after pregnancies complicated by IUGR had higher waist-to-hip ratios ($P <0.001$). After controlling for body mass index, both women with a history of preeclampsia or IUGR had higher waist circumferences ($P <0.001$) and waist-to-hip ratios ($P <0.001$) as well as smaller hip circumferences ($P < 0.001$).

Conclusion:

Despite differences in body mass index, both women with a history of preeclampsia and women after pregnancies complicated by IUGR have a metabolically adverse fat distribution, marked by an excess of fat deposition in the abdominal region relatively to the hip region. These findings may explain, at least partly, their increased cardiovascular risk.

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Persistent Renal and Central Hemodynamic Anomalies in Middle-Age Women with a History of Preeclampsia. Marc EA Spaanderman,[1] Timo H Ekhart,[2] Robert Aardenburg,[2] Louis LH Peeters.[2] *[1]Obstetrics and Gynecology, Radboud University Medical Center, Nijmegen, Netherlands; [2]Obstetrics and Gynecology, University Medical Center Maastricht, Maastricht, Netherlands.*

Background: A history of preeclampsia is associated with persistent short-term alterations in circulatory function and remote cardiovascular disease. In this study we tested the hypothesis at least 20 years after preeclamptic pregnancy renal and central hemodynamic function is impaired as compared to women with uncomplicated pregnancy.

Methods: In 20 formerly preeclamptic women (PE) and 24 healthy parous controls (Control) who were normotensive at 6 weeks post-partum follow up, we assessed at least 20 years after delivery blood pressure (mmHg), cardiac output (CO, doppler ultrasonography, L/min) and effective renal plasma

flow (ERPF, PAH clearance, ml/min/1.73m^2) after which we calculated total peripheral vascular resistance (.100 dyne.s/cm^5) and renal vascular resistance (.100 dyne.s/cm^5). Data were analyzed parametrically ($p<0.05$).

Results: Age and BMI were comparable between groups. Blood pressure was higher in formerly PE. Moreover, 40% of formerly PE women and 13% of Control were hypertensive ($p<0.05$). Although cardiac out was comparable between groups, total peripheral and renal vascular resistance were about 20% higher and ERPF 15% lower in formerly PE women as compared to Control.

Conclusion: At least 20 years after gestational hypertensive disease, women who were normotensive at direct follow up have impaired renal and central hemodynamic function and developed more often chronic hypertension. Long-term follow up may also be warranted in apparently healthy formerly PE women who are normotensive at post-partum follow up.

circulatory function in formerly preeclamptic women and healthy parous controls

	CO	ERPF	TPVR	RVR
Formerly PE	5.0±0.6	397±61*	18±2*	120±29*
Control	4.7±0.8	469±80	15±2	97±25

* = $p<0.05$

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Pregnancy Increases Blood-Brain Barrier Permeability Coefficient (L_p) to Lucifer Yellow: Role of Estrogen. Marchien J Wiegman,[1,2] Marilyn J Cipolla.[1] [1]*Neurology, Ob/Gyn and Pharmacology, University of Vermont, Burlington, VT, USA;* [2]*Ob/Gyn, University Medical Center Groningen, Groningen, Netherlands.*

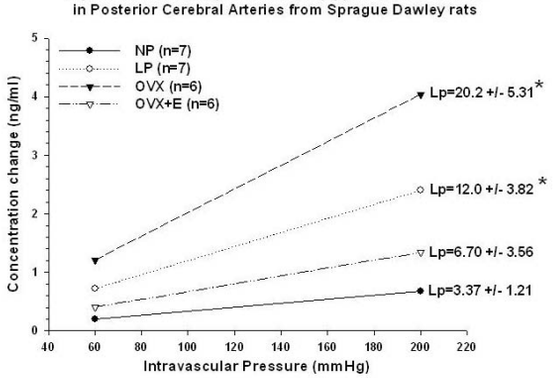
Background: Eclampsia is similar to posterior reversible encephalopathy syndrome in which an acute rise in blood pressure causes breakthrough of autoregulation, blood-brain barrier (BBB) disruption, and cerebral edema formation. We previously showed that late-pregnant (LP) animals developed cerebral edema during breakthrough, a response that was absent in nonpregnant (NP) animals. In the current study we hypothesized that pregnancy predisposes the brain to edema during acute hypertension by enhanced BBB permeability. We further hypothesized that the underlying effect of pregnancy on the BBB permeability is due to elevated estrogen levels.

Methods: Permeability coefficients (L_p) to Lucifer Yellow (LY), a polar compound that does not pass through tight junctions, were compared in posterior cerebral arteries (PCA) from 4 groups of Sprague Dawley rats: NP (n=7), LP (d20; n=7), ovariectomized and implanted with 17β-estradiol (0.5mg, 21-day release) and estriol (5.0mg, 21-day release) pellets for 14 days (OVX+E; n=6), and ovariectomized and implanted with placebo pellets for 30 days (OVX; n=6). PCAs were isolated, pressurized in an arteriograph, and perfused with 0.5mg/ml LY in saline. Concentration changes of LY outside the vessel wall were determined at pressures from 60-200mmHg. The slope of the pressure vs. permeability curve is the rate of flux, or L_p for LY.

Results: L_p for LY was significantly increased in PCAs from LP and OVX animals vs. NP ($p<0.05$; Figure 1). Estrogen was protective of the BBB only in ovariectomized animals, decreasing L_p 150% in OVX+E vs. OVX. However, pregnancy did not afford protection and had a L_p that was 256% greater than NP.

Conclusions: Pregnancy significantly increases BBB permeability to LY, an effect that may predispose the brain to edema formation during acute hypertension. These data also show that estrogen modulates L_p in ovariectomized animals differentially than pregnancy, suggesting that the increased BBB permeability in pregnancy is caused by a mechanism other than elevated estrogen levels.

Figure 1. Permeability Coefficients (Lp) for Lucifer Yellow in Posterior Cerebral Arteries from Sprague Dawley rats



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In Both Pre- and Early Pregnancy, the Sympathoinhibitory Response to Volume Expansion Is Blunted in Formerly Preeclamptic Women with Low Plasma Volume. Ineke Krabbendam,[1] Marc EA Spaanderman,[1] Dorette A Courtar,[2] Robert Aardenburg,[2] Ben J Janssen,[3] Fred K Lotgering,[1] Louis LH Peeters.[2] [1]*Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands;* [2]*Obstetrics and Gynecology, University Hospital Maastricht, Maastricht, Netherlands;* [3]*Pharmacology and Toxicology, University of Maastricht, Maastricht, Netherlands.*

Background: The circulation of formerly preeclamptic women with a low plasma volume (LPV) is characterized by sympathetic dominance. These women respond to a new pregnancy with an aberrant rise in atrial natriuretic peptide (ANP) and a 3 times higher chance to develop recurrent gestational hypertensive disease compared to their counterparts with normal plasma volume (NPV). ANP has sympathicomimetic capacity. We postulate that the sympathetic overdrive in LPV-women is associated with a reduced venous capacitance. To this end, we compared the response to volume expansion (VE) in women with LPV and NPV, both before and in pregnancy.

Method: In 24 non-pregnant normotensive formerly preeclamptic women, we measured PV (HSA I^{125} indicator dilution method) at least 6 months post partum. We intravenously infused 500 ml of iso-oncotic fluid over 30 minutes. During the infusion, we recorded changes in heart rate (HR, bpm), blood pressure (BP, mmHg), cardiac output (CO, l/min), sympathetic activity (LFsys, mmHg^2, low frequency component of spontaneous fluctuations in systolic BP, Portapress) and ANP (nmol/l). Eight women became pregnant within 1 year and were evaluated at 12 weeks gestation. Changes in circulatory and autonomic function between and within groups were analyzed non-parametrically ($p<0.05$).

Results: Before pregnancy, VE leads to comparable changes in HR, BP and CO in women with LPV (17/24) and NPV (7/24). In NPV, LFsys decreased 28%, but only 7% in LPV ($p<0.05$). ANP remained unaltered in NPV, but increased in LPV. In the pregnant group, 4 women had LPV and 4 had NPV. In both groups, pregnancy did not alter the response to VE.

Conclusion: Irrespective of pregnancy, the sympathoinhibitory response to VE is diminished in LPV. These data suggest that in these women VE leads to venous overfill, giving rise to ANP-release and consequently sympathetic activation, flattening the normal baroreceptor-mediated sympathoinhibitory response. We speculate that this mechanism contribute to circulatory maladaptation to pregnancy, sympathetic dominance and subsequent gestational hypertensive disease.

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Effect of Antihypertensive Treatment on Angiogenic Factors in Women with Hypertensive Disorders in Pregnancy. Asma Khalil,[1] Shanthi Muttukrishna,[2] Kevin Harrington,[1] Eric Jauniaux.[2] [1]*Obstetrics, Queen Mary, University of London, London, United Kingdom;* [2]*Obstetrics, University College London Hospitals, London, United Kingdom.*

Introduction

Anti-angiogenic factors, such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng), and pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), play an important role in the pathophysiology of pre-eclampsia (PE). It has recently been shown that antihypertensive drugs can alter cytokine release in normal and hypertensive pregnancy. These cytokines are known to stimulate secretion of both sFlt-1 and VEGF. It is not known if antihypertensive therapy can affect the secretion of angiogenic factors in PE.

Patients and methods

We recruited 129 women with hypertensive disorders in pregnancy (63 PE and 66 non-proteinuric hypertension [HT]) and 129 matched normotensive controls. PlGF, sFlt-1 and sEng levels, before and 24-48 hours after initiating antihypertensives, were measured using an ELISA. Having validated these assays for placental tissue, the same markers were measured in 84 placentas delivered at cesarean section at similar gestational age (29 PE, 24 HT and 31 controls).

Analysis

The three study groups were compared using ANOVA multiple comparisons with Bonferroni post hoc testing. The data were normally distributed after logarithmic transformation. Marker levels before and after antihypertensive therapy were compared using paired t-test. We compared placental concentrations between the group which received antihypertensive therapy and the group which did not, using an independent t-test. Data were analysed using SPSS®.

Results
In both PE and HT, serum sFlt-1 was increased, and PlGF reduced at all gestations ($p<0.0001$). sEng levels were also increased in PE. After 28 weeks (but not before) antihypertensive treatment was associated with a significant fall in serum sFlt-1 and sEng, in PE only. The concentrations of both sFlt-1 and sEng were significantly higher in the placentas of women with PE, but not HT, compared with controls ($p=0.0002$). Only sFlt-1 was significantly reduced in the placenta in women who received antihypertensive therapy.
Conclusion
In PE, antihypertensive therapy after 28 weeks' gestation is associated with a significant fall in serum sFlt-1 and sEng, and in placental sFlt-1. These findings raise the possibility that these drugs may have an effect on the pathophysiology of PE other than their known antihypertensive action.

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The Synergistic Effect of Soluble VEGF Receptor 1 Pre-Treatment and Small Doses of TNF-α on Endothelial Cells. Tereza Cindrova-Davies,[1] Debbie A Sanders,[2] Olivera Spasic-Boskovic,[1] Graham J Burton,[1] D Stephen Charnock-Jones.[2] *[1]Dept of PDN, University of Cambridge, United Kingdom; [2]Dept of Obstetrics and Gynaecology, University of Cambridge, United Kingdom.*
Introduction: Preeclampsia is marked by an enhanced endothelial inflammatory response manifested by maternal endothelial activation. Soluble fms-like tyrosine kinase-1 (sFLT-1, sVEGF-R1), a naturally occurring circulating antagonist of VEGF-A and PlGF, is one of the secreted factors implicated in the pathogenesis of preeclampsia. In women who develop preeclampsia, sFLT rises sharply, preceding the onset of the clinical disease. The aim of this study was to examine the effect of a combined treatment with recombinant sFLT-1 and TNF-α on the activation of human umbilical cord endothelial cells (HUVEC) by examining leukocyte adhesion, and the expression of ICAM, VCAM, endothelin and vWF.
Methods: HUVEC were seeded, grown overnight and pre-treated with recombinant sFLT (50-250 ng/ml) in a basic 2% FBS-DMEM medium for 24 hr. On day 3, low doses of TNF-α (0.5 ng/ml) were applied to pre-treated cells for 6 hr. Antagonism of the VEGF-A action was mimicked at the protein level by pre-incubating HUVEC with anti-FLT antibody (5-10 μg/ml), anti-KDR antibody (1 μg/ml), anti-VEGF antibody (5 μg/ml), or VEGF receptor inhibitor SU5614 (5 μM). At the RNA level, the effect of sFLT was mimicked by siRNA transfection of HUVEC with si*FLT* or si*KDR*. At the end of each experiment cells were either harvested for Western blotting, fixed in 2% PFA for immunofluorescence or incubated with labelled HL60 leukocyte cells, followed by fluorescent detection of adhesion.
Results: Pre-incubation of HUVEC with sFLT and subsequent treatment with low doses of TNF-α increased the adhesion of HL60 leukocytes and increased ICAM-1, VCAM-1, endothelin and vWF, compared to TNF-α treatment alone. Similar results were obtained when cells were pre-treated with SU5614, anti-FLT, anti-KDR or anti-VEGF. Transfection knock-down of *FLT* or *KDR* gene also significantly increased leukocyte adhesion when small doses of TNF-α were added.
Conclusions: Pre-incubation with recombinant sFLT, anti-FLT, anti-KDR, anti-VEGF, SU5614 or knocking-down *FLT* or *KDR* transcripts all antagonised the autocrine actions of VEGF and/or PlGF. This predisposes HUVECs to be more sensitive to the effect of TNF-α. Our study shows that sFLT and TNF-α combine to induce an enhanced synergistic effect, activating endothelial cells.
Supported by the Wellcome Trust.

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Varying Levels of Maternal Obesity Reveal a Reciprocal Relationship between Oxidative and Nitrative Stress in the Placenta. Jessica A Smith, Victoria HJ Roberts, Angela B Heizer, Leslie Myatt. *Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA.*
Maternal obesity is associated with increased production of inflammatory cytokines and risk of poor perinatal outcome. Inflammatory cytokines can stimulate production of reactive oxygen (ROS) and nitrogen (RNS) species, which can covalently modify protein function. Placental oxidative and nitrative stress are increased in pathological pregnancies and associated with altered placental function.
Objectives: Determine the effect of increasing body mass index (BMI) on placental nitrative stress, measured by the expression and localization of nitrated (nitrotyrosine) and oxidized proteins.
Methods: Placental tissue was collected at term (37-41 wks) from lean (BMI 18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (30-40 kg/m^2) patients (n=5 or 6/group). Tissue was sectioned for immunostaining with nitrotyrosine antibody. Protein samples were either dot blotted onto nitrocellulose membrane, probed with nitrotyrosine Ab, and nitrated proteins detected using ECL, or derivatized using 2,4-dinitrophenylhydrazine (DNPH) (OxyBlot® Kit), separated on SDS-PAGE, probed with anti-DNPH Ab (1:150) and oxidized proteins detected using ECL. Protein band intensity was measured by densitometry. Oxidized proteins were selected for MALDI mass spectrometry analysis.
Results: Nitrotyrosine residues were immunolocalized primarily in the fetal capillary endothelial cells and the villous stroma, but were almost absent in the syncytiotrophoblast. By Dot Blot, nitrotyrosine expression differed across the three groups ($p<0.003$, ANOVA) with expression in tissue from obese women being significantly increased compared to lean ($p<0.005$) and overweight ($p<0.05$, Tukey test). Several oxidized proteins were detected with significantly greater expression seen in the lean versus overweight groups ($p<0.02$, Mann-Whitney U). One oxidized protein was identified by MALDI-MS with four peptide matches and 19.2% coverage as 3-beta hydroxysteroid dehydrogenase (3bHSD).
Discussion: With increasing BMI, an increase in nitrative stress appears to occur in parallel with a decrease in oxidative stress. Oxidative stress is apparently reduced as ROS are consumed by the interaction with RNS to give nitrative stress. 3bHSD is involved in the biosynthesis of steroid hormones and glucocorticoids. Its activity and hence steroid metabolism in the placenta may be regulated by oxidation with implications for fetal development.

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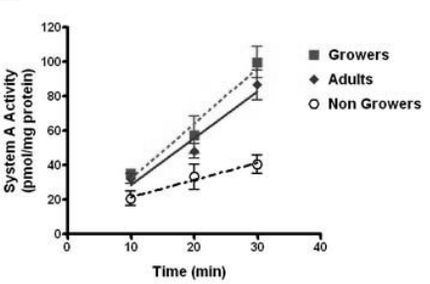
Impact of Maternal Growth on Placental Nutrient Transport in Teenage Pregnancies. Christina E Hayward, Susan L Greenwood, Philip N Baker, Colin P Sibley, John RG Challis, Rebecca L Jones. *Maternal and Fetal Health Research Group, University of Manchester, United Kingdom.*
Background Teenagers are susceptible to delivering small-for-gestational-age (SGA) infants. Previous studies implicate continued maternal growth as a causal factor[1]. Growing adolescent sheep have reduced fetal birthweight due to impaired placental development and nutrient transfer[2]. We hypothesized that placental function is impaired in human teenage pregnancy if there is maternal growth.
Methods Placentas were collected from 29 teenagers (15-18 years) and 21 adults. Activity of the amino acid transporter, system A, was quantified by the sodium-dependent uptake of ^{14}C-methylaminoisobutyric acid into placental fragments. Teenagers were defined as growing (>2mm increase in kneeheight per 90 days) or non-growing. System A activity was analysed in relation to individualised birthweight centile and maternal growth.
Results Placental system A activity was significantly lower in teenage compared to adult pregnancies ($p<0.05$). This was unrelated to birthweight; teenagers who delivered infants appropriate-for-gestational age (AGA) had significantly lower placental system A activity compared to AGA infants delivered to adults ($p<0.01$). Growing teenagers did not deliver lower birthweight infants than non-growing teenagers (median birthweight 3550g and 3290g respectively). Furthermore, system A activity in placentas from growing teenagers was significantly elevated compared to that in non-growing teenagers ($p<0.01$), and was similar to placental activity in adults.
Conclusions System A activity was reduced in placentas from teenagers compared to adults. This suggests that inherently lower placental function predisposes teenagers to SGA, but that other factors (e.g. adequate nutrition) can compensate in those teenagers delivering AGA infants. In contrast to our hypothesis, placental system A was elevated in growing teenagers and mimicked that of adults. This may be related to a hormonal-milieu in growing teenagers that is conducive to fetal growth, in part through stimulating placental transport.
[1]Scholl et al 1994 Am J Clin Nutr 60:183; [2]Wallace et al 2001 Reprod 122:347.



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Maternal Obesity and Overnutrition Increase Placental Fatty Acid (FA) Transport. Mei J Zhu,[1] Maggie S Strable,[2] J Thomas Brenna,[2] Min Du,[1] Bret W Hess,[1] Gary E Moss,[1] Peter W Nathanielsz,[1,3] Stephen P Ford.[1] *[1]Center for the Study of Fetal Programming, Department of Animal Science, University of Wyoming, Laramie, WY, USA; [2]Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA; [3]Department of Obstetrics and Gynecology, University of Texas Health Sciences Center, San Antonio, TX, USA.*

Introduction: Placental FA transport is important for fetal growth and development. FA transport across the placenta is mediated by the fatty acid transporters (FATP, 1 and 4) as well as fatty acid translocase (FAT)/CD36, a multi-ligand putative FA transport protein. Little information is available regarding the specific impact(s) of maternal obesity on placental FA transport.

Objective: To determine the impact of maternal obesity on placental FA transport and the expression of FATP1, FATP4 as well as FAT/CD36 in cotyledonary (COT) tissue at midgestation.

Methods: Nonpregnant ewes were randomly assigned to control (C, 100% of NRC recommendations) or obesogenic (OB, 150% of NRC) diets from -60 to 75 days of gestation (dG). Under general anesthesia, maternal and fetal blood was collected to measure FA concentrations by gas chromatography, and leptin by RIA (Linco Research, Missouri) from five twin bearing ewes. COT tissue was separated from caruncular tissue, frozen in liquid N_2 and stored at -80°C until western blot analysis.

results: At 75 dG, twin fetuses of OB ewes were heavier than C ewes (234±7 vs. 186±7 g; P<0.05). Maternal leptin levels were higher in OB ewes than C ewes (7.7±1.0 vs. 2.7± 0.2 ng/ml; P<0.01), and were positively correlated with body condition scores, an index of fatness ($R^2 = 0.85$; P<0.05). Maternal plasma FA profiles (%, w/w) showed that 18:0, 20:5n-3, and 22:6n-3 were lower and 18:2n-6 was higher in OB animals (P<0.05), whereas in fetal plasma 16:1n-9 and 18:2n-6 were lower and 18:2n-6, 22:4n-6, and 22:5n-6 were higher (P<0.05). FATP1 and FATP4 expression was higher (P<0.05) in COT from OB than C ewes (1.29±0.04 vs. 1.00±0.06 and 1.49±0.17 vs. 1.00± 0.09 Arbitrary Units, respectively); no difference was observed for FAT/CD36 in COT tissues.

Conclusions: OB ewes exhibited elevated leptin concentrations in association with altered net placental transport of FA into the fetal compartment, particularly critical n-3 and n-6 long chain polyunsaturated FA. This increase in FA transport was associated with enhanced expression of FATP1 and FATP4 in COT tissue.

NIH INBRE 1P20RR16474.

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Placenta-Mediated Fetal Hypoglycemia as the Cause of Altitude-Associated IUGR. Stacy Zamudio,[1] Tatiana Torricos,[2] Maria M Oyola,[1] Lourdes Echalar,[2] Enrique Vargas,[2] Nicholas P Illsley.[1] *[1]Obstetrics, Gynecology & Women's Health, UMD-New Jersey Medical School, Newark, NJ, USA; [2]Instituto Boliviano de Biología de Altura, Universidad de San Andreas Mayor, La Paz, Bolivia.*

Rationale: Human residence at >3000 m is associated with intrauterine growth restriction (IUGR). Placental hypoxia is present, as evidenced by up-regulation of HIF-1α and HIF-regulated gene products. But deficits in maternal oxygen delivery are not causally associated with altitude-associated IUGR, nor do they contribute to placental hypoxia. We asked whether glucose, the most important fetal substrate, might be reduced or preferentially consumed anaerobically, thereby sparing oxygen, but limiting energy available for growth.

Methods: Doppler/imaging ultrasound, hematology and blood gases were used to quantify fetal blood flow, oxygen delivery/consumption at elective Cesarean delivery (n=80 at 3600, 88 at 400 m). Plasma venous and arterial glucose concentrations were measured in both mothers and in cord blood from the doubly clamped cord using a hexokinase-G6PDH coupled enzyme assay. Data were analyzed by unpaired t-tests or ANOVA, as appropriate.

Results: Birth weight was decreased by >300 grams at 3600 m (p<.0001) despite similarity in placental weight, gestational age and maternal attributes. While umbilical blood flow was lower at 3600 m, umbilical venous oxygen delivery was similar (0.46±0.03 vs. 0.50±0.03 µmol/min/kg, mean± SEM, p=0.40), as was fetal oxygen consumption (0.26 ± 0.02 versus 0.26 ± 0.02 µmol/min/kg). Maternal A-V glucose extraction was increased at high altitude (1.25 versus 0.9 mM, p<0.05), but this did not translate into increased fetal glucose concentrations. High altitude neonates had reduced glucose concentrations in the umbilical vein (2.9±0.1 versus 3.6±0.1 mM, p<.0001) and artery (2.3±0.01 versus 2.8±0.01 mM, p<.0001). Fetal glucose consumption was reduced at high altitude (49.8±6.0 µmol/min/kg versus 69.0±5.6 µmol/min/kg at 400 m, p<0.05). In fetuses at the lowest quartile of O_2 consumption at 3600 m the glucose/oxygen quotient (mol of glucose consumed per mol of oxygen) was increased (2.4±0.7 vs. 1.0±0.7, p<.005), indicating increased anaerobic glucose consumption. A similar pattern was not seen at 400 m.

Conclusion: These data suggest that the placenta is consuming glucose, perhaps thereby sparing O_2 for the fetus, but at the cost of reduced placental transport of substrate for fetal growth.

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Homocysteine Inhibition of System A Amino Acid Transport Activity in Human Placenta. Eleni Tsitsiou, Susan L Greenwood, Colin P Sibley, Stephen W D'Souza, Jocelyn D Glazier. *Maternal and Fetal Health Research Group, St. Mary's Hospital, University of Manchester, Manchester, United Kingdom.*

BACKGROUND: Elevated plasma levels of the amino acid homocysteine (Hcy) during pregnancy are associated with vascular-related complications and adverse neonatal outcomes including a reduced birthweight. Fetal and maternal plasma Hcy concentrations are positively correlated suggesting placental transport of Hcy may be an important determinant of fetal plasma Hcy. The mechanisms involved in placental Hcy transport are uncharacterised. Evidence that the system A amino acid transporter, which transports neutral amino acids in a Na^+-dependent manner, is important in promoting fetal growth and that a reduced system A activity is associated with intrauterine growth restriction (IUGR), led to our hypothesis that system A provides one mechanism for placental Hcy transport. This hypothesis was tested by measuring the ability of Hcy to inhibit system A activity in isolated microvillous plasma membrane (MVM) vesicles and placental fragments.

MATERIALS AND METHODS: MVM vesicles and placental fragments were isolated from placentas of normal pregnancies at term. System A activity was measured at initial rate (30s and 20 min respectively) as Na^+-dependent ^{14}C-methylaminoisobutyric acid (MeAIB) uptake into MVM vesicles (0.165mM) or fragments (0.0085mM) in the absence (control) or presence of L-Hcy and DL-Hcy or model substrates.

RESULTS: 20mM L-Hcy (custom-synthesised) and DL-Hcy (commercial source) significantly (p<0.05) inhibited Na^+-dependent ^{14}C-MeAIB uptake into MVM vesicles compared to control; comparable in magnitude to other model substrates (MeAIB, L-Ala, L-Ser, L-Met; n=9, Kruskal-Wallis with Dunn's Multiple Comparison Test). L-Hcy, L-Met and MeAIB (0.05-20mM) caused a dose-dependent inhibition of Na^+-dependent ^{14}C-MeAIB uptake into MVM with EC50 values (in mM; mean ± SE) of 0.53 ± 0.04, 0.27 ± 0.02, and 1.13 ± 0.12 respectively, n=6). Na^+-dependent ^{14}C-MeAIB uptake into fragments was reduced substantially in the presence of 10mM L-Hcy or DL-Hcy causing a 85 ± 13 and 88 ± 12% reduction (mean ± SD, n=2-3) of control respectively.

CONCLUSION: These observations suggest that Hcy is a relatively high affinity substrate for system A in the human placenta. We speculate that inhibition of placental amino acid uptake by Hcy could impact on fetal growth and development.

Supported by the MRC and Action Research Endowment Fund.

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Glucose Regulates Placental mTOR Activity and Glucose Deprivation Down-Regulates Placental System L Activity in an mTOR-Dependent Manner. Sara Roos,[1] Theresa L Powell,[2] Thomas Jansson.[2] *[1]Department of Physiology, Institute of Neuroscience and Physiology, Gothenburg, Sweden; [2]Department of Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA.*

Placental amino acid transporters are down-regulated in intrauterine growth restriction (IUGR). We have previously shown that mammalian target of rapamycin (mTOR) regulates placental system L transporter activity and that placental mTOR activity is decreased in IUGR. However, the upstream regulators of placental mTOR are unknown. In IUGR, fetal hypoglycemia and reduced maternal glucose levels are common and the placenta may therefore be exposed to low glucose levels. Hypothesis: We hypothesized that glucose availability regulates placental amino acid transporter activity mediated by changes in mTOR signaling. Methods: Cytotrophoblast cells were isolated and cultured until syncytialization at 66 hours. Cells were cultured for an additional 24 hours in culture media containing 0.5 mM, 4.5 mM, or 16 mM glucose (control), which corresponds to standard culture media. At 90 hrs, the activity of the mTOR signaling pathway was assessed by measuring the protein expression of S6K1 phosphorylated at Thr-389, the primary site of mTOR phosphorylation. In another set of cells, system L activity was assessed by measuring the BCH-inhibitable uptake of 3H-leucine. Results: As compared to control, phospho-Thr-389-S6K1 expression was reduced by 24% in cells incubated in 0.5 mM

glucose (n = 5, P = 0.046, Repeated-Measures ANOVA with Dunnett's post hoc test). The activity of system L decreased in a dose-dependent manner in response to decreasing glucose concentrations; -14% at 4.5 mM and -22% at 0.5 mM when compared to control. It was however only with 0.5 mM glucose that the decrease in system L activity was statistically significant (n = 11, P = 0.024, Repeated-Measures ANOVA with Dunnett´s post hoc test). In 16 mM glucose, incubation with rapamycin, a specific mTOR inhibitor, decreased system L activity by 18% (n = 11, P = 0.03, Wilcoxon Signed Ranks Test). In addition, in the presence of rapamycin, decreasing glucose concentrations did not affect system L activity. Conclusions: We show that glucose deprivation down-regulates system L activity in an mTOR-dependent manner in cultured cytotrophoblasts. These data support our hypothesis that the placenta responds to ambient glucose concentrations by altering amino acid transporter activity through the mTOR signaling system.

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Cholesterol Efflux Mechanisms in Endothelial Cells of the Human Placenta. Ute Panzenboeck,[1] Jasminka Stefulj,[1] Cornelia Schweinzer,[1] Christian Wadsack,[2] Manfred Moertl,[2] Gernot Desoye.[2] *[1]Institute of Pathophysiology, Medical University of Graz, Graz, Austria; [2]Clinic of Obstetrics and Gynaecology, Medical University of Graz, Graz, Austria.*

Background: Cholesterol is essential for fetal development. A large proportion of the fetal cholesterol demand is covered by synthesis in fetal organs, but up to 40% of fetal cholesterol is derived form the mother or placenta, respectively. To pass the placenta, maternal cholesterol must be taken up on the apical syncytiotrophoblast membrane, released at the basal membrane, and subsequently traverse the fetal endothelial cell layer of the placenta. This requires the existence of efficient cholesterol efflux mechanisms in placental endothelial cells (PECs).

Hypothesis: The present *in vitro* study tested the hypothesis that PECs express cholesterol efflux transporters that allow efficient cholesterol efflux, which can be regulated by oxysterols via LXR (liver-X-receptor).

Methods: Primary arterial and venous PECs from human placenta were separately isolated. The capacity of PECs to efflux cholesterol to acceptors was measured using [^{3}H]-cholesterol pre-labeled PECs with and without stimulation with oxysterols. Expression of ABCA1, ABCG1 and ABCG4 was measured by Western blotting and qPCR.

Results**:** Cholesterol efflux to both HDL_3 and lipid-free apoA-I was significant and time- and dose-dependent. Induction of cholesterol efflux pathways upon oxysterol treatment suggested involvement of the LXR-regulated lipid transporters, ABCA1, ABCG1 and/ or ABCG4. This notion was supported by oxysterol-induced upregulation of cholesterol efflux that was paralleled by increased mRNA and protein expression levels of ABCA1 and ABCG1 in PECs pre-treated with LXR agonists. HDL enrichment with ApoE, thus mimicking fetal HDL, further augmented cholesterol efflux. Arterial and venous PEC did not significantly differ.

Conclusion: The apoA-I/ABCA1 and the HDL_3/ ABCG1 cholesterol efflux pathways are operative in PECs and can be modulated by LXR activation.

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Placental Transport of Essential Amino Acids in Human Fetal Growth Restriction. Henry L Galan,[1] Alex Cheung,[2] Anna Maria Marconi,[3] Paolini L Cinzia,[3] Barton C Staat,[1] Battaglia C Frederick.[2] *[1]Obstetrics and Gynecology, University of Colorado Health Sciences Center, Aurora, CO, USA; [2]Pediatrics, University of Colorado Health Sciences Center, Aurora, USA; [3]Obstetrics and Gynecology, Ospedale San Paolo, Milano, Italy.*

OBJECTIVE: To compare *in vivo* placental transport rates of all 9 essential amino acids (EAA) via fetal/maternal (F/M) EAA enrichment between normal and fetal growth restriction (FGR) pregnancies.

STUDY DESIGN: A nonsteady state protocol with stable isotopic (^{13}C or ^{2}H) methodology was used to examine the relative rates of placental transport for all EAA. 12 uncomplicated term patients (CTL) and 7 with FGR (<10th percentile EFW & abnormal umbilical artery (UA) Doppler studies) received a 20cc EAA infusions IV into a peripheral vein over 2 minutes after initiation of cesarean section (CS). 1cc venous samples were taken from the contralateral warmed (arterialized) hand before the EAA infusion and then every 2 minutes for 12 minutes. Umbilical vein (UV) and UA samples were obtained. CTL & FGR groups were compared for EAA concentrations using HPLC and EAA plasma enrichments with GCMS. F/M molar percent enrichment (MPE) ratios for each EAA were calculated for the UV and UA MPE vs the maternal MPE.

RESULTS: FGR pregnancies were delivered earlier (39±0.2 *v* 32±0.6w; p<0.01), weighed less (3.5±0.2 *v* 1.2±0.1kg; p<0.01) and had higher S/D ratios (2.2±0.1 *v* 5.3±1.1; p<0.01). EAA concentrations were similar between groups, but there were differences (non-parametric testing; p<0.05) in transport rates between groups with His crossing considerably faster and Ile crossing slower in FGR . The table shows fetal vein/maternal (FV/M) ratios standardized to the leu FV/M ratio for all 9 EAA for both groups. Amino Acids fell into 3 groups for FGR pregnancies: 1) His (ratio~1.38), 2) leu, phe, met (ratios~1), and 3) val, thr, ile, trp, lys with intermediate ratios(~0.6).

	CONTROL	IUGR	P VALUE
Leu	1	1	
Ile	1.02±0.01	0.89±0.05	0.004
His	0.86±0.09	1.38±0.48	0.03
Phe, Met, Val, Thr, Lys, His, Trp (data not shown)			NS

CONCLUSION: This is the 1st study to compare the relative rates of *in vivo* placental transport for all EAA between normal and FGR human pregnancies showing striking differences in the transport rates of two EAA. In the absence of EAA concentration differences between groups, the higher His transport rate in FGR suggests higher utilization by the placenta.

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An hCG-Antagonist Represents a Novel Contraceptive Agent. Pratibhasri A Vardhana, Martin A Julius, Susan V Pollak, Rhonda K Trousdale, Vuk P Jovanovic, Joyce W Lustbader. *Obstetrics and Gynecology, Columbia University Medical Center, New York, NY, USA.*

Objective: HCG plays a pivotal role in reproduction. It is produced by the developing embryo and sustains corpus luteum function in pregnancy. HCG increases VEGF and PECAM, two key angiogenic proteins necessary for follicular growth. HCG and LH are structurally similar and bind to the same receptor (LHR). Our laboratory has produced an hCG-Antagonist (hCG-Ant) that binds to the LHR but does not activate it, and therefore can prevent follicular development, ovulation, and early embryogenesis. We hypothesize that the hCG-Ant acts as a novel contraceptive agent.

Design: *In vivo* rodent model.

Methods: For conventional ovulation studies, 21d immature female Wistar rats were randomized into 3 groups. 11 rats received 10 IU pregnant mare serum gonadotropin (PMSG) ip for 1d, followed by 10 IU hCG or hCG-Ant 48h later. At 24h post-hCG, serum was obtained for E2, P4, and inhibin A quantification. For superovulation studies, 18 rats received 50 IU PMSG ip for 4d, followed by 30 IU hCG, hCG-Ant or hCG then hCG-Ant on day 5. The control group received 50 IU PMSG for 4d followed by saline (0.1mL). The ovaries were assessed for stigma formation. For hyperstimulation studies, animals received 10 IU of PMSG for 4d followed by 10 IU of hCG, hCG-Ant, hCG then hCG-Ant, or saline. The ovaries were assessed for VEGF, VEGFR2, and PECAM expression using RT-PCR, immunohistochemistry (IHC), and Western Blotting. Initial mating studies were performed using 20 mice treated with 5 IU of PMSG followed by 5 IU of hCG or hCG-Ant.

Results: Ovarian weight was significantly higher in the hCG-treated group *vs.* hCG-Ant (0.0361±0.014 g *vs.* 0.019±0.007 g) (p<0.05). E2 and P4 levels were significantly higher in the hCG *vs.* hCG-Ant treated group (91.45±30.84 pg/mL and 6.48±4.13 ng/mL *vs.* 53.12±18.55 pg/mL and 2.43±2.03 ng/mL) (p<0.05). Inhibin A levels were significantly higher in the hCG *vs.* hCG-Ant treated group (553.63±907.18 pg/mL *vs.* 247.2±204.19 pg/mL) (p<0.05). There were 80% less stigma on hCG-Ant treated ovaries *vs.* hCG. VEGF and VEGFR2 were present in ovaries treated with hCG and absent in ovaries treated with hCG-Ant by Western Blot. IHC showed decreased staining with anti-VEGF, VEGFR2, and PECAM antibodies after hCG-Ant treatment *vs.* hCG. Of 10 animals treated with hCG-Ant, none became pregnant.

Conclusion: These data suggest that the hCG-Ant may act as a contraceptive agent and can prevent pregnancy.

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Expression Profiling of Cardiovascular Disease and Endothelial Function Genes in Response to Estradiol, Equilin and Genistein in Human Coronary Artery Endothelial Cells. Michael L Sprague, Douglas D Taylor, Christine L Cook, Cicek Gercel-Taylor. *Obstetrics, Gynecology and Women's Health, University of Louisville School of Medicine, Louisville, KY, USA.*

Cardiovascular diseases are the number one cause of death in American women. Increased number of cardiovascular events are observed after menopause. Cardiovascular benefits of hormone therapy remain in debate with Women's Health Initiative data showing an increased incidence of cardiovascular events compared to placebo. A lower calcium burden was demonstrated in the coronary

vasculature of women who received exogenous estrogen compared to those who received placebo. The purpose of this investigation was to identify the gene expression in response to the estrogen, equilin, as the major component of conjugated equine estrogen and genistein, a phytoestrogen in human coronary artery endothelial cells.

Human coronary artery endothelial cells from a 43-year old female were used. Cells were treated with estradiol [E2], equilin [Eq] (0.1 nM) or genistein [Gen] (5 micromolar) for 48 hours. Focused oligomicroarrays for cardiovascular disease and endothelial cell function were used to study gene expression. RT-PCR was used to confirm the transcription of genes analyzed in oligoarrays.

Vascular adhesion molecules and genes involved in the inflammatory response were most affected with the three estrogen sources. Significant reduction of integrin alphaE was seen with all three 2.63, 2.06 and 16.74 for E2, Eq and Gen respectively. Similarly NfKB1 was also reduced 2.62, 2.07 and 6.33 fold compared to controls. Significant reduction of CCL2 was demonstrated with Eq (3.11 fold) and Gen (5.49 fold). TNFreceptor1A and 1B were only significantly decreased by Gen. VCAM-1 which is regulated by proatherogenic factors and upregulated mainly at atherosclerosis -prone sites, was significantly reduced (25.95-fold) with genistein, and a slight reduction was seen with E2 and no change was observed with Eq.

Our data support the clinical findings that estrogen has favorable effects on the genes involved in atherosclerotic disease. While E2 has been shown to be slightly more effective than equilin in the modulation of gene expression, genistein was significantly more effective in the favorable expression of genes involved in particularly the inflammatory response.

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Effect of Estrogen Replacement on Modulation of Vascular Reactivity by Matrix Metalloproteinases in a Rat Model of Menopause. Olga N Lekontseva, Sandra T Davidge. *Physiology and Obstetrics/Gynecology, University of Alberta, Edmonton, AB, Canada.*

Introduction: The prevalence of cardiovascular disease in women dramatically rises in the postmenopausal period. Although deficiency of estrogen has been implicated in the pathophysiology of systemic vascular dysfunction, the effects of estrogen on vasculature are complex and not completely understood. We have previously shown that estrogen exerts a beneficial effect on the aging vascular system by reducing circulating levels of the inflammatory cytokine TNFα. TNFα is a known regulator of matrix metalloproteinases (MMPs), proteolytic enzymes that may modulate vascular tone through cleavage of vasoactive peptides such as big endothelin-1 (ET-1). The role of estrogen in this pathway is unknown. We tested the hypothesis that in aging/estrogen deficiency, TNFα-induced MMP activity mediates greater vasoconstriction, in part, through the ET-1 pathway. We further hypothesized that estrogen replacement reduces vascular sensitivity to the constriction by preventing MMP activation.

Methods: Aged (12 month old) female Sprague Dawley rats were ovariectomized and treated with either placebo [OVX], 17β-estradiol [OVX+E_2], or the TNFα inhibitor etanercept [OVX+Etan]. After four weeks, resistance mesenteric arteries were isolated and studied on the pressure arteriograph. Concentration-response to exogenous big ET-1 (1nM-1μM) in the absence or presence of MMP inhibitor (GM6001, 10μM) was assessed in the vessels.

Results: Treatment of "menopausal" [OVX] rats with either estrogen or the TNFα inhibitor reduced sensitivity of arteries to big ET-1 (EC_{50}=0.07±0.01μM in OVX versus 0.15±0.04μM in OVX+E_2 or 0.16±0.03μM in OVX+Etan groups; $p<0.02$). Although MMP inhibition attenuated maximal constriction in all of the arteries, there was a significantly greater ($p<0.05$) role of MMPs in big ET-1-induced vasoconstriction in the OVX+E_2 group (reduction in max constriction=70.3±11.8%) compared to OVX (40.7±9.1%) and OVX+Etan groups (35.4±3.9%).

Conclusions: Both estrogen and TNFα inhibition reduced big ET-1 vasoconstriction. However, contrary to our hypothesis, TNFα is not contributing to MMP modulation of ET-1 vasoconstriction. Interestingly, our study demonstrates a novel role for estrogen to increase MMP contribution to big ET-1 vasoactivity with the net effect being less vasoconstrictive. Understanding this unique pathway of regulation by estrogen in the aged vasculature will allow for development of new therapeutic options for women.

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Potential Modulation of the Lysyl Oxidase Gene by Methylation in Pelvic Organ Prolapse. Micheline J Wong,[1] Qing Ji,[2] Joseph Campeau,[1] Barry Starcher,[3] Juan C Felix,[4] Frank Stanczyk,[1] Carl Klutke,[5] John J Klutke.[1] *[1]Obstetrics and Gynecology, Los Angeles County Medical Center/Women's and Children's Hospital-University of Southern California, Los Angeles, CA, USA; [2]Surgery, University of Southern California/Keck School of Medicine, Los Angeles, CA, USA; [3]Biochemistry, University of Texas Health Center, Tyler, TX, USA; [4]Pathology, Los Angeles County Medical Center/Women's and Children's Hospital-University of Southern California, Los Angeles, CA, USA; [5]Urology, Washington University School of Medicine, St. Louis, MO, USA.*

Introduction: The etiology of pelvic organ prolapse is multifactorial, with both inherited and acquired components. The molecular mechanisms of prolapse have not been established yet. We have previously shown that lysyl oxidase (LOX) expression is suppressed in uterosacral ligaments of women with pelvic organ prolapse. It has also been shown that LOX is a tumor suppressor gene inactivated by methylation in human gastric cancers.

Hypothesis: The aim of this study was to analyze the DNA sequence of the promoter region of the lysyl oxidase gene in tissues from women with pelvic organ prolapse and identify whether methylation is present. Our hypothesis is that the promoters of the LOX gene in women with pelvic organ prolapse have significantly more methylation sites than women without prolapse.

Materials and Methods: Genomic DNA was isolated from the uterosacral ligaments of eight women with pelvic organ prolapse and 8 women without prolapse (controls). Genomic DNA samples were treated with EZ DNA Methylation Kit (ZEMO Research, Orange, CA). The LOX gene promoter region of –246 to +74 was amplified by PCR and then cloned into PCR2.1-TOPO (Invitrogen, Carlsbad, CA) and transformed into an *E. coli* DH5a strain. Amplified plasmid DNA samples containing the LOX gene promoter region from each woman were sequenced and methylated CpG islands were identified by sequence comparison.

Results: A total of 66 methylated CpG sites were found in the patient group with pelvic organ prolapse while only 1 methylated CpG site was found in the non-prolapse control group.

Conclusion: These findings suggest that methylation in the promoter region suppresses LOX gene expression in women with pelvic organ prolapse.

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Lactation and Incidence of Premenopausal Breast Cancer: A Prospective Cohort Study. Alison M Stuebe,[1] Walter C Willett,[2] Karin B Michels.[1] *[1]Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA, USA; [2]Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA.*

Background: Reports from case-control and cohort studies have suggested an inverse association between lactation and breast cancer risk, but findings have been inconsistent.

Methods: We conducted a prospective observational cohort study of 57,585 parous women participating in the Nurses' Health Study II from 1997 to 2005. Our primary outcome was incident premenopausal breast cancer.

Results: During the study period, 621 cases of premenopausal breast cancer were diagnosed during 355,871 person-years of follow-up. Women who had ever breastfed had a 24% lower incidence of premenopausal breast cancer (95% confidence interval [CI] 5-40%) compared with women who never breastfed, adjusting for parity, age at first birth, year of first birth, height, body mass index (BMI), BMI at age 18, family history, personal history of benign breast disease, participant birth weight, preterm birth, age at menarche, oral contraceptive use, physical activity, and alcohol consumption. No trend was observed with duration of lactation (p=0.52). The association between ever-breastfeeding and premenopausal breast cancer was modified by use of medication to suppress lactation (p=0.05); in analyses restricted to women who had never used suppressive medication, ever-breastfeeding was associated with a 46% (95%CI 23-63%) reduction in incident disease. The association between lactation and premenopausal breast cancer was further modified by family history of breast cancer (p=0.03). Among women who had never used suppressive medication and reported a family history, those who had breastfed had a 67% lower covariate-adjusted risk of premenopausal breast cancer (95% CI 38-82%) than women who had never breastfed. Among women without a family history, ever-breastfeeding was not associated with breast cancer incidence (Hazard Ratio 0.68, 95% CI 0.42-1.09). Among women who had ever breastfed, we observed no association between breast cancer risk and duration of lactation amenorrhea or exclusive breastfeeding.

Conclusion: In a large, prospective cohort study, ever-breastfeeding was inversely associated with risk for premenopausal breast cancer. At the durations

observed in our cohort, we observed no trend with duration of breastfeeding or lactation amenorrhea. The inverse association with ever-breastfeeding was stronger among women with a family history of breast cancer.

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Regulatory and NKT Cells at the Maternal-Fetal Interface. Thomas F McElrath,[1] Rachael A Clark.[2] *[1]Maternal-Fetal Medicine, Brigham & Women's Hospital, Boston, MA, USA; [2]Harvard Skin Disease Research Center, Brigham & Women's Hospital, Boston, MA, USA.*

Introduction: Pregnancy represents an immunologically challenging event requiring maternal tolerance of the fetal semi-allograft. An increase in decidual $CD4^+CD25^+$ T cells has been documented but it is unclear if these represent $FOXP3^+$ T cells (Tregs). The possibility also exists that other $CD3^+$ lineages with potential regulatory function exist within the decidua parientalis. We examined if $CD4^+CD25^+$ cells are true $FOXP3^+$ Tregs and evaluated the frequency of other T cell subsets with possible regulatory potential.

Methods: We extracted T cells from term deciduas of planned cesarean deliveries. Cells were stained with directly conjugated monoclonal antibodies and were analyzed on a six color flow cytometer.

Results: We found that only a subset (median 33%) of $CD25^+$ T cells were true $FOXP3^+$ regulatory T cells. From 65 donors, $FOXP3^+$ Tregs accounted for a median of 7.1% of all $CD4^+$ cells. These cells were memory $CD45RO^+$ T cells lacking CCR7, and L-selectin but expressing CD25, CTLA-4, GITR, and HLA-DR. Additionally we found that a median 21% of $CD3^+$ T cells expressed the NKT marker CD161. These cells were a mixture of CD4 and $CD4^-CD8^-$ T cells, with variable numbers of CD8 T cells, suggesting they do not represent merely recently activated T cells. There was enrichment for T cells with NKT markers after culture on HELA cells expressing CD1d, suggesting that these cell represent true NKT cells. NKT cells were of the non-classical type, with a diverse T cell repertoire (0.4% iV24JQ) and also expressed CD69, HLA class II, CD45RO but were CCR7 and L-selectin low.

Comments: We find that only a minority of CD25-expressing T cell in the decidua are true $FOXP3^+$ Tregs. Because much of the work on Treg in pregnancy has used CD25 as a Treg marker, this suggests additional studies are needed to confirm the role of these cells in pregnancy. We find that a novel population of non-classical NKT cells exists in the human decidua. NKT cells can either promote or antagonize tolerance, depending on the immunologic context. The large number of these cells in the decidua suggests they may play a role equal or exceeding that of regulatory T cells.

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Thy-1 Downregulation in Vaginal Tissue May Contribute to Dysregulation of Extracellular Matrix Synthesis and Repair in Women with Pelvic Organ Prolapse. Marsha K Guess,[1] Kathleen A Connell,[1] Richard Bercik,[1] Lloyd G Cantley.[2] *[1]Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA; [2]Internal Medicine/Neprhology, Yale University School of Medicine, New Haven, CT, USA.*

Objectives: Thy-1 is a cell surface glycoprotien expressed in human fibroblasts, neurons, hematopoetic stems cells and endothelial cells. Thy-1 expression affects fibroblast proliferation and migration, cell-cell, as well as, cell-matrix interactions. Moreover, Thy-1 expression has been shown to play a critical role in fibroblast dedifferentiation into myofibroblasts, as well as in extracellular matrix (ECM) production and fibrosis. Women with pelvic organ prolapse (POP) have alterations in vaginal ECM protein expression and metabolism, as well as decreases in smooth muscle fractional content. In the current study, we evaluated Thy-1 as well as the smooth muscle markers alpha-smooth muscle actin (aSMA) and desmin expression in women with pelvic organ prolapse compared to women with normal pelvic support (controls).

Methods: Anterior apical vaginal wall specimens from women with POP and controls were collected at the time of hysterectomy. Messenger RNA and protein expression of Thy-1, aSMA and desmin were evaluated using semi-quantitative RT-PCR, real-time PCR and western blot analysis. GAPDH and beta actin were used as internal controls.

Results: RT-PCR demonstrated the presence of Thy-1, aSMA and desmin in vaginal tissue from women with POP and controls. Further, Thy-1 mRNA expression was downregulated 57% in women with POP compared to controls ($p = 0.004$). A parallel decrease in Thy-1 protein was seen in women with POP compared to controls ($p=0.02$). Although a 44% and a 37% decease were seen in aSMA and desmin mRNA, these differences were not statistically significant. Similarly, no differences were seen in aSMA and desmin protein expression.

Conclusion: We demonstrate that there is significantly less Thy-1 expression in vaginal tissue from women with POP compared to controls. Differential expression of Thy-1 in prolapsed vaginal tissues suggests that Thy-1 may have a functional role in mediating ECM metabolism in the female genitourinary tract.

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HuR Expression Is Altered in Ectopic Endometrium. S Karipcin, T Altun, UA Kayisli, E Seli. *Ob & Gyn, Yale U., New Haven, CT, USA.*

Introduction: Cytokines and growth factors contribute to cyclic turnover of the normal endomterium and to the pathogenesis of endometriosis. Cytokine and growth factor messenger RNAs (mRNAs) undergo rapid turnover that is primarily mediated by AU-rich elements (ARE) that consist of multiple stretches of adenylate and uridylate residues located in the 3' untranslated region (3'-UTR) of their mRNAs. HuR is a ubiquitously expressed RNA-binding protein that stabilizes ARE containing mRNAs and prolongs their expression. We hypothesized that HuR may play a role in the regulation of cytokine expression during normal menstrual cycle and in endometriosis.

Methods: Tissue sections obtained from normal (n=14) and ectopic (n=7) endometrium were immunostained for HuR. Staining intensity was evaluated by HSCORE and grouped according to menstrual cycle phase. Statistical analysis was done with one-way ANOVA. Cultured stromal cells isolated from normal endometrium were treated with vehicle, estradiol (E2; 10^{-8} M), or progesterone (P, 10^{-8} M) for 24 and 48h, and HuR expression was determined using Western analysis and normalized to ß-actin.

Results: HuR immunostaining was nuclear in endometrial cells. HuR immunoreactivity was significantly lower in the early proliferative and late secretory phases (157.5 ±11.08 and 190.0 ±15.2, respectively), compared to the mid-late proliferative (270.0±8.0) and early-mid secretory phases (256.6±20.2)($p<0.01$). Moreover, HuR expression was significantly lower in ectopic endometrial cells when compared to normal endometrium in mid-late proliferative and early and mid-secretory phases ($p<0.01$). Progesterone suppressed HuR levels significantly in cultured endometrial stromal cells at both 24 and 48 h compared to control ($p<0.05$) while estrogen did not cause a significant change.

Discussion: Decreased HuR levels in the late secretory and early proliferative phases are likely to contribute to degradation of cytokines and result in lower cytokine levels observed mid-cycle. Late secretory decrease in HuR levels may be mediated by progesterone as suggested by in vitro findings. In ectopic endometrium, persistent low expression of HuR compared to normal endometrium most probably results from elevated cytokine levels associated with endometriosis. The effect of lower HuR expression in ectopic endometrium on other ARE-containing transcripts, and on the pathogenesis of endometriosis remains to be elucidated.

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A Novel Promoter Controls *Cyp19a1* Gene Expression in Mouse Adipose Tissue. Hong Zhao, Joy Innes, Scott Reierstad, Mehmet Bertan Yilmaz, Serdar E Bulun. *Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL, USA.*

Background: Aromatase is the key enzyme for estrogen biosythesis, and is encoded by the *Cyp19a1* gene. Thus far, only three unique untranslated first exons associated with distinct promoters in the mouse *Cyp19a1* gene were described (brain-, ovary and testis-specific exon I). However, it remains unknown whether aromatase is expressed in other mouse tissues via previously unknown tissue-specific promoters activating new exon Is.

Methods: Real–time PCR was used to examine the aromatase expression levels in various C57BL6 mouse tissues. 5'-rapid amplification of cDNA ends (5'-RACE) was used to determine the transcriptional start sites of *Cyp19a1* transcripts. Promoter activity was measured using serial deletion mutants of DNA fused to the luciferase reporter gene.

Results: Real-time PCR results showed that aromatase was expressed in male gonadal fat and the expression level is lower than that in testis. The adipose tissue-specific untranslated exon I of *Cyp19a1* transcript was isolated using 5'-RACE and this novel gonadal fat-specific exon I of *Cyp19a1* mRNA did not show sequence similarities to previously reported ones. This new adipose-specific exon I was mapped to 75 kb upstream of the translation start site in the coding exon II. The genomic region upstream of the adipose-specific exon I was cloned into luciferase plasmids. Transfection of murine 3T3-L1 cells with these plasmids showed that promoter activity was conferred by the sequence located at -1 to -343 bp upstream of the transcriptional start site. Dexamethasone significantly induced activity of the adipose-specific promoter region.

Conclusion: Taken together, our results suggest that a novel *Cyp19a1* transcript is regulated by a tissue-specific promoter in male murine gonadal fat. These

results expanded the 5'-untranslated region of *Cyp19a1* up to 75 kb upstream of the translational start site. Aromatase expression in mouse gonad fat may have important physiological roles.

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Triclocarban Potentiates Effects of Testosterone on Accessory Sex Organs in Castrated Male Rat. Mohamed I Ahmed,[1] Jiangang Chen,[2] Nancy A Gee,[2] Kurt Benirschke,[3] Bill L Lasley,[2] Antoni J Duleba.[1] *[1]Ob/Gyn, University of California Davis, Sacramento, CA, USA; [2]Center for Health and the Environment, University of California Davis, Davis, CA, USA; [3]Pathology, University of California Davis, Davis, CA, USA.*

Objectives: Triclocarban (TCC) is an antimicrobial agent widely used in various personal care products such as soaps and toothpaste; it is also identified as a ubiquitous environmental contaminant persisting during wastewater treatment. Recent in vitro studies identified TCC as a novel endocrine disruptor amplifying the transcriptional activity of androgens. This study evaluated in vivo effects of TCC using a castrated male rat model.

Methods: Forty-eight Sprague-Dawley rats were castrated at the age of 42-46 days. At the age of 48-52 days, animals were randomly assigned to four treatment groups: control ©, TCC (0.25% in diet), testosterone propionate (TP; 0.2 mg/kg/day), and TP+TCC. Following a 10-day treatment the animals were sacrificed; reproductive and selected other organs were removed, weighed and evaluated histologically by an observer blinded to treatment.

Results: The table below shows that TCC augmented effects of TP on weights of accessory sex organs. Histological assessment revealed that TCC induced greater glandular distention with more secretions compared to the effects of TP alone; furthermore, mitotic figures were seen only in prostates from rats exposed to TP+TCC.

Conclusions: TCC is a newly identified endocrine disruptor with unique and novel actions resulting in potentiation of androgen effects on sex organs. These observations underscore possible environmental risks related to exposure to TCC.

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Organ	C	TCC	TP	TP+TCC
Body weight (g)	217.7 ± 8.6	222.7 ± 11.1	217.1 ± 6.4	217.0 ± 8.1
Kidney (g)	2.07 ± 0.26	1.98 ± 0.26	1.96 ± 0.27	1.91 ± 0.21
Liver (g)	10.7 ± 0.9	12.5 ± 1.2 a	11.4 ± 1.3	11.7 ± 1.2
Seminal vesicle (mg)	105.4 ± 28.2	132.2 ± 39.7	323.1 ± 70.0 a	576.5 ± 73.4 b
Ventral prostate (mg)	58.5 ± 18.9	85.8 ± 22.2 a	136.7 ± 8.5 a	228.0 ± 23.5 b
Glans penis (mg)	72.3 ± 12.5	78.1 ± 8.5	83.7 ± 8.5	113.3 ± 14.1 b
Cowper's gland (mg)	15.1 ± 3.2	18.1 ± 2.5	32.0 ± 3.77 a	36.3 ± 4.5 b
Levator ani/ bulbocavernosus muscle (mg)	129.3 ± 6.0	133.9 ± 7.4	323.9 ± 7.3 a	366.9 ± 12.2 b

Values are mean±SD; a P<0.05 vs. C; b P<0.05 vs. C and TP.

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Activin and Related Transforming Growth Factor-β Superfamily Members in the Endometrium: Roles in Decidualization. Chelsea J Stoikos,[1,2] Craig A Harrison,[1] Lois A Salamonsen,[1] Evdokia Dimitriadis.[1] *[1]Prince Henry's Institute of Medical Research, PO Box 5152, Clayton, Victoria, Australia; [2]Department of Obstetrics and Gynaecology, Monash University, Clayton, Victoria, Australia.*

BACKGROUND: Blastocyst implantation is dependent on the differentiation of human endometrial stromal cells (HESC) into decidual cells. Transforming growth factor family members have well defined roles in cell differentiation and proliferation. Activin A, a TGFβ superfamily member, enhances HESC decidualization and localizes to decidualized cells in human endometrium. Other TGFβ superfamily members, including BMP2, BMP4, BMP7, GDF5, GDF8 (myostatin), GDF11 and Nodal, may also be present in decidual cells and therefore may also play a role during this important process. This study aimed to determine whether activin is the major family member driving decidualization or whether other family members contribute to the process.

METHODS: Broad ranging activin inhibitors (Activin-M108A and SB431542) that effect receptor-ligand interactions of other TGFβ superfamily members were used in HESC decidualization. Protein localization was examined in secretory phase endometrium and first trimester decidua by immunohistochemistry and mRNA expression was examined in an ex vivo model. The secretion of candidate proteins was measured during HESC decidualization and certain recombinant proteins added during decidualization to examine their effect.

RESULTS: M108A (25nM) and SB431542 (10μM) significantly reduced decidualization (60% and 77% respectively) demonstrating that activin and possibly other TGFβ family members are involved in decidualization in vitro. BMP2, GDF5 and TGFβ1 protein were detected in decidual cells of mid-late secretory endometrium and first trimester decidua whilst all ligands except Nodal, were expressed by HESC. Both BMP2 and TGFβ1 secretion increased during HESC decidualization and administration of both these proteins significantly enhanced decidualization in vitro.

CONCLUSIONS: These data support a role for activin A, BMP2 and TGFβ1 in HESC decidualization. This is important as the elucidation of factors involved during decidualization will aid in better understanding implantation and fertility.

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Abnormal Chromatin Remodeling in Diabetic Murine Oocytes. Laura Lawrence, Ann Ratchford, Cybill Esguerra, Qiang Wang, Kelle Moley. *OB/GYN, Washington University, St. Louis, MO, USA.*

Background: Diabetic women experience increased miscarriages and adverse pregnancy outcomes. Previous studies suggest adverse diabetic outcomes may occur earlier than the preimplantation period, particularly during oogenesis. We hypothesize that diabetes affects chromatin remodeling and chromosomal condensation in murine oocytes.

Methods: MII oocytes from diabetic and control mice were fixed with 4% PFA, permeabilized with 0.5% Triton X-100 for 15 min, and immunostained against a-tubulin and the nucleus for 1 hr at RT. Images were obtained with Laser Confocal Scanning Microscope. Protein expression levels of chromatin with dimethyl H3K9 modifications were measured in nondiabetic and diabetic denuded murine oocytes at 44 hours post PMSG (0 hour) and at six hours post HCG (6 hour) via Western immunoblots. Mature nondiabetic denuded oocytes were fixed in 4%PFA and permeabilized with 0.5% Triton X-100. They were stained via immunohistochemistry against histone protein H3 at Lysine 9 (H3K9me2) and heterochromatin.

Results: Immunohistochemistry reveals that diabetic MII oocytes have aberrant spindle formations and metaphase chromosome alignment. Approximately 10/50 (20%) diabetic oocytes examined had abnormal spindles and metaphase alignments compared with only about 1/70 normal oocytes (1.4%). Western blot demonstrated 3 times higher expression of dimethylated chromatin in diabetic oocytes at time 0 compared with nondiabetic oocytes. At time 6 hours, diabetic oocytes had significantly fewer H3K9 modifications than the controls. When staining mature murine nondiabetic oocytes for dimethylation of H3K9me2 by immunohistochemistry, we demonstrated H3K9me2 expression in a condensed heterochromatin ring surrounding the nucleolus, consistent with transcriptional silencing.

Conclusions: Diabetic MII oocytes have a significant increase in abnormal spindle formation and metaphase chromosome alignment. They also have increased dimethylation compared with normal oocytes at a time point when they should be transcriptionally active, storing maternal mRNA in preparation for the silencing period. In addition, after HCG injection to trigger maturation and gene silencing, the diabetic oocytes had decreased dimethylated chromatin changes. Our findings suggest that diabetic oocytes may be exiting the transcriptionally active period prematurely and may ultimately experience decreased, partial, and incomplete gene silencing.

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Identification and Characterization of Human Embryonic Poly(A) Binding Protein (ePAB). SA Pauli, O Guzeloglu-Kayisli, H Demir, MD Lalioti, D Sakkas, E Seli. *Ob & Gyn, Yale U., New Haven, CT, USA.*

Hypothesis: Transcriptional silencing begins with oocyte maturation and persists during the initial mitotic divisions of the embryo. Gene expression during this period depends on the translational activation of maternal mRNAs by cytoplasmic polyadenylation, and requires an embryonic poly(A) binding protein (ePAB), which stabilizes maternal transcripts and promotes their translation. ePAB has been identified in Xenopus and mouse, where its expression pattern reflects its function: ePAB is only expressed in oocytes and early embryos until zygotic genome activation (ZGA) when it is replaced by the somatic cytoplasmic poly(A) binding protein (PABPC1). In this study we sought to identify human ePAB and characterize its expression.

Methods: Nucleotide and protein sequence databases were searched using the NCBI BLAST server. Pairwise and multiple alignments of the human, mouse,

and Xenopus ePAB genes and proteins were performed. Expression of human ePAB and PABPC1 mRNA was tested in ten different somatic tissues, testes, and ovaries by RT-PCR. Amplification with actin primers provided a positive control and allowed semi-quantitative analysis. ePAB and PABPC1 expression in human prophase I (PI) and metaphase II (MII) oocytes, 8-cell embryos and blastocysts was evaluated using quantitative real time PCR.
Results: Human ePAB is a 625 aa protein with 77% identity and 84% similarity to mouse ePAB and contains 4 RNA recognition motifs and a PABP domain. Human ePAB mRNA is detected in ovaries and to a lesser extent in testes and several somatic tissues including kidney, liver, and muscle. Similar to its mouse orthologue, human ePAB mRNA is expressed in PI and MII oocytes, but not in 8-cell embryos or blastocysts. PABPC1 mRNA is ubiquitously present in all tissues as well as 8-cell, and blastocyst stage embryos. However, its levels are significantly lower than that of ePAB in oocytes.
Conclusions: In this study we report the identification of human ePAB. Similar to that observed in Xenopus and mouse, human ePAB is the predominant poly(A) binding protein in oocytes and it is replaced by PABPC1 following ZGA, which occurs at 4- to 8-cell stage in human. Our findings suggest that the unique translational regulatory pathways that control gene expression during oogenesis and early embryo development may be common between model organisms and humans.

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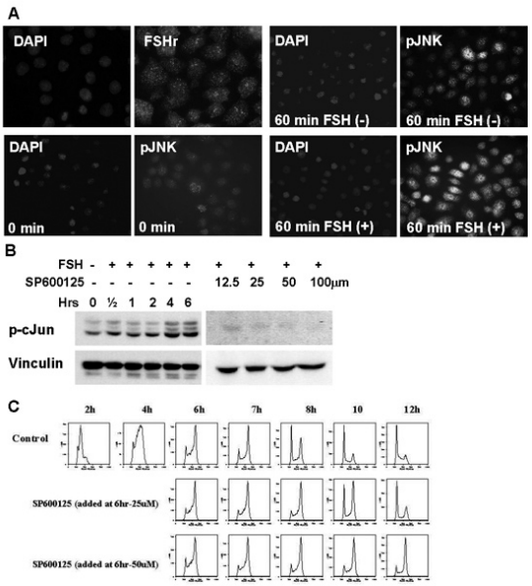
Follicle Stimulating Hormone Regulates Granulosa Cell Mitosis through c-Jun N Terminal Kinase (JNK) Pathway. Ozgur Oktem, Kutluk Oktay. *Obstetrics and Gynecology, New York Medical College, Valhalla, NY, USA.*
Objective: c-Jun NH2-terminal kinase (JNK), a member of mitogen-activated protein (MAP) kinase family, is involved in cell proliferation, differentiation, and survival. FSH is required for granulosa cell proliferation and antral follicle growth but its mechanism of action in preantral stages is not well defined. We previously showed that pharmacological inhibition of JNK pathway halts in-vitro growth of murine preantral follicles in serum free media supplemented with FSH (100mIU/mL). SIGCs (spontaneously immortalized rat granulosa cell line) have characteristics similar to preantral granulosa cells and hence they were used in this study to determine whether the JNK pathway plays a key role in preantral granulosa cell proliferation. Our specific aims were to determine whether: a) FSH activates JNK pathway in granulosa cells; b) The inhibition of JNK pathway blocks cell cycle progression.
Material and methods: SIGCs were treated with 100mIU/mL recombinant FSH in serum free media two days after serum starvation. Activation of JNK pathway was analyzed with IF and WB using phospho-JNK and phospho-cJun expression, respectively. FSH receptor expression (FSHr) was analyzed with IF. The inhibition of JNK pathway on cell cycle progression was analyzed by FACS using a JNK inhibitor SP600125.
Results: FSHr protein was expressed in SIGC indicating that they can respond to FSH (Fig 1A). Likewise by IF, phospho-JNK expression was significantly increased in SIGC 1 hour post FSH exposure (Fig-1A). Similarly on WB, phospho-cJun expression increased as early as 30 min after FSH exposure and peaked at 4 hrs. cJun phosphorylation was abolished 1 hr after treatment with SP600125 (50mM) (Fig 1B). FACS analysis showed that the inhibition of JNK by SP600125 resulted in cell cycle arrest at G2/M transition in a dose dependent fashion (Fig 1C).
Conclusion: These results strongly suggest that the proliferative effect of FSH on immature granulosa cells is mediated through the activation of JNK pathway. This is the first experimental observation implicating JNK signaling in granulosa cell cycle control. (NICHD 043339-01).



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The Induction of 17{alpha}-Hydroxylase (CYP17) Expression in Granulosa Cells. Satin S Patel,[1] Victor E Beshay,[1] William E Rainey,[2] Bruce R Carr.[1] *[1]Reproductive Endocrinology and Infertility, University of Texas at Southwestern Medical Center at Dallas, Dallas, TX, USA; [2]Physiology, Medical College of Georgia, Augusta, GA, USA.*
According to the traditional two-cell two-gonadotropin hypothesis of the ovary, androgen production arises exclusively from theca cells. The granulosa cells, in turn, utilize androstenedione, which is aromatized eventually to estradiol. Studies involving immunohistochemical analysis of normal ovaries have shown that granulosa cells express significantly higher levels of the activator protein-1 (AP-1) transcription factor, cFos compared to theca cells, where cFos expression is virtually absent. We hypothesize that cFos functions to inhibit the expression of CYP17 in granulosa cells, thereby suppressing androgen production. Hence, the inhibition of cFos activity might result in CYP17 expression in the granulosa cell. Our objective was to define the role of cFos, in the regulation of CYP17 expression in granulosa cells. Transformed human luteinized granulosa (HGL5) cells were utilized for all experiments. HGL5 cells were cultured in monolayer for 72 h. Cells were treated for 48 h with and without PD98059 (PD), a MAPKK inhibitor, which also blocks cFos expression. RNA was isolated and real time RT-PCR was performed for CYP17. cFos RNA interference experiments were carried out using RNAifect, cFos *SMART*pool siRNA and scrambled siRNA for 48 h. RNA was isolated and RT-PCR was also performed for CYP17. Immunochistochemical studies were performed on normal ovaries, staining for cFos and CYP17. Treatment of HGL5 cells with the MAPKK inhibitor PD for 48 h, resulted in a 10-fold increase in CYP17 mRNA expression compared to basal conditions. In cFos gene silenced cells, CYP17 mRNA expression also increased by 10-fold compared to control siRNA conditions. Immunohistochemical staining for cFos and CYP17 showed significant staining of cFos in the granulosa cell layer, but absent staining for CYP17. Conversely, the theca cell layer did not stain for cFos, but staining was evident for CYP17. These results suggest that the AP-1 transcription factor, cFos, may play a role in the inhibition of CYP17 expression in granulosa cells. This may provide an explanation for the lack of CYP17 expression in granulosa cells.

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CLIP-170 Functions Downstream of mTOR Kinase during Local Ovarian Responses to Stress. Niels Galjart,[2] Joshua Johnson.[1] *[1]Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA; [2]Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands.*
Introduction: Cytoplasmic linker protein 170 (CLIP-170; CLIP1) is a plus-end binding protein that has been shown to regulate the function of the microtubule cytoskeleton. CLIP-170 has been shown to be required for the stability and proper orientation of the mitotic spindle. Our group has recently shown that the stress and nutrition-responsive kinase mTOR (mammalian Target of Rapamycin) is involved in granulosa cell--and follicle--growth, acting during

the G2/M stages of the granulosa cell cycle. As CLIP-170 has been identified as an mTOR substrate, we hypothesized that its function at the mitotic spindle would be positively regulated by mTOR during the late G2 and M phases of the cell cycle in granulosa cells. During periods of stress (e.g., mTOR inhibition), mTOR would fail to phosphorylate CLIP-170, leading to spindle checkpoint failure and follicle undergrowth.
Objectives: The expression of CLIP-170 and mTOR were evaluated. Computational analysis of potential CLIP-170 phosphorylation sites and comparison with residues on known mTOR targets were performed. CLIP-170 Threonine 182 and Serine 186 were chosen and evaluated as *bona fide* mTOR phosphorylation sites. A preliminary assessment of the effects of mTOR inhibition upon CLIP-170 function was performed.
Methods: For protein expression analyses, Western blots, immunostaining of tissues and primary granulosa cells in culture were performed. Computational analysis of potential CLIP-170 phosphorylation sites was followed by *in vitro* assessment of mTOR kinase activity upon CLIP-170 and a peptide substrate.
Results: CLIP-170 was expressed in the ovarian stroma, blood vessels (including the endothelial cells of both arteries and veins), granulosa cells, and in the oocytes of primordial and growing follicles. Overlapping expression was found between CLIP-170, mTOR, and the mTOR cofactors Raptor and Rictor in granulosa cells. This expression was conserved between the mouse and the human. Evaluation of CLIP-170 phosphorylation supported Thr 182 as a *bona fide* mTOR target.
Conclusions: CLIP-170 was supported as an mTOR substrate protein during granulosa cell mitosis. The mechanism of mTOR action during granulosa cell growth and survival is likely to include the phosphorylation of CLIP-170 and subsequent positive regulation of mitotic spindle function.

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The Effect of a Selective Oxytocin Antagonist (Barusiban) in Threatened Preterm Labour: A Randomised, Double-Blind, Placebo-Controlled Trial. Steven Thornton,[1] Thomas M Goodwin,[2] Gorm Greisen,[3] Morten Hedegaard,[4] Joan-Carles Arce.[5] *[1]Warwick Medical School, University of Warwick, Coventry, United Kingdom; [2]Maternal-Fetal Medicine, University of Southern California, Los Angeles, USA; [3]Dept of Neonatology, Rigshospitalet, Copenhagen, Denmark; [4]Dept of Obstetrics, Rigshospitalet, Copenhagen, Denmark; [5]Clinical Research & Development, Ferring Pharmaceuticals, Copenhagen, Denmark.*
Objective: A mixed oxytocin/vasopressin V_{1a} antagonist, atosiban, has been shown to reduce uterine contractions in placebo-controlled clinical trials and is useful in the management of preterm labour. The objective of this study was to determine the effect of a selective oxytocin antagonist, barusiban, in delaying delivery and reducing uterine contractions in women with threatened preterm labour at a late gestational age and relatively high risk of delivery.
Methods: This was a randomised, double-blind, placebo-controlled multi-centre study in 6 countries. A total of 163 women between 34+0 and 35+6 weeks gestation, and with ≥6 uterine contractions of 30sec duration during 30min, cervical length ≤15mm, and cervical dilatation >1 and <4cm were randomised to receive a single intravenous bolus dose of either barusiban 0.3mg, 1mg, 3mg, 10mg or placebo. Rescue tocolytics were prohibited. The primary end-point was percentage of women who did not deliver within 48h. Uterine contractions were monitored by cardiotocography. Obstetrical and neonatal outcomes were determined.
Results: There were no significant differences between the placebo and any barusiban group in percentage of women who did not deliver within 48h (72% in the placebo group and 65% to 88% in the barusiban groups). There was no dose-effect relationship nor an effect at 12 or 24h. None of the barusiban groups were associated with a significant reduction in number of uterine contractions compared to placebo at any time point up to 48h post-dosing. Postpartum blood loss and time to established lactation were not significantly increased with barusiban. Barusiban was well tolerated and was not associated with safety concerns for the women, fetus or neonates.
Conclusion: A single intravenous bolus of a selective oxytocin antagonist, barusiban (dose range 0.3-10mg), did not delay delivery or reduce uterine contractions compared to placebo in women with preterm labour at late gestational age and with short cervical length. The results contrast those of the mixed oxytocin/vasopressin V_{1a} antagonist, atosiban.

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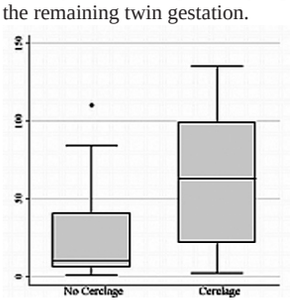
Prolonged Delivery Intervals in Triplet Gestations. Tracy A Manuck, Heather L Mertz, Leah Passmore, David C Merrill. *Obstetrics and Gynecology, Wake Forest University Health Sciences, Winston-Salem, NC, USA.*
Objective: Delayed interval delivery is one management strategy for previable preterm labor affecting multiple gestations. Prior reports of asynchronous deliveries have examined twins and higher-order multiples as a group. This study was conducted to analyze the unique situation of asynchronous triplet deliveries.
Study Design: Cases of asynchronous triplet deliveries resulting in an ongoing twin gestation were ascertained through Medline. Data were abstracted and combined with two similar previously unpublished cases. Patients were grouped by management with and without rescue cerclage. Variables compared included use of tocolytics, antibiotic administration, gestational age at delivery of each fetus, interdelivery interval, delivery mode, birthweights, and short and long term outcomes. Chi-square or t-test analyses were used where appropriate.
Results: Fifty-one cases of asynchronous triplet deliveries met inclusion criteria and were analyzed. Twenty-three patients (45.1%) underwent placement of a rescue cerclage following delivery of the first infant. These patients delivered the first fetus at a significantly earlier gestational age as compared to those patients without a cerclage (20.3 +/- 3.4 weeks vs. 23.5 +/- 3.5 weeks, p=0.0019). Patients with a rescue cerclage had a significantly longer prolongation of the remaining twin gestation (59.4 +/- 41.3 days vs. 25.9 +/- 30.1 days, p=0.0016). No significant differences in use of tocolytics or antibiotics, gestational age at delivery of triplets "B" and "C," mode of delivery, short term outcome (alive at 24 hours), or long term outcome (alive at discharge) were noted, despite delivery of triplet "A" at a significantly younger gestational age.
Conclusion: Rescue cerclage, particularly when placed following previable delivery of a first triplet, may significantly prolong the delivery interval for the remaining twin gestation.



Mean pregnancy prolongation (days).

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Amnioinfusion and Preterm Premature Rupture of the Membranes (PPROM): Indications and Perinatal Outcome. Elisa Valmori, Patrizia Bozzetti, Anna Maria Marconi. *Obstetrics and Gynecology, University of Milano, DMSD San Paolo, Milano, Italy.*
Objective The aim of our study was to evaluate the role of amnioinfusion in pregnancies complicated by PPROM.
Materials and Methods We studied 52 singleton pregnancies with PPROM at <25 weeks gestation. All patients were managed conservatively with bed-rest, prophilactic antibiotics, tocolytics and steroids. Only patients without vaginal bleeding and/or contractions were included: 11 patients showed an amniotic fluid pocket (AFP) persistently ≥2 cm and did not undergo amnioinfusion (Group B) whereas 41 had a maximum AFP < 2 cm and were offered amnioinfusion to restore an adequate amount of amniotic fluid (Group A). In 17 patients of Group A amnioinfusion was successful (AFP ≥2 cm for 48 hours following the procedure: Group A1) whilst in 24 it was unsuccessful (AFP<2 for 48 hours: Group A2) and repeated. Results were analized with the Student t test for unpaired samples and with the $\chi 2$ when appropriate. p values <0.05 were considered significant.
Results The group where amnioinfusion was not successful (Group A2) showed the worst outcome (see table). There were 6 intrauterine deaths, all in this group. Pulmonary hypoplasia was present in 11/52 (22.5%) newborns (both survived and deceased) newborns, 10/11 in Group A2. No maternal complications were recorded.
Conclusions Our data confirm that a conservative-active management with amnioinfusion can be considered a reasonable option in women with PPROM. In our series it was effective in preventing both neonatal death and pulmonary hypoplasia.

Study Results

	Group A1 (infusion successful)	Group A2 (infusion unsuccessful)	Group B (no infusion)	p A1vsA2	p A2vsB	p A1vsB
N° of cases	17 (32.7%)	24 (46.15%)	11 (21.15%)	-	-	-
Gestational Age at PPROM (weeks)	18.7±3.5 (19.43)	18.7±2.5 (18.86)	20.2±3.5 (20.14)	NS	NS	NS
Latency (days)	70.8±39.8 (65)	40.7±25.2 (34)	65.2±56.5 (35)	0.005	NS	NS
Gestational Age at Outcome (weeks)	28.8±3.4 (29.86)	24.6±3.2 (25)	29.5±6.4 (29.3)	<0.001	0.004	NS
N° of born alive	16 (94%)	11 (45.8%)	9 (81.8%)	0.001	0.04	NS
Neonatal Mortality	1 (5.9%)	9 (37.5%)	2 (18.2%)	0.02	NS	NS
Alive at discharge	15 (88.2%)	1 (4.2%)	6 (54.5%)	<0.001	<0.001	NS
N° of pulmonary hypoplasia	1 (5.9%)	10 (42%)	0 (0%)	0.04	0.04	NS

Values are numbers or mean ± SD; median or % in brackets

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Prolonged Stretch Stimulates Human Myometrial Contractility In Vitro and Blocks the Inhibitory Effect of Prolonged Incubation in Synthetic Progestogen. Yolande Cordeaux, Dharmintra Pasupathy, Stephen Charnock-Jones, Gordon C Smith. *Dept. Obstetrics & Gynaecology, University of Cambridge, Cambridge, United Kingdom.*

Background.

Synthetic progestogens are effective in reducing the risk of spontaneous preterm birth in high risk singleton, but not multiple, pregnancy. We hypothesized that myometrial stretch may inhibit the response of human myometrium to progestogens.

Methods.

Myometrial strips obtained with written consent at the time of term planned cesarean section were studied using a modification of the method of Young and Zhang (JSGI 2004;11:478-82). Strips were maintained in individual tubes in tissue culture media in an incubator for a period of three days. The effect of prolonged stretch was assessed by comparing strips connected to a 0.6g weight with those connected to a 2.4g weight. The effect of prolonged exposure to progestogen was studied by adding medroxyprogesterone acetate (MPA, 100nM or 1000nM). Following the 3 day incubation, myometrial strips were transferred to an organ bath containing Kreb's solution. All were placed under 2g tension and responses obtained to 50mM potassium then oxytocin. Contractility was expressed as the ratio of the maximum response to potassium or oxytocin to the wet weight of the tissue (units = g.tension per gram), summarized as the mean (SEM) and compared using Student's paired t test.

Results.

Prolonged stretch increased the maximum response to both potassium (0.6g weight = 24.6 [5.3]; 2.4g weight = 43.1 [6.5], n=6, P=0.01) and oxytocin (0.6g weight = 30.4 [8.0]; 2.4g weight = 50.3 [7.6], n=6, P=0.01). In strips with a 0.6g weight, incubation in MPA for three days reduced the maximum response to potassium (vehicle = 30.1 [5.5]; MPA = 23.2 [5.2], n=5, P=0.02) and there was a trend towards a reduced maximum response to oxytocin (vehicle = 37.6 [8.2]; MPA = 31.5 [7.2], n=5, P=0.1). In strips with a 2.4g weight, incubation in MPA for three days had no effect on either the maximum response to potassium (vehicle = 38.1 [13.4]; MPA = 42.4 [16.9], n=3, P=0.4) or oxytocin (vehicle = 44.7 [15.1]; MPA = 51.0 [20.0], n=3, P=0.4).

Conclusions.

1. Prolonged stretch increases human myometrial contractility in vitro. 2. Prolonged exposure to a progestogen inhibits the contractility of human myometrium but this effect is blocked by prolonged stretch. These properties of human myometrium may explain the failure of 17OH progesterone caproate to reduce the incidence of spontaneous preterm birth in multiple gestations.

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Comparing Uterine Electromyography Measurements to Other Parturition Factors in Pregnant Patients in Preterm Labor with and without Tocolytics. Sangeeta Jain, William L Maner, Janet L Brandon, Gary DV Hankins, Robert E Garfield. *Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, TX, USA.*

Objective: To determine if transabdominal uterine electromyography (EMG) correlates with parturition factors such as measurement-to-delivery time (MTDT), cervical dilation (CD), cervical effacement (CE), and station (S) for preterm labor patients with and without tocolysis. **Materials and Methods:** 17 pregnant preterm labor women were included. Uterine electromyography (EMG) was measured for 30 minutes. CD, CE, and S were assessed at or near the time of uterine EMG measurement. The power density spectrum peak frequency (PDSPF) was calculated on EMG. Patients were grouped (G1: tocolysis, N= 4; G2: no tocolysis, N=13). Pearson-product-moment test was used for correlation. Significant differences were sought between groups using Student-*t* test. P<0.05 significant. **Results:** There was a significantly higher uterine EMG activity (PDSPF: 0.453 ± 0.051 vs. 0.379 ± 0.016), but no difference in CD (5.125 ± 1.727 vs. 3.714 ± 1.976), for patients delivering within 6 days of EMG recording compared to those who delivered later, regardless of tocolysis. There was no apparent difference in uterine EMG in tocolytic vs. non-tocolytic patients, regardless of MTDT (**TABLE 1**). **Conclusions:** Uterine EMG activity is significantly greater in patients in preterm labor who delivered within 6 days of measurement, making it a viable alternative diagnostic parameter for assessing the state of parturition. Tocolytics may not affect uterine EMG, but this should be further verified with larger studies. Supported by grant NIH R01- HD037480.

	EMG	GA	MTD	MA	CD	CE	S
Tocolytic	-0.364	-0.309	0.539	-0.396	-0.215	0.360	-0.258
	0.165	0.227	0.0255	0.116	0.441	0.206	0.418
EMG		0.284	-0.586	-0.0817	0.494	-0.192	0.812
		0.286	0.0170	0.764	0.0727	0.529	0.00238
GA			-0.291	0.0727	0.0190	-0.408	0.0826
			0.257	0.782	0.946	0.147	0.799
MTD				-0.184	-0.666	-0.137	-0.605
				0.479	0.00670	0.641	0.0370
MA					0.262	0.0925	0.0190
					0.346	0.753	0.953
CD						0.214	0.547
						0.462	0.0657
CE							-0.149
							0.645

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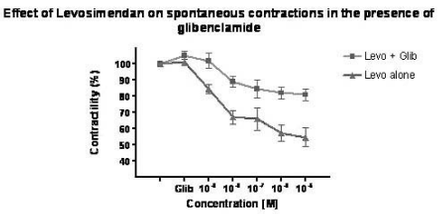
Levosimendan and the K-ATP Channel: Effects on Human Uterine Contractility. Mark P Hehir, Audrey T Moynihan, Terry J Smith, John J Morrison. *Department of Obstetrics and Gynaecology, National University of Ireland, Galway, Ireland.*

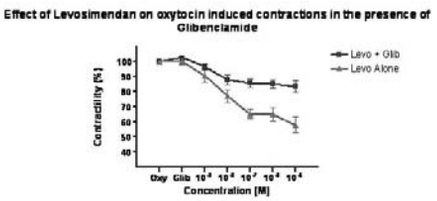
Objective: Calcium sensitizers are a novel class of drugs with unique molecular and phsiological actions. Levosimendan, the best characterized of these compounds and is used in the treatment of acute and chronic heart failure. Levosimendan can exert an inotropic effect via sensitization of myofilaments to calcium. It also exerts a relaxant effect on vascular smooth muscle through the opening of ATP-dependent potassium channels and has been shown to be a potent inhibitor of human uterine contractions in vitro. For these reasons we investigated the effects of levosimendan on uterine contractions, both spontaneous and agonist induced, in the presence of glibenclamide, a K-ATP channel blocker.

Method: Biopsies of human myometrium were obtained at elective caesarean section (n=20). Dissected myometrial strips suspended under isometric conditions, undergoing spontaneous and oxytocin-induced contractions, were exposed to glibenclmide (100mmol) followed by cumulative additions of levosimendan in the concentration range of 1 nmol/L to 100 mmol/L. Control experiments were performed simultaneously.

Results: Levosimendan exerted an inhibitory effect on spontaneous and oxytocin induced contractions in human m yometrium in vitro, in comparison to control experiments. The effect of levosimendan was significantly antagonized by glibenclamide with the mean maximal inhibition seen due to levosimendan greatly reduced (n=6, p<0.05).

Conclusion: The calcium sensitizer levosimendan exerted a potent relaxant effect on human uterine contractility *in vitro*. This action was antagonized by glibanclamide and this study demonstrates that the effect of levosimendan on uterine smooth muscle is mediated at least in part through the K-ATP channel.





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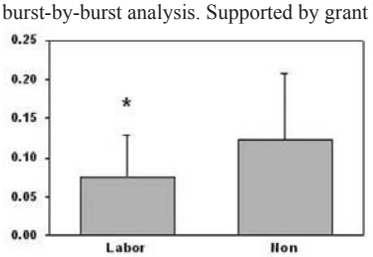
Using Chaos (Lyapunov) Analysis of Uterine Electromyography Signals To Discriminate between Labor and Non-Labor Patients. William L Maner, Lynette B MacKay, Cecilia Recabarren, Robert E Garfield. *Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, TX, USA.*

Introduction: The determination of the beginning and ending points for "bursts" of electrical activity occurring during uterine contractions is sometimes difficult. If bursts cannot be discerned, the preferred burst-by-burst analysis cannot be performed. One solution to is to analyze any given electrical recording in its entirety. But this approach has often lead to meaningless results when traditional analytic methods are applied. Chaos analysis, using Lyapunov exponents, may provide an answer. **Materials and Methods:** 46 term patients were included in the analysis: 27 were in labor (group 1), while19 were non-labor (group 2). 30 minute recordings were analyzed using "Lyapunov exponent." For each pair of subsequent trajectories in phase space, only the most positive Lyapunov exponent was calculated. The mean largest exponent was found by averaging over all of the trajectories in the recording. The Lyapunov exponent is given in units of bits per data sample. Thus a value of +1 means that the separation of nearby orbits doubles on the average in the time interval between data samples. The mean largest exponent was found for each patient recording. These values were compared using t-test ($P < 0.05$ considered significant). **Results:** The mean and SD of the Lyapunov exponent for all the patients was 0.0955 ± 0.0701. Moreover, the Lyapunov exponent calculated for each patient was positive. Comparing Lyapunov exponents of the two groups showed a statistically low value (low chaos) for the laboring group, compared to the non-laboring group (**Figure**). **Conclusions:** There is a chaotic component associated with uterine EMG traces, since small but non-negative Lyapunov exponents were found in all the traces observed. The Lyapunov exponent indicated significantly lower chaotic behavior in the whole EMG traces of patients who were in labor than found in those who were not in labor, implying that this measure could be a good diagnostic parameter for labor, possibly eliminating the need for tedious burst-by-burst analysis. Supported by grant NIH R01- HD037480.



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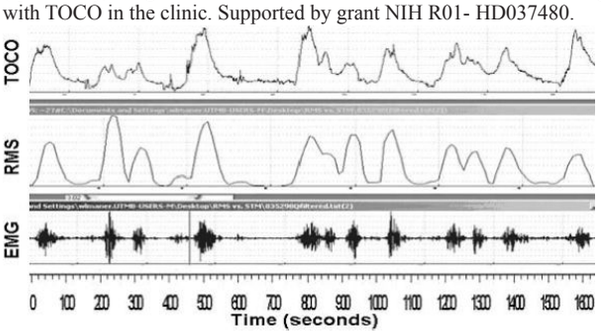
Comparing Uterine EMG to Tocodynamometer for Monitoring Contractions. Robert E Garfield, Lynette B MacKay, Sangeeta Jain, William L Maner. *Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, TX, USA.*

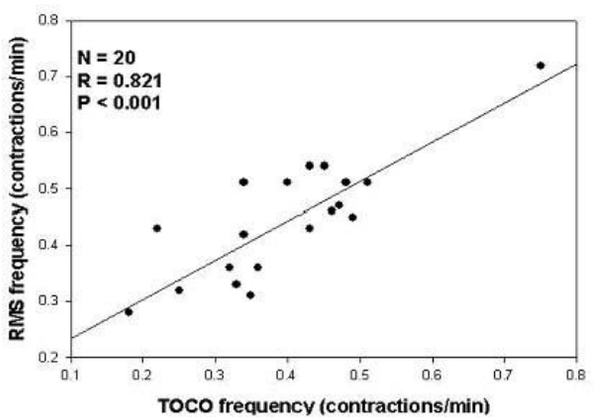
Objective: To determine whether uterine electromyography (EMG) plots contractions similarly to tocodynamometer (TOCO).

Study design: 20 pregnant term labor patients were recorded using both uterine EMG and TOCO simultaneously. Uterine EMG signals were sampled at 100Hz and band-pass filtered in the 0.34 to 1.00 Hz range. Root-mean-square (RMS) function was calculated from the uterine EMG signals in order to produce a "TOCO-like" trace from the original EMG trace. EMG-generated RMS contraction plots were then compared to TOCO contraction plots using the following criteria: Contractions were assigned a marker value of "1." In-between contraction periods were assigned a "0." From these marker values, contraction rates were established. Correlation was found between the contraction rates of RMS and TOCO. Temporal overlap of contractions plotted by the two methods was used to find overall percent agreement (OPA), positive percent agreement (PPA), and negative percent agreement (NPA). These parameters were corrected for within-patient variation using a bootstrap method.

Results: Uterine RMS contraction plots were seen to correspond with TOCO contraction plots (**Fig. 1**). Corrected OPA, PPA, and NPA were high at 90.68%, 84.52%, and 95.77%, respectively. There was a large, statistically significant correlation between uterine EMG and TOCO contraction frequency (**Fig. 2**).

Conclusions: The similarity between TOCO and uterine EMG contraction plots (specifically, using RMS to convert) will allow EMG to be used interchangeably with TOCO in the clinic. Supported by grant NIH R01- HD037480.





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Low Concentrations of Thrombin-Inhibitor Complexes and the Risk of Spontaneous Preterm Delivery. David N Hackney, Hyariv N Simhan. *Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA.*

Background: Concentrations of thrombin-antithrombin (TAT) complexes in maternal plasma increase during pregnancy. High concentrations have been associated with adverse outcomes, including preterm delivery, presumably secondary to chorion-decidual bleeding. Recently, however, low concentrations of maternal TAT have been associated with placental histologic lesions, and may be reflective of impaired placental thrombin activation.

Methods: A secondary analysis of a multicenter case-control study nested within an observational cohort was performed. Subjects with spontaneous preterm birth at less than 36 weeks were matched to controls by race and parity. Maternal plasma TAT concentrations were previously measured at 24 and 28 weeks. Differences in TAT concentrations between cases and controls were analyzed with Mann-Whitney U. The relationship between maternal TAT concentrations less than the 25% percentile and preterm birth was determined with crude and adjusted Odd Ratios (OR). Logistic regression was employed to control for potential confounders.

Results: Maternal plasma concentrations of TAT were available from 169 cases at 24 weeks and 136 cases at 28 weeks, and controls. There was no correlation between TAT concentrations and 1st or 2nd trimester vaginal bleeding (p=0.43). Concentrations of TAT were lower in cases then controls at 28 weeks (p=0.01). A non-significant trend towards lower TAT concentrations was present at 24

weeks (p=0.08). The OR for spontaneous preterm birth with TAT concentrations <25% at 28 weeks was significant (2.13, 95% CI 1.11-4.06, p=0.02) when adjusted for smoking, prior preterm birth, vaginal bleeding, fetal fibronectin, bacterial vaginosis, body mass index and cervical length.

Conclusion: Concentrations of TAT at 28 weeks were lower in cases of spontaneous preterm birth than in matched controls, and remained significant when controlled for multiple potential confounders. This is in contrast to other studies of TAT and preterm delivery risk but is in line with in vitro data that associate placental thrombotic and inflammatory lesions with lower maternal TAT concentrations. In the absence of acute bleeding events, low TAT concentrations may represent impaired placental thrombin activation and be pathogenic.

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Impact of Body Mass Index (BMI) on Plasma Concentrations of 17 α Hydroxyprogesterone Caproate (17-OHPC). Steve N Caritis,[1,3] Raman Venkat.[2] *[1]Department of Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA; [2]Department of Pharmaceutical Sciences, University of Pittsburgh, School of Pharmacy, Pittsburgh, PA, USA; [3]Maternal-Fetal Medicine Units Network, NICHD, Bethesda, MD, USA.*

Introduction

The 250 mg IM weekly dose of 17-OHPC utilized for the prevention of preterm birth (PTB) is empiric and based on the dose reported to be effective in randomized clinical trials. Whether maternal plasma concentrations are affected by maternal BMI has not been evaluated.

Objective

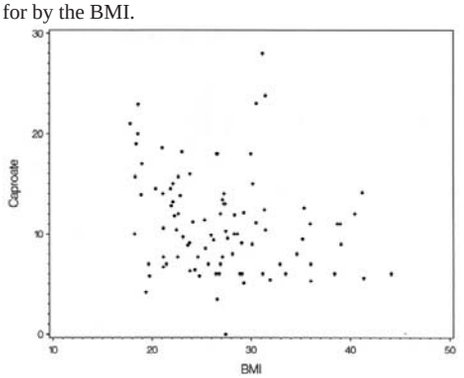
To evaluate the relationship between maternal BMI and plasma concentrations of 17-OHPC in women with twin gestation.

Methods

This is a secondary analysis of patients with twin gestation who participated in a 14 center placebo controlled trial evaluating the impact of 17-OHPC in PTB prevention. Women with twin gestation were randomized between 16-21 weeks to receive either 17-OHPC or placebo weekly until 35 weeks gestation. A plasma sample was collected at 24-29 weeks after at least five consecutive injections had been administered to assure steady state concentration. The sample was drawn just prior to the next injection and analyzed for 17-OHPC by HPLC-mass spectrometry. The lower limit of quantification of 17-OHPC was 1ng/ml. The relationship between 17-OHPC concentration in women who received active drug and with an available pre-pregnancy BMI, was assessed by multiple linear regression, adjusting for race/ethnicity and gestational age at sample test.

Results

A total of 94 women were in the analysis cohort. Concentration of 17-OHPC was negatively correlated with the BMI (p = 0.03). Although the relationship was significant, the overall contribution of BMI to the variability in 17-OHPC concentration was small, ($r^2 = 0.06$.) i.e. only 6% of the variability is accounted for by the BMI.



Conclusion

The variation in plasma 17-OHPC concentrations seen in this group of subjects who received 250 mg 17-OHPC weekly was minimally affected by the BMI.

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Relationship of 17α Hydroxyprogesterone Caproate (17-OHPC) Concentrations and Gestational Age at Delivery in Twins. Steve N Caritis,[1,2] Hyagriv Simhan.[1,2] *[1]Department of Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA; [2]Maternal-Fetal Medicine Units Network, NICHD, Pittsburgh, PA, USA.*

Introduction - This is a secondary analysis of women participating in a 14 center randomized placebo controlled trial (RCT) evaluating the impact of 17-OHPC in preterm birth (PTB) prevention among women with twins.

Objective - To evaluate the relationship between plasma 17-OHPC concentrations and gestational age (GA) at delivery in women with twins receiving weekly injections of 17-OHPC.

Methods - Women with twins were randomized between 16-21 weeks to receive weekly IM injections of either 17-OHPC (250 mg) or placebo until 35 weeks. After a minimum of five consecutive injections had been administered to assure steady state concentrations a plasma sample was collected between 24-29 weeks. The sample drawn just prior to the next scheduled injection was analyzed for 17-OHPC by HPLC-MS in a blinded manner. The lower limit of quantification of 17-OHPC was 1 ng/ml. We conducted univariate analyses to assess the association of 17-OHPC concentration and GA at delivery. We also conducted a proportional hazards model to evaluate the time from sample draw to spontaneous delivery (censoring indicated preterm deliveries), and a logistic regression to evaluate PTB<35 weeks; in both analyses we adjusted for BMI, race and GA at sample draw.

Results – 96 women assigned to 17-OHPC were included; all received all of their scheduled injections. The concentration of 17-OHPC was significantly higher in women delivering < 35 weeks compared with those women delivering ≥35 weeks (p=0.002, Table). Concentration of 17-OHPC was significantly correlated with GA at delivery (r = -0.38, p=0.0001). Each unit increase of 17-OHPC was associated with a 30% increased odds of delivering < 35 weeks (odds ratio 1.3, 95% CI,1.14-1.50, p=0.001) and a 12% increase in hazard of spontaneous delivery (hazard ratio 1.12, 95% CI, 1.05-1.19, p=0.0004) after adjusting for confounders.

Gestational Age at Delivery	Mean (SD) - ng/ml
< 35 weeks (n=34)	13.9 (5.9)
>35 weeks (n=62)	9.4 (3.5)

Conclusion

Plasma 17-OHPC concentrations after weekly injections were inversely related to GA at delivery in women with twins. Since 17-OHPC induces its own metabolism it is possible that higher concentrations during initial treatment are associated with lower plasma concentrations and reduced efficacy in later pregnancy . Clearly more studies are needed.

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The Effect of 17-alpha Hydroxyprogesterone Caproate (17-OHPC) on Maternal Plasma CRP Levels in Twin Pregnancies. Hyagriv N Simhan,[1,2] Steve N Caritis.[1,2] *[1]Department of Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA; [2]Maternal-Fetal Medicine Units Network, NICHD, Bethesda, MD, USA.*

Objective: The mechanism of action of 17-OHPC with respect to prematurity prevention has not been elucidated. Recent in vitro work suggests the possibility of an anti-inflammatory effect of 17-OHPC. We sought to determine if 17-OHPC exerts a systemic anti-inflammatory effect among women with twin pregnancies as represented by plasma C reactive protein (CRP)

Methods: This is a planned ancillary analysis of a multicenter RCT of 17-OHPC for the prevention of preterm birth among women with twins. Women received either 17-OHPC intramuscularly 250 mg once weekly or placebo. A single maternal plasma sample was collected once at each of two epochs, 24-28 weeks and 32-36 weeks and assayed for CRP (using a commercially available highly sensitive ELISA method). All CRP samples were drawn more than 2 weeks prior to delivery. For this analysis we only included women who had at least 5 consecutive injections prior to the first blood sample to assure steady state concentrations. Multivariable analysis was a general linear model (GLM) of $\log_{10}$CRP versus treatment group, adjusted for maternal BMI, smoking, race, chorionicity and gestational age at sampling.

Results: In the primary trial, there was no difference in preterm birth by treatment group. CRP concentration was not associated with preterm birth < 35 weeks. After adjustment for covariates, the difference in log CRP concentration between treatment groups was significant at both time points.

Concentration of CRP by treatment group

Gestational Age Epoch	Placebo Group CRP*	17-OHPC Group CRP*	P-value in GLM
24-28 weeks, n=210	7955 (4315-13995), n=116	10561 (5729-19582), n=94	0.02
32-36 weeks, n=145	6369 (1960-13762), n=74	9264 (5103-15701), n=71	0.008

*Median and interquartile range (ng/mL)

Conclusions: Women with twin gestations who receive 17-OHPC therapy have higher plasma concentrations of CRP compared to placebo-treated women. Whether this effect is of clinical significance is uncertain.

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Fetal Progesterone Withdrawal: A Potential Mechanism for Preterm Parturition. Yana Filipovich,[1] Marci G Adams,[1] Emmet Hirsch.[1,2] *[1]Department of Ob/Gyn, Evanston Northwestern Healthcare, Evanston, IL; [2]Department of Ob/Gyn, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

Objective: In many non-human species, maternal circulating progesterone levels fall prior to delivery, leading to the theory that in humans progesterone withdrawal occurs on a local and/or functional level. Our objective was to characterize maternal and fetal progesterone in human preterm and term labor.

Methods: Women between 23.7 and 34.7 weeks' gestation (cases) or term controls (37-41 weeks) with either labor with intact membranes or premature rupture of the membranes prior to labor (PROM) were enrolled in a prospective case-control study. Progesterone was measured by Immulite assay in maternal serum collected upon enrollment and again within 60 minutes after delivery and in umbilical cord serum obtained at delivery. Maternal progesterone treatment was not used in any subjects.

Results: 20 cases and 20 controls were studied (see Table for comparisons).

	Cases	**Controls**	**P value**
GA at enrollment, weeks	29.4 ± 3.2	39.3 ± 1.1	<0.0001
Interval to delivery, days (median, range)	2 (0 - 25)	0 (0 - 1.4)	<0.01
Maternal progesterone at enrollment, ng/ml	125 ± 87	185 ± 64	<0.02
Maternal progesterone after delivery, ng/ml	55 ± 57	47 ± 27	0.6
Cord progesterone, ng/ml	878 ± 602	762 ± 457	0.5

Among cases, fetal but not maternal progesterone was significantly lower in preterm labor with intact membranes (475 ± 372 ng/ml, n = 8), as compared to PROM (952 ± 417, n = 12), p<0.02. This difference increased further when cases of clinical chorioamnionitis were excluded.

Conclusions: Serum progesterone in laboring patients prior to delivery is higher at term than in the preterm period, which may be attributable to increased placental mass in late pregnancy. This disparity disappears shortly after delivery of the fetus and placenta. Fetal progesterone levels are several-fold higher than peripartum maternal levels. Preterm labor with intact membranes is associated with diminished fetal progesterone, a phenomenon unrelated to clinical infection. These findings suggest the possibility of fetally regulated progesterone withdrawal as a mechanism underlying preterm labor with intact membranes.

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Systematic Review and Meta-Analyses of Preterm Birth Genetic Association Studies. Ramkumar Menon,[1] Mario Merialdi,[2] Ana Pilar Betran,[2] Tomas Allen,[2] Bruce Lin,[3] Judith Eckardt,[1] Muin Khoury,[4] John P Ioannidis,[5] Lars Bertram,[6] Mads Hollegaard,[7] Digna R Velez,[1] Siobhan Dolan.[8] *[1]The Perinatal Research Center, Nashville, USA; [2]World Health Organization, Geneva; [3]March of Dimes, New York, NY; [4]Center for Disease Control, Atlanta, GA; [5]Univ of Ioannina School of Medicine, Greece; [6]Massachusetts General Hospital, Boston, MA; [7]NANEA, Aarhus, Denmark; [8]Albert Einstein College of Medicine, New York, NY.*

OBJECTIVE: Growing body of literature reveals the genetic contributions to PTB. Many studies have reported associations between single nucleotide polymorphisms [SNPs] and PTB. However, many of these studies are inconclusive and non reproducible. The challenge of identifying robust associations between genetic variation and either susceptibility or protection from PTB is enormous. A systematic review of literature followed by meta-analysis was performed to understand true associations between SNPs and PTB.

METHODS: For systematic review, articles were chosen based on Medline and EMBase searches (1990–April 2007) and relevant articles were chosen based on stringent inclusion criteria. Primarily, studies reporting genetic associations between SNPs in maternal DNA in singleton pregnancies and spontaneous PTB were included. Other criteria included, but not limited to, provided genetic data in a complete enough format so that it could be evaluated in meta-analysis and defined the clinical outcome clearly. Meta-analysis was performed wherever >3 replication data sets were available

RESULTS: A total of 5422 abstracts were reviewed and 88 were selected for full text review. Data were extracted from 50 articles. Over 100 associations were reported between SNPs on various candidate genes and PTB; however only 32 had replication dataset. Meta-analysis documented significant association between PON2A148G (Odds ratio [OR]=1.64 (95%CI 1.18-2.26), PON1(rs#662)(OR=1.14; CI-1.007-1.34), TNFRSF6-670A/G (FAS) (OR=1.52; CI-1.11-2.08) and PTB. Two SNPs PON2S311C (OR=0.56; CI-0.36-0.85) and IFN gamma (rs2430561; OR-0.62; CI-0.43-0.91) documented protective effect.

CONCLUSIONS: Systematic review concludes significant heterogeneities leading to lack of reproducible data in genetic association studies of PTB. Heterogeneities are contributed predominantly by lack of adequate power, poor phenotype selection, and population admixture. The functional relevance of the risk and protective alleles needs to be verified.

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Survival in Triplet Pregnancies Complicated by Twin Twin Transfusion Syndrome (TTTS). Jignesh Parvadia,[1] Mounira Habli,[2] Jeff Livingstone,[2] William Polzin,[3] Foong Lim,[1] Timothy Crombleholme.[1] *[1]Pediatric and Thoracic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [2]Obstetric and Gynecology, University of Cincinnati, Cincinnati, OH, USA; [3]Obstetric and Gynecology, Good Samaritan Hospital, Cincinnati, OH, USA.*

Objective

Little is known about the response of TTTS to treatment either by amnioreduction or selective fetospopic laser in triplet pregnancy, particularly the survival of the bystander fetuses. In order to define the response of triplet pregnancies to treatment for TTTS we reviewed our experience with higher order multifetal gestations complicated by TTTS.

Study design

Retrospective chart review of patients diagnosed with in high order gestation from 2004-2007 was performed.

Results

Among 254 cases of TTTS 11/254(4.3%) patients with high order gestations were identified (n=11) with a mean GA at diagnosis of 20.3±0.8 weeks. 9 pregnancies (81.8%) were dichorionic triamniotic and 2(18.1%) were monochorionic triamniotic. Cincinnati modification of Quinterro staging was utilized to characterize recipient cardiomyopathy as mild (stage IIIa, n=3), moderate (stage IIIb, n=1) and severe (stage IIIc or IV, n=3) categories. 4/11 (36.3%) were treated with amnioreduction alone (AR), 2/11(18.1%) with selective fetoscopic laser photocoagulation (SFLP) alone, 2/11(18.1%) with AR followed by SFLP and 1/11(9.09%) with AR followed by intrafetal radio frequency ablation (RFA). 1/11(9.09%) patient had a cervical cerclage. 2/11(18.1%) patients were treated but remain undelivered. Mean GA at delivery was 29.5±1.04 weeks. Overall survival was 21/27(77.7%) with bystander survival was 9/9(100%), donor survival 6/9(66.6%), recipient survival was 6/9(66.6%).

Conclusion

Triplet pregnancies treated for TTTS have 100% survival rate for bystander fetuses and have 66.6% survival rates for donor and recipients comparable to twins treated for TTTS.

GA at diagnosis	20.3±0.8weeks
Cincinnati modification of Quinterro	1(I), 1(II), 3(III), 3(IIIA), 1(IIIB), 2(IV)
GA at delivery	29.5±1.04 weeks
Live birth	
- Donor	6/9(66.6%)* #
- Recipient	6/9(66.6%)*#
- Bystander	9/9(100%) #
Birth weight	
- Donor	1141±348 gms
- Recipient	1010.1±128gms
-Bystander	1339.7±201gms
* Fetus treated with RFA excluded	# Undelivered patients excluded

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Breast Feeding Improves Perinatal Outcome Independent of Maternal Antenatal Methadone Dose. Mounira Habli, David Pizzaro, Marquia Grier, Jeffery Livingston. *Obstetrics and Gynecology, University Of Cincinnati, Cincinnati, OH, USA.*

OBJECTIVE

To assess the effect of breast feeding (BF) on perinatal outcome in relation to maternal antenatal methadone dose.

STUDY DESIGN

A retrospective chart review study of 170 methadone dependent mother and infant pairs. Patients were categorized into 3 groups based on maternal dose at time of delivery: group 1: dose ≤ 50 mg, group 2: dose 51-100mg, group 3: dose >100 mg. The Finnegan's scoring system was used to monitor neonatal abstinence syndrome(NAS). Treatment for NAS was initiated if there were 2 scores of ≥ 8. Neonatal outcome data included:% NICU admission, % of babies discharged(D/C) at time of maternal d/c, % NAS, % treated for NAS and total hospital stay. Data were analyzed by T-test and Fisher's exact test.

RESULTS

Maternal characteristics between the 3 groups were similar. Regardless of maternal methadone dose, BF infants have shorter hospital stays and higher rates of d/c at time of maternal d/c, lower incidence of NAS and fewer NICU admission(Table). In all three groups, breast feeding did not impact the severity of NAS as reflected in Finnegan's score(FS).(Table)

CONCLUSION

Regardless of maternal methadone dose, breast feeding improves perinatal outcome but did not affect the severity of NAS.

Dose(mg)	0-50(n=55)		51-100(n=59)		>100(n=46)	
	no-BF(n=23)	BF(n=32)	no-BF(n=29)	BF(n=30)	no-BF(n=23)	BF(n=23)
NICUAdmission n(%)	7(30.4)	4(12.5)	14(48.2)	2(6.7)***	9(39.1)	2(8.7)*
D/C home n(%)	7(30.4)	26(81.3)***	5(17.2)	18(62.1)**	4(17.4)	17(73.9)***
NAS-n(%)	16(69.5)	7(21.8)**	22(75.9)	11(35.5)*	13(56.5)	5(21.7)*
Methadone treated n(%)	6(26.1)	15(65.2)	21(72.4)	7(23.4)	12(52.2)	3(13.1)
Total hospital stay(d)	10.9±1.8	4.8±6.5**	7.1±11	4.07±3.5***	18.2±15.5	3.8±3.6***
FS at 24 hours	6.1±2.86	4.9±2.7	7.6±4.4	5.8±2.78	8.2±5.2	5.7±4.4
FS at 48 hours	6.3±3.5	6.82±3.2	5.8±3.3	6.4±3.3	7.9±4.1	4.8±2.3*

Data(mean±SD).*P<0.05,**P,0.01***P<0.001

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Sealing Fetal Membrane Defects with Collagen Following Fetoscopic Endoluminal Tracheal Occlusion (FETO) Treatment for Severe Congenital Diaphragmatic Hernia (CDH). Roland Devlieger, Leonardo Gucciardo, Elisa Done, Tim Van Mieghem, Jan Deprest. *Department of Obstetrics and Gynaecology, University Hospitals Leuven, Leuven, Belgium.*

Objective: To evaluate the effects of preventive collagen plugging of the fetoscopic access port at the time of balloon removal on pregnancy outcome in fetoscopic endoluminal tracheal occlusion pregnancies.

Study design: Fifty-one pregnancies involving fetuses with severe congenital diaphragmatic hernia (CDH) were studied. All patients underwent FETO between 26-29 weeks gestational age (GA) and fetoscopic balloon removal around 34 weeks GA. At the time of balloon removal, a purified dried collagen plug was inserted through the fetoscopic access port in 23 consecutive pregnancies but not in the first 28 pregnancies considered as controls. All patients underwent post-plugging ultrasound and magnetic resonance imaging studies to evaluate for membrane dehiscence, amniotic fluid volume and fetal well being. GA at delivery, incidence of premature rupture of the membranes (PPROM), bleeding at port retrieval and adverse fetal effects were compared in both groups.

Results: Mean (SD) GA at FETO [28.2 (1.8) vs. 27.5 (1.5) weeks; P= NS] and balloon removal [33.9 (0.7) vs. 33.7 (1.1) weeks; P= NS] was similar in the treatment and in the control group. Incidence of PPROM following the second fetoscopy was 2/23 in the study group compared to 9/28 in the control group (P<0.05). Mean (SD) GA at delivery was 37.0 (1.8) weeks in the study group, compared to 36.4 (2.0) in the control group (P=0.28). Bleeding from the trocar insertion site occurred in 4 cases in both groups, but clinically significant bleeding occurred only in one of the controls. Membrane dehiscence was noted in 4 patient in the treatment group compared to 6 in the control group (P=NS).

Conclusion: Preventive collagen plugging of the fetal membrane defect created by the fetoscopic access resulted in a significant reduction in PPROM rates and a trend towards increased GA at birth without adverse fetal effects in FETO pregnancies. Wider application of this technique should be considered, but needs evaluation in larger, randomized trials.

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Hydrops Fetalis: Etiology and Outcome 1995-2005. Seonaid M Mulroy,[1] Emma Dudman,[2] Jan E Dickinson,[2] John P Newnham,[2] Craig E Pennell.[2] *[1]King Edward Memorial Hospital, Perth, WA, Australia; [2]School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia.*

Hydrops fetalis is an uncommon fetal condition characterised by the abnormal accumulation of fluid in two or more body cavities, traditionally associated with a poor prognosis. The relative rarity of this presentation has meant that published case series have consisted of small numbers.

A retrospective review of case notes of all cases managed at KEMH between 1995 and 2005 was performed. In Western Australia, KEMH is the only tertiary maternity hospital incorporating a Maternal-Fetal Medicine unit. Cases were obtained from the MFM database.

In the period 1995 to 2005 there was a total of 60 pregnancies affected by hydrops (incidence 0.2 per 1000 births). The average maternal age was 29 years. In 5 cases a fetal abnormality had occurred in a previous pregnancy. The median gestational age at diagnosis was 18 weeks (range 11-36 weeks). In just over half (53%) of cases, the diagnosis was confirmed prior to delivery. A post-mortem was performed on all but 2 of the babies not born alive. Edema was present in at least 3 cavities in over half of cases (n=36).

Chromosomal anomalies included Trisomy 21, Trisomy 18 and Turners syndrome. In all cases of infection, Parvovirus B19 was implicated. Cardiac arrythmias included SVT and atrial flutter. Cases classified as other included alpha thalassemia and syndromic disorders.

In 33 cases an interruption of pregnancy was performed at a mean gestational age of 19 weeks. Of those who did not elect to terminate the pregnancy, there were 15 fetal deaths in utero, 11 live borns with 1 neonatal death. For the live borns, the median gestational at delivery was 36.3 weeks (range 33 to 40.7 weeks). The causes of hydrops in live birth cases included cardiac arrythmia (n=3), infection (n=2), chromosomal abnormality (n=1), unknown (n=4) and other (n=1).

Of live born infants, there was one neonatal death, with all other cases surviving into infancy.

The poor outcome for fetuses affected by hydrops has again been demonstrated in this series with less than 20% of cases being live born.

Outcome by etiological classification

	TOP	FDIU	Live birth
Chromosomal	73.1%	23.1%	0.04%
Cardiac arrhythmia	0%	0%	100%
Infection	25%	25%	50%
Other	50%	16.7%	33.3%
Unknown	50%	35%	15%

Etiological classification

Etiology	Percentage
Chromosomal	43.3
Cardiac arrythmia	35
Infection	5
Other	10
Unknown	6.7

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MR Imaging Detection of Deep Medullary Veins Involvement in Fetal Cerebral Damage. Laura Avagliano,[1] Andrea Righini,[2] Cecilia Parazzini,[2] Chiara Doneda,[2] Fabio Triulzi,[2] Luca Ramenghi,[3] Gaetano P Bulfamante.[1] *[1]DMCO, Medical School, University of Milan, Milan, Italy; [2]Department of Radiology, Children's Hospital V. Buzzi, Milan, Italy; [3]Neonatal Intensive Care Unit, IRCCS-Policlinico, Mangiagalli, Regina Elena, Milan, Italy.*

Introduction

Deep medullary veins (DMV) have been noted to play a role in the development of hemorrhagic and periventricular leukomalacic lesions in premature babies. Since deep vein drainage system is relatively more prominent in the developmental brain than adult brain, we investigated if DMV anomalies could be associated with clastic lesions in-utero.

Methods

Two senior neuroradiologists reviewed 900 fetal brain exam performed between 2001 and 2007, seeking for unequivocal anomalies in DMV, such as periventricular venular engorgement. All MR scanning is performed at 1.5 Tesla, using surface abdominal coils and single-shot fast spin-echo T2-weighted 3-4 mm thick sections, with 1.1-1.25 mm in planar spatial resolution.

Results

We found 6 cases with DMV anomalies (tab.1). Most of the DMV engorgement is located at frontal lobes level.

Conclusions
From this limited preliminary series it appears that DMV involvement plays a role in the development of periventricular leukomalacia and periventricular hemorrhagic necrosis. The observation that these lesions are mostly located at frontal level may suggest that some of the term neonates carrying sequelae of atypically located leukomalacia (i.e. deep frontal lobe) might have developed these lesions in-utero. It is of interest to notice that most of our cases were related to heart failure. Therefore, central venous hypertension affecting immature deep cerebral venous system has to be taken into account.

Cases with DMV engorgement

Gestational age (weeks)	Pregnancy disorders	Prenatal MRI
23	Fetal heart failure. Parvovirus B19 infection	Periventricular bilateral hemorrhagic necrosis and edema
31	Fetal heart malformation and failure	Severe global brain edema, dural sinuses engorgement
22	Fetal heart malformation and failure	CSF cortical space enlargement, subcutaneous edema
24	Co-twin death in monochorionic pregnancy. In survivor heart malformation and failure	Periventricular bilateral hemorrhagic necrosis-ischemia, intraventricular hemorrhage
25	Fetal intracranial hemorrhage, anemia, heart failure. Placental abruption	Unilateral intraventricular hemorrhage, hydrocephalus
27	Severe IUGR, thorax hypoplasia	Brain edema, CSF cortical spaces obliteration, generalized veins congestion

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Response to Genetic Counseling after a Positive First Trimester Combined Screening Tests in Women Younger Than 35: The Boston Experience. Joaquin Santolaya-Forgas, Louise Wilkins-Haug. *Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA, USA.*

INTRODUCTION: In our center, patients with an estimated risk for chromosomal abnormalities at term greater that 1 in 220 after 1st trimester combined screening test (FT) are offered non-directive genetic counseling. The aim of this study was to evaluate the responses of women younger than 35 attending this genetic counseling session.

MATERIAL AND METHODS: Data from patients referred for a positive FT from September 1, 2003 to July 1, 2007 was retrieved from our database. Information concerning women younger than 35 years of age at the estimated date of delivery was extracted and tabulated.

RESULTS: During the study period 591 women had genetic counseling for positive FT. Thirteen patients were excluded from further analysis (8 had incomplete clinical documentation and 5 had spontaneous miscarriages prior to 16 weeks gestation). Four hundred and twenty-five patients were older than 35 and 153 were younger than 35 at the estimated date of delivery. **Table 1** depicts summary statistics for studied variables in this younger group of women.

CONCLUSIONS: Overall this data suggests that approximately 25% of this younger group of women opted for chorionic villous sampling (CVS), 40% for amniocentesis and more than 34% declined prenatal genetic testing. Moreover, this data also suggests that: 1) these women opted for CVS when the FT risk (mean = 1 in 58) almost doubled the CVS procedure related risk quoted at 1% and, 2) when the FT risk is between 1 in 100 and 1 in 220 almost half (53 out of 115) declined not only the 1st trimester CVS but also the 2nd trimester amniocentesis. We believe that understanding our patient population is important to optimize both the efficiency and efficacy of the alternative prenatal screening programs.

Acceptance for prenatal genetic testing after a positive first trimester combined screening test in women of less than 35 years of age

	CVS	Amniocentesis	Declined testing	Total
Number of cases (%)	38 (24.8)	62 (40.5)	53 (34.6)	153 (100)
Estimated Risk after FT	1 in 58	1 in 108	1 in 124	

FT= 1st trimester combined screening test. CVS = Chorionic villous sampling

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Prenatal Imaging in the Detection of Pulmonary Hypoplasia in Fetuses with Congenital Diaphragmatic Hernia: A Systematic Review and Meta-Analysis of Data. Ellen M Knox,[1] David Lissauer,[1] Khalid Khan,[2] Mark D Kilby.[1] *[1]Department of Fetal Medicine, Division of Reproduction and Child Health, Birmingham Women's Hospital, University of Birmingham, Birmingham, West Midlands, United Kingdom; [2]Division of Reproduction, Child Health & Epidemiology, Birmingham Women's Hospital, University of Birmingham, Birmingham, West Midlands, United Kingdom.*

Objective: To determine the optimal diagnostic test using prenatal ultrasonography and MRI for predicting pulmonary hypoplasia in fetuses with congenital diaphragmatic hernia.

Methods: Relevant papers were identified by searching MEDLINE (1966-2007), EMBASE (1988-2007) and the Cochrane Library (2004 issue 2). In addition, the specialist literature on the topic and reference lists were hand searched for relevant articles. Studies were selected if they examined diagnostic tests for the prenatal prediction of pulmonary hypoplasia in fetuses with congenital diaphragmatic hernia. The primary outcome measure was perinatal survival. Study selection, quality assessment and data abstraction were performed independently and in duplicate by separate observers.

Results: Of a total number of 8581 articles (published studies), there were eighteen studies that fulfilled the entry criteria. Six examined entirely unique heterogeneous parameters. Of the remainder, all 12 examined the ultrasound measurement of lung to head ratios (LHR) at a 'cut-off of greater than or less than the thresholds of 0.6, 1.0, 1.4, 1.6. In addition, the presence of liver in the fetal thorax was included (if present) as a co-variable (liver "up"). A LHR $\geq$1.0 compared to <1.0 provided the strongest association with perinatal survival (Peto OR 5.73, 95% CI 3.38-9.71). The finding of "liver up"in the fetal thorax had a negative association with survival (Peto OR 0.25, 95% CI 0.16-0.39). Only three studies provided data for LHR in conjunction with the presence of liver in the fetal thorax (Peto OR survival for LHR $\geq$ 1.0 compared to <1.0 was OR 7.65, 95% CI 3.42-17.10). Data was also available for liver up and LHR $\geq$ 1.4 which had a Peto OR of 2.2 (95% CI 0.83-5.82).

Discussion: The data supports the view that LHR may be a useful prognostic indicator of perinatal survival in fetuses with congenital diaphragmatic hernia. However, heterogeneity still exists regarding the timing of ultrasound measurement and the use of MRI. The majority of studies have small sample sizes.

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Non-Invasive Techniques To Screen for Fetal Anaemia: A Systematic Review and Meta-Analysis. Samantha J Pretlove, Khalid S Khan, Mark D Kilby. *Fetal Medicine, Birmingham Women's Hospital, Birmingham, West Midlands, United Kingdom.*

Objective:
To estimate, in fetal anaemia, the diagnostic value of fetal ultrasonography and Doppler blood flow in the evaluation of fetal anaemia

Methods:
Literature from 2000 to 2007 was identified using MEDLINE and EMBASE, the Cochrane Library and relevant specialist register of the Cochrane Collaboration, and by checking reference lists of known primary studies and review articles. Studies were selected if the accuracy of the fetal ultrasound parameters or Doppler studies of blood flow in the fetal vessels was estimated compared with a reference standard. Diagnostic tests evaluated were ultrasound measurement of the fetal spleen and liver length and Doppler studies from the umbilical vein and middle cerebral artery. Data from the selected studies were abstracted as 2x2 tables comparing the diagnostic test result with the reference standard. Results were pooled where appropriate. Diagnostic accuracy was expressed as sensitivity and specificity.

Results:
Twenty-nine primary studies were identified containing suitable data. Twenty-one of these gave data on middle cerebral artery Doppler peak systolic velocity (MCA-PSV) and 15 could be pooled in the meta-analysis giving a sensitivity of 0.75 (0.63-0.86) and a specificity of 0.89 (0.84-0.95) for 1078 cases in detecting severe anaemia. Four studies gave data on spleen perimeter and it was possible to pool three of these giving a sensitivity of 0.20 (0.10-0.35) and a specificity of 0.28 (0.18-0.41) for 112 cases. Three studies had data for liver length measurements and two were pooled. The sensitivity was 0.16 (0.05-0.36) and the specificity was 0.77 (0.46-0.95) but only 38 cases were used in the analysis. There were three studies on umbilical vein maximum velocity Doppler studies and all were suitable for meta-analysis giving a sensitivity of 0.73 (0.61-0.83) and a specificity of 0.54 (0.33-0.73) with 97 cases analysed.

Two studies gave data on middle cerebral artery time-averaged mean velocity score giving 52 cases. The sensitivity was 0.95 (0.83-0.99) and specificity was 0.85 (0.55-0.98).
Discussion:
Middle cerebral artery peak systolic velocity Dopplers remain the gold standard for non-invasive screening of fetal anaemia. Middle cerebral artery time-averaged mean velocity scores require further investigation. Other tests perform poorly when diagnostic accuracy is assessed.

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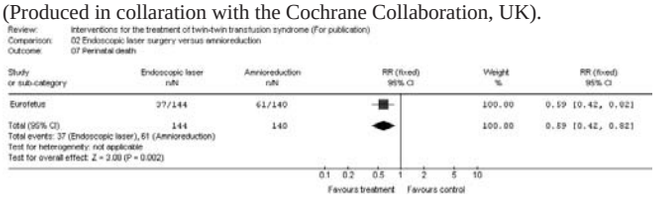
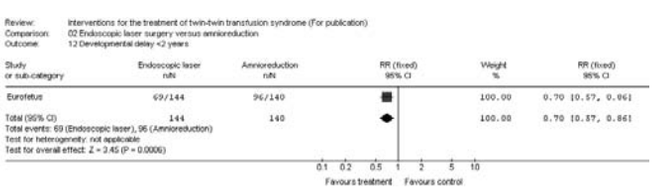
Cochrane Review of Treatment in Twin to Twin Transfusion Syndrome. Devinder Roberts,[1] James Nielson,[1] Mark D Kilby.[2] [1]*Department of Obstetrics and Gynecology, Liverpool Women's Hospital, University of Liverpool, Liverpool, North West, United Kingdom;* [2]*Department of Fetal Medicine, Bimringham Women's Hospital, University of Birmingham, Birmingham, West Midlands, United Kingdom.*
Background:
Twin-twin transfusion syndrome is a condition affecting monochorionic twin pregnancies associated with a high risk of perinatal mortality and morbidity.
Objectives:
The objective of this review was to evaluate the impact (maternal,fetal and pediatric)of treatment modalities in twin-twin transfusion syndrome.
Methodology: We searched The Cochrane Pregnancy and Childbirth Trials Register and Cochrane Controlled Trials Register. We also searched conference proceedings and made personal contact with experts active in the area of the review. Randomised and quasi-randomised studies of amnioreduction versus laser coagulation, septostomy versus laser coagulation or septostomy versus amnioreduction. Eligibility was assessed by one reviewer. Study authors were contacted for additional information.
Main results
Two studies were included. This review shows that laser coagulation of anastomotic vessels results in less fetal (RR 0.71; 95% CI 0.57, 0.89) and neonatal deaths (RR 0.30; 95% CI 0.17, 0.56) than amnioreduction (Figure 1). There is no difference in perinatal outcome between amnioreduction and septostomy. More babies in the laser arm are alive without neurological abnormality at six months of age (developmental delay at <2 years RR 0.70, 95% CI 0.57, 0.86)(Figure 2).
Conclusions: Endoscopic laser coagulation of anastomotic vessels should be considered in the treatment of all stages of twin twin transfusion syndrome to improve perinatal outcome. Further research on the effect of treatment on milder forms of twin twin transfusion syndrome (Quintero stage 1 and 2) are required.
(Produced in collaration with the Cochrane Collaboration, UK).





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Population-Defined Epidemiological Data of First Trimester Chorionic Villus Sampling Performed in the West Midlands Region of the United Kingdom during 2005. Ann Tonks,[1] Michael J Wyldes,[1] Mark D Kilby.[2] [1]*Congenital Anomaly Registry Team, West Midlands Perinatal Institute, Birmingham, West Midlands, United Kingdom;* [2]*Department of Fetal Medicine, Division of Reproduction & Child Health, Birmingham Women's Hospital, University of Birmingham, Birmingham, West Midlands, United Kingdom.*
Background: A recent systematic review investigating the world literature relating to procedure-related complications of chorionic villous sampling (CVS) concluded that although the risks of pregnancy loss are relatively low, lack of adequate controls tends to underestimate the true added risk of prenatal invasive procedures (Obstet Gynecol.2007;110(3):687-94).
Methods: The West Midlands is a large region within the UK containing approximately 12% of the total UK population. The Congenital Anomaly Register for this region is able to monitor pregnancy outcomes with accurate deminator data.
Results: There were 371 first trimester CVS performed, by ten operators, in the West Midlands (UK) in 2005. This equates to 5.4 procedures per 1000 births. Significantly higher rates were noted in areas of high socioeconomic status. The median number of procedures performed per operator was 35 (range 9-81). All operators were performing other invasive tests such as amniocentesis or cordocentesis,etc. The most common indication for CVS was: i) fetal anomaly on dating scan (24.25%) ii) abnormal (>1 in 300) risk on combined first trimester screening (22.4%) iii) molecular genetic diagnosis (18.1%) and iv) maternal request (35.25%). Using a combination of first trimester scanning and CVS, 33% had abnormal karyotype/structural anomaly.The corrected loss rate (background and procedure-related) following CVS in normally formed, singleton pregnancies was 3.6% (95thCI 1.1-6.0%) up to 7 days postnatal (perinatal loss) and 2.7% (95thCI 0.60-4.8%) with 28 days of procedure.
The proportion of CVS in which an adequate sample was not obtained was 1.1% (95th CI 0.0-2.1%).
Conclusions: This epidemiological study using accurate demoninator data provide interesting statistics relating to the uptake and prenatal risks of first trimester CVS.

West Midlands CVS 2005 : loss rates by gestation/outcome

Gestation	Crude %	Corrected %
Loss < 7 days	1.1 (0.0 - 2.3)	0.4 (0.0 - 1.3)
Loss < 14 days	1.8 (0.2 - 3.3)	0.9 (0.0 - 2.1)
Loss < 28 days	5.0 (2.4 - 7.5)	2.7 (0.6 - 4.8)

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Maternal Gastric Bypass and Fetal Rhizomelic Chondrodysplasia Punctata: A Case Report. V Sachar, J Moldenhauer, E Wang. *Dep't of OB/Gyn, Univ of Chicago, Chicago, IL, USA.*
With the increasing prevalence of obesity in the last two decades, we have seen a tremendous increase in bariatric procedures in reproductive aged women. Malnourishment and vitamin deficiency are common complications after gastric bypass which may impact on fetal development. We present the case of a 27 yo who underwent a duodenal switch procedure in 2005. She was discovered to be pregnant during evaluation for persistent malnutrition in 2006. Multiple prenatal ultrasounds were performed; the first at 15 weeks gestation was unremarkable. The 19 week sono revealed a male fetus with shortened femurs and humeri bilaterally, nasal bridge hypoplasia, macroglossia, poorly defined hands, and possible clubbed feet. Amniocentesis revealed a normal karyotype. 3D ultrasound redemonstrated the abnormal facial findings. A fetal echocardiogram was normal. The lagging long bone measurements continued to worsen, ultimately with femurs 6 weeks behind. The fetal thoracic circumference was two standard deviations below the mean, giving rise to concern for pulmonary sequelae. Growth restriction was noted at the 33 wk sonogram. Delivery by cesarean section was at 33 5/7 weeks secondary to nonreasuring fetal status. The birthweight was 1468gm; Apgars were 1, 4, and 6. Postnatal radiographs confirmed antenatal ultrasonographic findings and demonstrated evidence of epiphyseal stippling. The infant remained intubated until 12 weeks of life, after which it died secondary to respiratory complications associated with pulmonary hypoplasia. Gene mapping studies have not found any point mutations on the recessive gene as an etiology of this disorder. Rhizomelic chondrodysplasia punctata refers to a heterogeneous group of bone dysplasias with a familiar radiographic phenotype involving punctate calcifications and epiphyseal stippling. The etiology of this may be secondary to chromosomal abnormalities, mendelian gene disorders, or teratogens, notably warfarin. This case may be explained by vitamin K deficiency of the embryo due to maternal malabsorption after bariatric surgery. The maternal vit K level was <.03 ng/dl at time of delivery(normal > 0.10). The teratogenic effects of vitamin K deficiency in this instance highlight the need for strict counseling and screening for vitamin deficiency in those women undergoing bariatric surgery since previous obesity-related anovulation is reversed as patients lose weight, resulting in unexpected pregnancies and potentially preventable fetal abnormalities.

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Term Preeclampsia: Any Risk for the Neonate? Sindhu K Srinivas, Jamie Bastek, Christina M Andrela, Emmanuelle Pare, Michal A Elovitz. *Obstetrics and Gynecology; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*
Introduction: Preeclampsia continues to be a major contributor to maternal morbidity and mortality worldwide. Preeclampsia at term is not associated with

the same risk of neonatal morbidty and mortality as preterm preeclampsia . However, neonatal outcomes in term women with preeclampsia have not been adequately studied. We sought to compare short term neonatal outcomes in term infants born to women with and without preeclampsia.

Methods: This study was part of a large case control study. Women with preeclampsia (n=228) and term controls (n=563) were prospectively identified. Infants with congenital anomalies were excluded. Hospital length of stay (LOS), admission or transfer to the NICU, and use of mechanical ventilation or CPAP within first week of life were assessed. Associations between neonatal outcomes and preeclampsia were evaluated using chi-square analysis and Wilcoxon rank sum test. Significant confounders were controlled for using multivariable logistic regression.

Results: Discharge day of life was significantly different between neonates born to women with preeclampsia (median =2; mean =3.6) versus those born to women with uncomplicated term deliveries (median =2; mean =2.8, p<0.001). This difference persisted even when neonates with IUGR and those born to diabetic mothers were excluded (p<0.001). Term infants born to women with preeclampsia have a higher odds of being admitted or transferred to the NICU (AOR=2.16 [1.36-3.43], p=0.001) after controlling for IUGR, delivery mode, race, and gestational age at delivery. These infants also have a higher odds of mechanical ventilation (AOR=8 [1.43-44.6], p=0.018) and CPAP use (AOR=2.6 [1.23-5.32], p=0.012) after controlling for the same confounders. There was no difference in IVH or NEC between the two groups.

Conclusion: Neonates born to women with preeclampsia have differences in short-term morbidity when compared to neonates born to women without preeclampsia, despite being born at term. Whether this increase in neonatal morbidity is attributable to medications used in preeclampsia, such as magnesium sulfate, is unclear. These findings may have implications for patient counseling as well as hospital resource allocation. Further investigation correlating these findings with long-term morbidity is warranted.

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Could Confined Placental Mosaicism Account for Adverse Perinatal Outcomes in IVF Pregnancies? Benoit C Jacod,[1] GH Schuring-Blom,[2] KD Lichtenbelt,[2] JSE Laven,[3] D van Opstal,[4] MJC Eijkemans,[1] Nick S Macklon.[1] *[1]Reproductive Medicine & Gynaecology, University Medical Centre, Utrecht, Netherlands; [2]Clinical Genetics, University Medical Centre, Utrecht, Netherlands; [3]Obstetrics & Gynaecology, Erasmus Medical Centre, Rotterdam, Netherlands; [4]Clinical Genetics, Erasmus Medical Centre, Rotterdam, Netherlands.*

BACKGROUND: IVF singletons have poorer perinatal outcomes than singletons from spontaneous conceptions. This may be due to the influence of ovarian stimulation on the chromosomal constitution of the embryos which could be translated into localized chromosomal anomalies in the placenta. **AIM:** To compare the incidence of confined placental mosaicism (CPM) in IVF/ICSI pregnancies and spontaneous conceptions. **METHODS**: Multi-centre retrospective analysis of karyotype results obtained by chorionic villus sampling (CVS) performed because of advanced maternal age (≥36 years at 18 weeks of gestation) in the Netherlands between 1995 and 2005. **RESULTS**: From a total of 322246 pregnancies, 20885 CVS results were analysed: 235 in the IVF/ICSI group and 20650 in the control group. The mean age of women in both groups was 38.4 years (mean difference -0.08, 95% CI -0.35 – 0.18). Foetal karyotype was missing in 152 cases of possible CPM, all in the control group. When taking into account missing data, the incidence of CPM was lower in the IVF-ICSI group than in the control group, 1.3% vs. 2.2% (odds ratio 0.58, 95% CI 0.18 – 1.81) whereas the incidence of foetal chromosomal anomalies was increased 4.3% vs. 2.4% (odds ratio 1.82, 95% CI 0.96 – 3.46) although both differences are not significant. **CONCLUSIONS**: The incidence of confined placental mosaicism is not increased in IVF/ICSI pregnancies compared to spontaneous conceptions. CPM probably does not account for the adverse perinatal outcomes following IVF/ICSI.

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Fetal Rh D, Cc and Ee Genotyping Using Fetal DNA from Maternal Blood Is Not Impaired by the Presence of Maternal Alloimmunization. Chad A Grotegut,[1] Stacey L Jeronis,[2] John P Gaughan,[3] Enrique Hernandez,[2] Ossie Geifman-Holtzman.[2] *[1]Obstetrics and Gynecology, Duke University, Durham, NC, USA; [2]Obstetrics, Gynecology and Reproductive Sciences, Temple University, Philadelphia, PA, USA; [3]Biostatistics, Temple University, Philadelphia, PA, USA.*

Objective:
This study was conducted to assess the impact of maternal alloimmunization on the accuracy of fetal Rh D, Cc and Ee genotyping from maternal blood.

Methods:
We performed a literature search of English-written articles describing fetal Rh D, Cc and Ee determination from maternal blood (Am J Obstet Gynecol 2006;195:1163-73). Using this database, we determined the accuracy of Rh D, Cc and Ee genotyping in the presence of maternal alloimmunization. For each subgroup, 95% confidence intervals for a proportion were calculated and compared between groups.

Results:
We found 37 English-written publications reporting non-invasive RhD genotyping and 4 reporting non-invasive Rh CE genotyping from maternal blood. Fourteen (37.8%) of the Rh D articles and three (75%) of the Rh CE articles provided accuracy results in the setting of alloimmunization. The accuracy results are reported as follows:

	N correct	N total	Percent correct	95% CI
Rh D				
Alloimmunization	719	783	91.8	89.7, 93.6
Overall accuracy	2980	3261	91.4	90.4, 92.3
Rh Cc				
Alloimmunization	21	31	67.7	50.0, 81.5*
Overall accuracy	103	115	89.6	82.5, 94.0
Rh Ee				
Alloimmunization	39	41	95.1	83.0, 99.5
Overall accuracy	138	144	95.8	91.0, 98.3

For Rh Cc, only 67.7 % of the samples were determined correctly in the presence of alloimmunization.* This accuracy was significantly lower than the accuracy reported for all Rh Cc samples. When only studies utilizing free fetal DNA for Rh Cc genotyping were used (vs fetal cells), fetal Cc genotype was determined correctly in 11/11 (100%, 95% CI 70.0, 100), which was similar to the overall success rate for Rh Cc determination. Overall, there were no differences in the success of Rh D, Cc or Ee determination in the setting of alloimmunization compared to the overall accuracy seen when free fetal DNA was used.

Conclusions:
The presence of maternal alloimmunization does not reduce the accuracy of fetal Rh D, Cc or Ee non-invasive genotyping from maternal blood utilizing free fetal DNA. Further research into structure and rearrangements of the Rh D, Cc and Ee genes may further improve diagnostic accuracy of free fetal DNA from maternal blood.

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Detection of Aneuploidy Using Methylation-Sensitive DNA Amplification Followed by Oligonucleotide Microarray Analysis of Chromosome Copy Number. Lucia Y Brown,[1] Gregory Brown,[2] Stephen Brown.[1] *[1]Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA; [2]Pathology, Columbus Regional Hospital, Columbus, IN, USA.*

Background:
Fetal DNA, most likely of trophoblast origin, is present in both the plasma of pregnant women and provides a potential basis for non-invasive fetal diagnostic tests. However, fetal DNA in maternal plasma is highly contaminated with maternal DNA, and this contamination is the main technical challenge in trying to accomplish non-invasive detection of fetal chromosome abnormalities.

Methods:
Existing methods for the selective amplification of fetal DNA have generally relied on specific sequence differences between the mother and fetus. As an alternative, we have developed a method for selective amplification of fetal DNA that makes use of observation that trophoblast DNA is globally hypomethylated in comparison with DNA from other sources. In this method, a DNA mixture is first digested with a methylation sensitive restriction enzyme and then amplified by linker-mediated PCR. After an initial amplification, a second isothermal rolling-circle amplification is performed. This procedure results in the differential amplification of short, relatively hypomethylated fragments. After amplification, the resulting "representations" are comparatively hybridized to a microarray consisting of oligonucleotides that correspond to restriction fragments generated by the initial digest. Copy number differences are then detected through statistical analysis of array addresses that show significant amplification.

Results:
To test the feasibility of this method for detecting aneuploidy, we have prepared mixtures of peripheral blood DNA and first trimester trophoblast DNA from either normal or from samples with trisomy 18 and trisomy 21. We present data showing that aneuploidy can be detected even when 90% of the starting DNA sample was derived from a euploid source and only 10% was from an aneuploid trophoblast sample. Two different approaches to data analysis are presented. One relies on prior analyses of trophoblast methylation and the

second is independent of any prior knowledge or analysis. Both methods provide similar ability to detect aneuploidy. Future work will focus on testing whether this approach can be used for non-invasive prenatal diagnosis.

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Chromatin Immunoprecipitation and EMSA Analysis of NF-κB and C/EBP Synergism on the OTR Promoter. Shirin Khanjani, Yun S Lee, Vasso Terzidou, Mark R Johnson, Phillip R Bennett. *Institute of Reproductive & Developmental Biology, Imperial College, London, United Kingdom.*

OBJECTIVES:

We have shown, the transient transfections of the transcription factors NF-κBp65 and C/EBPβ leads to a synergistic increase in OTR promoter activity in human myocytes. This effect is mediated through a 20bp region of the promoter between -712 to -692 from TSS. We now report that this sequence binds both NF-κBp65 and C/EBPβ in vitro and ChIP studies show binding of both transcription factors to be increased by IL-1β in vivo.

MATERIALS AND METHODS:

EMSA studies were performed using a 33 bp oligonocleotide sequence (-712 to -672), containing the 20 bp region responsible for the synergistic activation of the OTR promoter and nuclear extracts from primary human myocytes treated with IL-1β 1ng/ml for 6 hours. For ChIP analysis, DNA protein complexes were crosslinked and antibodies recognizing P65, C/EBPβ and H4 (positive control) and IgG (negative control) were used for immunoprecipitation. Primers were designed to amplify the region -744 to -593, which includes the 20 bp response sequence. To further confirm the interaction between NF-κBp65 with C/EBPβ a 6xNF-κB consensus/LUC reporter construct was cotransfected with an expression vector for either NF-κBp65 or C/EBPβ.

RESULTS:

Specific NF-κBp65 and C/EBPβ binding was seen in the EMSA study. Pre-incubation with antibodies to NF-κBp65 and C/EBPβ led, not to supershift, but to elimination of DNA binding for both NF-kB p65 and C/EBPβ. ChIP analysis confirmed increased in vivo binding of NF-κB p65 and C/EBPβ to this region of the OTR promoter following stimulation with IL-1β.

Transfection of the NF-κB/Luc reporter construct with an expression vector for C/EBPβ caused a significant reduction in the basal promoter activity suggesting that C/EBPβ is binding to NF-kB. This interaction was further confirmed using a TF-TF interaction array.

CONCLUSION:

These data support the role of NF-κB and C/EBPβ in regulation of OTR. The 20 bp region contains a C/EBPβ but not a NF-κB DNA binding site suggesting that C/EBPβ primarily binds to this part of the OTR promoter but also interacts with NF-κB.

The EMSA data shows that the 20bp region binds both NF-κB and C/EBPβ. The loss of the supershift observed in previous EMSA studies suggests that the antibodies inhibit the interaction between C/EBPβ and NF-κB, therefore inhibiting DNA binding. ChIP analysis supports the concept that IL-1β leads to binding of NF-κB and C/EBPβ to the 20bp region.

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Regulation of Pro-Labour Genes by C/EBP, NF-κB and AP-1 in Human Uterine Myocytes. Suren R Sooranna,[1] Shirin Khanjani,[2] Yun S Lee,[2] Phillip R Bennett,[2] Mark R Johnson.[1] *[1]Imperial College Parturition Research Group, Imperial College, London, United Kingdom; [2]IRDB, Imperial College, London.*

Introduction: The transcription factors C/EBP (LAP), NF-κB (p65) and AP-1(c-Fos and c-Jun) are implicated in inflammatory processes such as parturition. The promoter regions of the pro-labour genes IL-8, PGHS-2 and OTR contain putative transcription factor-binding sites for these transcription factors. Our aim was to determine the effect of transfecting these transcription factors either alone or in combination into uterine myocytes and to determine their effects on the expression of pro-labour genes including IL-8, PGHS-2, OTR, connexin-43 and FP.

Methods: Primary cultures of human uterine myocytes (n=6) were grown from myometrial samples obtained at the time of elective LSCS. Cells were cultured in 24 well plates to 80% confluence at which point expression constructs for C/EBP (LAP), NF-κB(p65), AP-1 (c-Fos and c-Jun) were transfected either alone or in different combinations. The empty expression vector pSG5 was used as a filler construct. Cells were cultured for 24 and 72h after which culture medium was collected for ELISA and cells frozen at -80°C prior to RNA extraction. Copy numbers of IL-8, PGHS-2, OTR, FP, connexin-43 and GAPDH were measured by qPCR using a Rotor-GeneTM (Corbett Research).

Results: 24h post transfection with C/EBP (LAP), NF-κB (p65), c-Fos and c-Jun alone or in combination showed no significant changes in PGHS -2, OTR and connexin-43 expression. Over expression of p65 alone or together with either c-Fos or c-Jun increased FP expression by 56, 76 and 73% respectively (p=0.028). NF-κBp65 consistently increased IL-8 expression either alone (by 1108%; p=0.018) or in combination with LAP, c-Fos or c-Jun (by 1110, 1385 and 527% respectively; n=6; p=0,028). Rel A, LAP, c-Fos and c-Jun together also increased IL-8 expression (by 502%; p=0.018) and a small but significant increase was seen with a combination c-Fos and c-Jun (by 137%; p=0.018). The changes observed in IL-8 expression were reflected in the medium IL-8 concentration at 72h post transfection. In the presence of RelA and c-Fos IL-8 increased from 3.7 ± 0.2 to 71.0 ± 16.7pg/mL of culture medium (mean ± SEM; n=4).

Conclusions: NF-κB (p65) consistently increased FP and IL-8 expression in human myometrium. The data suggest that PGHS-2 activation has greater dependence upon other transcription factor(s) in addition to p65.

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True Identity of Myometrial PR-C: Fact or Fiction? Yun S Lee, Suren R Soorana, Mark R Johnson, Jan Brosens, Phillip R Bennett. *Imperial College Parturition Research Group, Hammersmith Campus, London, United Kingdom.*

Progesterone is thought to be central to maintenance of pregnancy. Multiple progesterone receptor (PR) isoforms underlie complex and diverse biological action of progestins. Previously two human PR isoforms have been identified: PR-B and PR-A. PR-A is N-terminally truncated form of PR-B (initiation site methionine 165). In some cells PR-A inhibits PR-B. It has been proposed that increased expression of PR-A in myometrium underlies a 'functional progesterone withdrawal'. The breast cancer cell T47D contains a 60 kDa progestin-specific binding protein that is not found in PR negative cells. It was proposed that there is a downstream methionine (Met595) which serves as a translation initiation site for the generation of a PR isoform of 60 kDa. Based on such findings Condon et al (Mol Endo 2006) have focused on the possible role of 60kDa PR-C in human parturition. They found that expression of "PR-C" using PR antibody (sc-538 Santa Cruz, SC) is increased in upper segment myometrium with labour and that overexpression of this protein inhibits PR-B function.

We cloned the same human PR cDNA into pSG5 expression vector. In vivo translation produced a protein of only 39kDa. Furthermore overexpression of PR-C595 did not significantly decrease PR-B activity in human myocytes. We examined other downstream initiation sites, which may produce a 60 kDa protein. We constructed potential PR isoforms in pSG5 vector with initiation sites at Met 289 and 301. In vivo translation produced proteins of approximately 70 and 62 kDa respectively. To determine the effect of PR isoforms on PR-B function, myocytes were co-transfected with PR-A, PR-C289, PR-C301 and PR-C595 with constant amounts of PR-B. The progesterone dependent PRE/LUC was used as reporter. Unlike PR-C595 both PR-C289 and PR-C301 significantly inhibited ligand dependent PR-B mediated transcriptional activity. We found in Western analysis that the antibodies PGR-312 (NC) and the sc-539 (SC) detected both endogenous and overexpressed PR-A and PR-B but none of the PR-C isoforms. The sc-538 antibody detected only PR-A and PR-B very poorly.

Our data suggests that the sc-538 antibody would not detect any PR-C protein and that none of the commercially available antibodies in the UK do so. Great care needs to be taken when over-expressing PR isoforms to ensure that proteins are of the expected size. If PR-C does exist in vivo then the 60kDa but not the 39kDa isoform might inhibit PR-B function.

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Tumer Necrosis Factor alpha (TNFα) Stimulates Matrix Mentalloproteinase-9 (MMP-9) Production in Cultured Human Chorion Trophoblast Cells through TNFα Receptor 1 Signalling to IKKβ, ERK1/2, NF-kB. Wei Li, John RG Challis. *Dept. of Physiology and Obstetrics & Gynecology and Medicine, University of Toronto, Toronto, ON, Canada.*

Objective: Previous studies have shown that TNFα induces production of MMP-9. That is critical for membrane rupture and later events in the parturition cascade. However, the TNFα signalling cascades involved in TNFα-induced MMP-9 production in human chorion trophoblast (CT) are unknown. **Methods:** Purified human chorion trophoblast cells were cultured in the presence of antibodies or chemical inhibitors that specifically block/inhibit distinct TNF receptors and kinase pathways. The media and cell protein were used for zymography and western blot analysis. **Results:** TNFα-induced MMP-9 production (analyses by zymography) was significantly blocked/inhibited by

TNF receptor 1 antibody (TNF RI ab), NF-κB inhibitor (NF-κB activation inhibitor) and ERK inhibitor (U0126) ($p<0.01$), but not by TNF receptor 2 antibody (TNF RII ab), p38 MAPK inhibitor (SB203580) and JNK inhibitor (SP600125). By western blot analysis, we found that the protein level of TNF receptor associate factor 2 (TRAF2) was higher than that of TNF receptor associate factor 1 (TRAF1) (TRAF2>TRAF1) in untreated CT cells. However, after TNFα treatment for 4h to 24h, TRAF1 protein level was increased, but TRAF2 protein level was reduced (TRAF1>TRAF2). The increase of TRAF 1 and decrease of TRAF2 were blocked by TNF RI ab, but not by TNF RII ab. We also found that TNFα rapidly (within 5-30 min) and significantly increased phosphorylation of IKKβ, ERK1/2 and JNK1/2/3 and the phosphorylation of these protein kinases by TNFα was reduced significantly by TNF RI ab, but not by TNF RII ab. Moreover, we found that the changes of increased TRAF1 and decreased TRAF2 in CT cells (TRAF1>TRAF2) resulted in a dramatic deficiency in phosphorylation of the above protein kinases induced by TNFα compared with the normal CT cells (TRAF2>TRAF1). Nuclear localization of NF-κB p65 in TNFα treated cells was increased compared to untreated controls.
Conclusion: We have demonstrated for the first time that TNFα stimulates MMP-9 production in CT cells through TNF RI-TRAFs-IKKβ/ERK-NF-κB signaling pathways, but not through the JNK/p38-AP-1 pathway. These studies reveal steps within this pathway as possible therapeutic targets to inhibit MMP-9 expression potentially attenuating TNFα-induced degradation of extracellular matrix and pre-term rupture of the fetal membranes.

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Activation of the Fibrinolytic Cascade Is Elevated among Women with Spontaneous Preterm Birth. Janet M Catov,[1,2] James M Roberts,[1,2] Hyagriv N Simhan.[1] [1]*Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA;* [2]*Magee-Womens Research Institute, Pittsburgh, PA, USA.*
Objective: Women with preterm birth are at elevated risk of cardiovascular disease, but mechanisms that might relate these conditions are not understood. We hypothesized that women with spontaneous preterm vs. term births may have early gestation evidence of activation of the fibrinolytic cascade, as measured by the thrombin-antithrombin III (TAT) complex. We also tested if this relation may be associated with inflammation.
Methods: TAT was measured in plasma collected <21 weeks gestation (mean 10.2 weeks, SD 3.9) among women without chronic medical conditions, preeclampsia or growth restriction who delivered singleton liveborn infants. Inflammation was assessed by C-reactive protein (CRP) measured in serum from the same samples. Women with spontaneous preterm birth (sPTB) <34 weeks (n=32) and 34-<37 weeks (n=72) were compared to women with term births >=37 weeks (n=215). High TAT was defined as >5.2 ng/ml (highest quartile among women with term births) and high CRP was defined as >=8 ug/ml. Multinomial logistic regression was utilized to relate elevated TAT and inflammation to risk of sPTB subtypes.
Results: Women with sPTB were more likely to have TAT concentrations in the highest quartile compared to women with term births (<34 weeks, 53.1%; 34-<37 weeks, 30.7%; >=37 weeks 24.11%, $p<0.01$). Women with high TAT concentrations had a 3.3-fold (95% CI: 1.5-7.3) increased risk for sPTB <34 weeks, after adjustment for body mass index, race, age and gestational age at sampling. There was no relation between high TAT and sPTB 34-<37 weeks (OR 1.2, 95% CI 0.7-2.3). Additional adjustment for elevated CRP (>=8ug/ml) did not effect the estimates associated with TAT, and elevated CRP was independently related to risk for both sPTB subtypes (<34 weeks, OR 2.9 [1.1-8.0]; 34-<37 weeks, OR 2.7 [1.3-5.6]). Thus, women with high TAT and elevated CRP appeared to be at particularly elevated risk of sPTB <34 weeks (OR 8.3, 95% CI 1.8-38.1).
Conclusions: The thrombin-antithrombin III complex was elevated early in gestation among women with sPTB <34 weeks, perhaps secondary to microvascular injury. This effect was independent of inflammation, suggesting that the elevated fibrinolytic cascade may function independently from inflammation among women with sPTB.

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Plasma Cortico-Releasing Hormone and Cortisol Concentrations and Psychological Stress among Pregnant Women. Katherine P Himes, Hyagriv N Simhan. *Obstetrics and Gynecology, University of Pittsburgh Medical Center, Magee Womens Hospital, Pittsburgh, PA, USA.*
Objective: Many studies have found an association between psychological stress and preterm birth. We sought to determine if women with greater psychological stress during pregnancy had higher concentrations of plasma cortico-releasing hormone (CRH) or cortisol.
Study Design: This is a secondary analysis of a multicenter case-control study, nested within an observational cohort. Of 3,073 participants, plasma CRH and cortisol concentrations at 24 and 28 weeks gestation were available in 178 controls who delivered after 37 weeks and 159 cases who delivered before 37 weeks. The Abbreviated Scale for the Assessment of Psychosocial Status in Pregnancy (ASAPS) was available for all women. Concentrations of CRH and cortisol were compared between women above and below the lowest quartile score on the ASAPS among cases and controls. The same analysis was done for the portion of the scale related to psychological stress. Concentrations of CRH and cortisol and psychological stress were also compared between black and non-black cases and controls. Univariate analysis was performed with Kruskal Wallis or Chi-square.
Results: There was no difference in CRH or cortisol concentrations at 24 or 28 weeks among women above or below the lowest quartile on the ASAPS (controls:p=0.17-0.66 cases:p=0.47-0.97). Greater psychological stress was not associated with higher concentrations of CRH or cortisol at 24 or 28 weeks(controls:p=0.33-0.63 cases:p=0.17-0.78). CRH concentrations were not different between blacks and non-blacks. Among both cases and controls, cortisol concentrations at 24 and 28 weeks were lower in black women than non-black women (controls:$p<0.01$ cases:$p< 0.01$). The median cortisol concentration among control black women was 11.0 g/ml at 24 weeks compared to 14.4 g/ml among non-black women and 14.4 g/ml compared to 18.6 g/ml at 28 weeks. Black women reported less psychological stress than non-black women ($p= 0.001$)
Conclusion: We found no relationship between psychological stress and plasma CRH or cortisol. Furthermore, while stress is hypothesized to play a role in the racial disparity of preterm birth, black women reported less psychological stress and had lower cortisol concentrations than non-black women. Improved assessments of psychological stress and additional biomarkers involved in the stress response may broaden our understanding of how stress contributes to preterm birth.

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Expression, Tissular Traffic and Activation of MMP-9 in Human Fetal Membranes during Labor. Rodrigo Vega-Sanchez, Arturo Flores, Marisol Castillo, Nardhy Gomez, Felipe Vadillo-Ortega. *Direction of Research, Instituto Nacional de Perinatologia, Mexico City, DF, Mexico.*
Introduction. Rupture of the fetal membranes (FM) during human labor occurs as a consequence of extracellular matrix degradation. This process is controlled by increased secretion and activity of matrix metalloproteinases, particularly MMP-9.Several evidences suggest that MMP-9 is mainly produced by infiltrating choriodecidual leukocytes that could arrive from placental circulation. Characterization of the synthesis, transport and activation of MMP-9 within the FM is critical to understand the process of membrane rupture during human labor.
Objectives. Expression and secretion of MMP-9 in placental leukocytes, trafficking of the enzyme through the FM and one possible mechanism for its activation were analyzed.
Methods. Leukocytes were isolated from placental blood of women after active labor. Maternal leukocytes were used as controls. Cells were cultured for 96 h. Relative expression of MMP-9 by RT-PCR and enzyme secretion by ELISA and zymography were followed at different times. To analyze the traffic of MMP-9 through the FM, fluorescein-conjugated proMMP-9 was added to the choriodecidual side of the FM in an *in vitro* system that allows the separation of amnion and chorion. Labeled MMP-9 was localized at distinct times by confocal microscopy. The protease responsible for the activation of MMP-9 was identified using neutralizing antibodies and specific inhibitors.
Results. No difference in the relative expression of MMP-9 in leukocytes throughout the culture was found. However, secretion of the enzyme significantly increased since 24 h ($p<0.05$). Experiments using labeled MMP-9, repeatedly showed that after 12 h in culture, the enzyme was mainly localized within the amniotic epithelium. Specific inhibition of MMP-3 significantly decreased the activation of pro-MMP-9.
Conclusions. Our results demonstrate that the increased secretion of MMP-9 by placental leukocytes is not associated to increased gene expression, suggesting that homing of a specific leukocyte subpopulation to the choriodecidua is occurring during labor. MMP-9 can be trafficked from the choriodecidua to the amnion, suggesting a transmembranal pathway that may regulate the tissular localization of the enzyme to the area of the FM with high content of connective tissue. Once secreted by the placental leukocytes, activation of MMP-9 depends mainly on MMP-3, which seems to be derived from the same leukocytes.

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Histone Deacetylase Inhibitors and an Inhibitory Effect on Human Uterine Contractility. Mark P Hehir,[1] Aidan M Sharkey,[1] Audrey T Moynihan,[1] GN Europe-Finner,[2] Stephen C Robson,[2] John J Morrison.[1] [1]*Department of Obstetrics and Gynaecology, National University of Ireland, Galway, Ireland;* [2]*School of Surgical and Reproductive Science, University of Newcastle Upon Tyne, United Kingdom.*

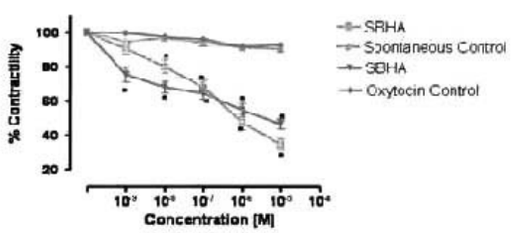
Objective: Preterm labour is a major problem in terms of perinatal morbidity and mortality. The histone-deacetylase inhibitor (HDACi), Trichostatin A (TSA) has been shown to have an inhibitory effect on myometrial contractility. The aim of this study was to evaluate the effect of the HDACi's Suberic bishydroxymate (SBHA) and Valproic Acid (VPA) on human uterine contractions and hence their potential role as tocolytic agents.

Methods: Biopsies of human myometrium were obtained at elective caesarean section (n=18). Dissected myometrial strips suspended under isometric conditions, undergoing spontaneous and oxytocin-induced contractions, were exposed to cumulative additions of SBHA in the concentration range of 1 nmol/L to 100 mmol/L and VPA (100nmol/L-1mmol/L). Control experiments were run simultaneously.

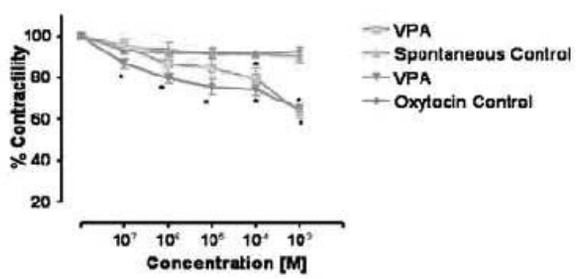
Results: SBHA and VPA exerted a potent and cumulative inhibitory effect on spontaneous and oxytocin-induced contractions, compared to control strips. The mean maximal inhibition (MMI) values for SBHA were 65.35% for spontaneous contractions (n=6; P< 0.05), and 53.53% for oxytocin-induced contractions (n=6; P<0.05). The MMI values for VPA were 36.57% for spontaneous contractions (n=6; P< 0.05), and 35.20% for oxytocin-induced contractions (n=6; P<0.05).

Conclusion: These results raise the possibility that HDACi's may have tocolytic potential, in addition to their current clinical indications. The inhibitory effect observed may be linked to the ability of HDAC inhibitors to induce the expression of genes involved in the maintenance of myometrial quiescence via epigenetic mechanisms but may potentially also involve non-epigenetic pathways.

Effects of Suberic bishydroxamate (SBHA) on spontaneous (green) and oxytocin-induced (blue) myometrial contractility



Effects of Valproic acid (VPA) on spontaneous (green) and oxytocin-induced (blue) myometrial contractility



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Progestin Suppresses thrombin-Enhanced Interleukin-6 Expression in Term Decidual Cells: Implications for Abruption-Induced Preterm Delivery. Edward Kuczynski, Lynn F Buchwalder, Frederick Schatz, Charles J Lockwood. *Obstetrics/Gynecology & Reprod. Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Background and Objective: Decidual hemorrhage (abruption) promotes binding of Factor VII to decidual cell (DC)-expressed tissue factor to generate thrombin. Thrombin in turn induces several biological effects leading to preterm delivery via activation of cell surface protease-activated receptors (PARs). Interleukin-6 (IL-6) is a pleiotropic proinflammatory cytokine induced by PARs. This study assessed the separate and interactive effects of thrombin and medroxyprogesterone acetate (MPA) on IL-6 expression in term DCs.

Methods: Term decidua from stripped fetal membranes were isolated and the DCs were purified on a Percoll gradient, grown to confluence and passaged until leukocyte-free. Confluent DCs were primed in 10^{-8}M estradiol (E2) of E2 +10^{-7}M MPA for 7 days, then incubated in a defined medium (DM) with corresponding steroids ± thrombin. After 24 hours, IL-6 levels in conditioned DM were measured by ELISA and Western blotting. In parallel 6-hour incubations, IL-6 mRNA levels were assessed by quantitative RT-PCR and normalized to β-actin mRNA.

Results: Secreted IL-6 levels were similar in cultures maintained in E2 alone (0.43 ± 0.14) and E2 + MPA (0.33 ± 0.06 pg/ml/ug protein; mean ± SEM; n = 9). The addition of thrombin (2.5 U/ml) enhanced secreted IL-6 levels by 14.3 ± 2.4 fold ($p<0.05$) in incubations with E2 and by 8.2 ± 1.4-fold ($p<0.05$) in incubations with E2 + MPA. The inhibitory effect of MPA was statistically significant ($p<0.5$). In confluent DCs incubated with E2 + MPA, exogenous thrombin (0.05-2.5 U/ml) elicited a concentration-dependent increase in secreted IL-6 levels. Hirudin acted as a pure thrombin antagonist, exerting no agonist effects alone, but counteracting thrombin-enhanced IL-6 secretion. Western blotting confirmed the ELISA results. Quantitative RT-PCR confirmed that IL-6 m-RNA levels corresponded to protein changes.

Conclusion: Thrombin enhances IL-6 mRNA and protein expression in term DCs and progestin blunts these effects. Since thrombin-generating abruption is closely associated with preterm delivery, anti-inflammatory effects induced by progestin inhibition of DC-derived IL-6 may contribute to the protection against PTD, and may explain the reported protective effects of administration of 17α-OH-progesterone in recent clinical trials.

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Inhibition of Catechol-O-Methyl Transferase Activity Dramatically Reduces PGE2 Production by Human Amniotic Explants. Melissa J Wentz,[1] Hassan M Harirah,[1] Ayman Al-Hendy.[1,2] [1]*OB/Gyn, UTMB, Galveston, TX, USA;* [2]*Present affiliation: Dep of OB/Gyn, Meharry Medical College, Nashville, TN, USA.*

Introduction: Catechol-O-methyltransferase (COMT) catalyzes the methylation of the phenolic hydroxyl groups in a variety of catechols. During estrogen metabolism, this enzyme converts the catechol estrogen, 2-hydroxyestrogen (2OHE2), to 2-ethoxyestrogen (2MeOHE2). The COMT substrate, 2-OHE2, can exhibit an anti-estrogenic effect in multiple biologic assays while the 2-methoxyestrogen (2-MeOE2) can exhibit an estrogenic effect. The biologic activities of these estrogen metabolites (2OHE & 2MeOE) depend upon their concentrations and tissue type. Since COMT activity ultimately controls levels of these metabolites, it appears to be a key factor in regulating the cellular estrogenic milieu. We have recently reported that amnion layers of human fetal membranes from laboring women exhibited 3 folds higher COMT mRNA expression when compared to non-laboring women (Wentz et. al. SGI 2007).

Objective: To investigate the impact of COMT inhibition on prostaglandin E2 (PGE2) production by human amniotic membrane explants.

Study Design: Explants consisting of 2-cm circular sections of the amnion layer (obtained from term pregnant women who underwent elective repeat cesarean section) were prepared and placed in tissue culture explants media at 37ºC. After a 4-hour incubation, explants were treated with the selective COMT inhibitor RO 41-0960, at 10 and 50 μM concentrations. The incubation media was harvested after 6 and 24-hour intervals. The levels of prostaglandin E2 (PGE2) in the media were measured by sensitive ELISA and were normalized against total protein concentration. **Results:** RO 41-0960 COMT inhibitor induced major reductions in PGE2 production in media collected from amnion explants of human fetal membranes. In the group treated with 10 μM of RO 41-9640 for 24 hours there was 72%±8% reduction of PGE2 after 24 hours compared to untreated control ($P<0.001$). In the amnion explants treated with 50 μM of RO41-9640, there was 74%±9% after 6 hours and 84%±11% after 24 hours of treatment compared to untreated control ($P<0.001$). **Conclusions:** This finding indicates that COMT activity in the amnion layer of human fetal membranes affects PGE_2 production. By facilitating a pro-estrogenic milieu in human fetal membranes in late gestation, increased COMT activity may indirectly increases production of factors associated with labor such as PGE_2.

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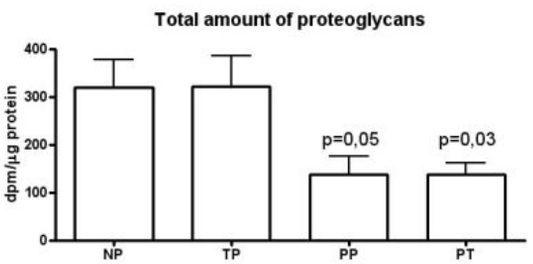
Proteoglycan Characterization of Human Cervical Fibroblasts from Non Pregnant, Term Pregnant, Partal and Preterm Partal Women. Anna E Holmberg,[1] Aurelija Dubicke,[2] Maria Sennstrom,[2] Gunvor Ekman-Ordeberg,[2] Anders Malmstrom.[1] [1]*Department of Medicine, Department of Experimental Medical Science, Lund, Sweden;* [2]*Department of Woman and Child Health, Department of Obstetrics and Gynecology, Stockholm, Sweden.*

Hypothesis: An extensive remodeling of the human cervical connective tissue takes place throughout pregnancy with a decrease in the total concentration of collagen and proteoglycans due to an altered higher metabolic turnover. We hypothesize that the profound changes in proteoglycan production in the human pregnant cervix can be seen in corresponding cervical fibroblasts as well. We also hypothesize that proteoglycan production in cervical fibroblasts from preterm partal women are simmilar to the production in fibroblasts from partal women.

Method: Cervical biopsies were obtained from 5 non-pregnant women, 5 women during elective cesarean section, 6 woman after spontaneous parturition and 4 after a preterm vaginal delivery. By explant technique fibroblasts were cultured from the biopsies. Produced proteoglycans were metabolically labeled with S^{35} during 24 hours and then purified by ion-exchange chromatography and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Results: The total proteoglycan production decreases with approximately 50% in partal and preterm partal cell cultures. The reduction of proteoglycans in preterm partal and partal cell cultures is significant compared to non pregnant fibroblast cultures. The distribution of biglycan and perlecan are similar in partal and preterm partal cells. Biglycan are significantly reduced by around 40% and perlecan are significantly increased by around 60% compared to non pregnant cultures. Preterm partal cervical fibroblasts secreates significantly more heparan sulfate proteoglycans compared to non pregnant cultures.



Conclusion: The changes in total proteoglycan production in the human pregnant cervix can be seen in corresponding cervical fibroblasts as well. Both partal and preterm partal cell cultures differ in their proteoglycan production compared to their non pregnant counterpart, suggesting a role for proteoglycans in cervical ripening.

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Oxytocin Receptor Expression and Function in Human Amnion. Vasso Terzidou,[1] Salwa Taher,[1] Sheri Lim,[1] Yun S Lee,[1] Shirin Khanjani,[1] Steven Thornton,[2] Philip R Bennett.[1] [1]*Parturition Research Group, Institute of Reproductive and Developmental Biology, Imperial College, London, United Kingdom;* [2]*Department of Biological Sciences, University of Warwick, Conentry, United Kingdom.*

BACKGROUND:

The role of the oxytocin/oxytocin receptor system is not well defined in human amnion. Previous studies in rabbit amnion have demonstrated an up-regulation of oxytocin receptors in the end of pregnancy and have shown that there is a large increase in the ability of OT to stimulate PGE_2 production. We and others have previously shown a role for NF-κB in OTR regulation. In whole genome array analysis of human amnion we found that OTR was the gene with the second highest increase associated with activation of NF-κB (the highest being COX-2). The present work was directed towards further understanding of OTR expression and function in human amnion at term.

METHODS & RESULTS:

We have shown that PGE_2 release by pre-labour primary human amnion cells is significantly increased after oxytocin treatment for 6 hrs (OT: 10-7 M; n=3; triplicate samples; 40 fold increase $p<0.05$).

The expression of OTR in labour (+) and labour (-) primary amnion cells was measured with real time RT-PCR. We found a significantly higher level of expression in the labour (+) cells (n=3; duplicate samples; $p<0.001$). Western blot analysis confirmed the upregulation of OTR in labouring amnion.

Treatment with IL-1b resulted in a significant upregulation of OTR which peaked with a 17 fold induction after 6 hours ($p<0.001$). IL-1β caused a 16 fold increase in OTR mRNA levels in labour (–) cells, bringing the expression level up to that found in labour (+) cells.

CONCLUSION:

Our findings provide further evidence for a role of OTR in human amnion. Expression of OTR in human amnion is significantly increased after the onset of labour. Term non laboured amnion can be stimulated with IL-1β to increase OTR expression to levels of laboured amnion. One of the functions of OTR in amnion cells is stimulation of prostaglandin synthesis.

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Expression of Antioxidant Defence Proteins in Human Myometrium before and after the Onset of Labour. Vasso Terzidou, Mandeep S Kandola, Shirin Khanjani, Jan J Brosens, Phillip R Bennett. *Parturition Reserach Group, IRDB, Imperial College, London, United Kingdom.*

Background

Oxidative stress is a result of an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defence mechanisms present in biological systems. Parturition and infection-induced preterm labour resemble inflammatory processes that are linked to the production of ROS including super-oxide (O_2^-), hydrogen peroxide (H_2O_2) and peroxynitrite. Low concentrations of ROS can act as second messengers in the regulation of several cellular functions. In an attempt to maintain redox homeostasis cells are equipped with machineries to both produce and scavenge ROS. Enzymatic scavengers include superoxide dismutase (SOD), glutathione peroxidise and catalase. SOD is the first enzymatic step in the defence system against oxidative stress.

Methods and Results

Nuclear factor-kappa B (NF-κB), a transcription factor family classically associated with inflammation, is activated in response to infection and pro-inflammatory cytokines, such as those prevalent during labour. Labour is associated with an increase in NF-κB activity in human myometrium and in fetal membranes. NF-κB is known to regulate a range of genes associated with the onset of labour. In a study using Affymetrix whole genome arrays analysis NF-κB overexpression was associated with increased expression of SOD-2 (3.5 fold). To determine changes in ROS scavenging potential upon onset of labour, lower segment myometrial biopsies were taken at term before and after the onset of labour (n=7; each group). cDNA was extracted from the tissues and real time RT-PCR was performed for SOD-2, serum/glucocorticoid-induced protein kinase-1 (SGK-1) and the DNA repair enzyme GADD45a. We found that labour onset is associated with a two fold increase in the levels of expression of each.

Conclusion

Oxidative damage at the fetomaternal interphase has been extensively studied in the placenta as part of investigations for first trimester pregnancy losses, IUGR and pre-eclampsia. The role of ROS is less well defined in human decidua and the underlying myometrium. Our results suggest a role for oxidative stress and redox homeostasis in the maintenance of myometrial quiescene during pregnancy and onset of labour.

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Plasma Anandamide Levels Increase during Labour Induction and Appear to Delay Labour Progression. Vijianitha Nallendran, Anthony H Taylor, Patricia MW Lam, Stephen C Bell, David J Taylor, Justin C Konje. *Cancer Studies and Molecular Medicine, University of Leicester, Leicester, United Kingdom.*

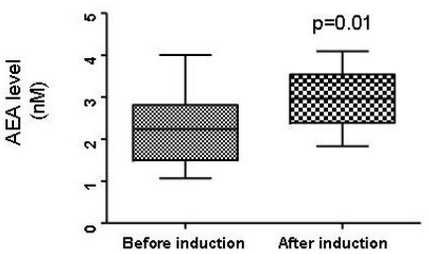
Background: The evidence for a role of the endocannabinoid, anandamide (AEA) in labour is conflicting. We previously showed elevated AEA plasma levels in labouring women[1] whilst another group showed that activation of functional receptors for anandamide actually inhibited uterotonin-induced contractions through an adenylate cyclase pathway[2]. The aim of this study was to explore further the relationship between labour and plasma AEA levels.

Methods: Plasma AEA levels in 20 women undergoing induction of labour for various indications at term, were measured by a sensitive isotope dilution method using HPLC- MS/MS. Each volunteer had an assessment of her cervix prior to the start of the induction when the first sample for AEA was collected. Once active labour was established (cervix ≥3cm dilated; contractions every 3-4 minutes), a second sample was collected.

Results: Seventeen (85%) of the subjects were multigravida. The inductions were for postdates(n=7); decreased fetal movements(n=3), fetal growth restriction(n=2), symphysis pubis dysfunction(n=2), diabetes mellitus(n=1), pre-eclampsia(n=1), fetal cardiac complication(n=1), spontaneous rupture of

fetal membranes(n=1). Plasma AEA levels increased significantly once labour was established (Figure) and demonstrated in 19 (95%) of the 20 cases. The median (interquartile range) plasma AEA level increased from 1.35nM (0.99-1.61) at induction to 2.24nM (1.49-2.82); *P=0.01; Mann-Whitney U-test) at active labour. There was a positive correlation between plasma anandamide levels taken before induction and the time taken for the women to enter into active labour (r^2=0.41, P=0.06 Pearson correlation).

Comparison of Plasma AEA levels During Induction



Conclusion: Plasma AEA levels were higher in labouring women compared to non-labouring women after the induction of labour confirming our previous observations and suggesting a direct role for this endocannabinoid in labour. Further studies are required to elucidate this role.

References:

1. Habayeb, OMH, *et al.*, (2004) J. Clin. Endocrinol. Metab.**89:** 5482-7.
2. Dennedy, MC, *et al.*, (2004) Am. J. Obstet. Gynecol.**190:** 2-9.

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Affymetrix GeneChip Microarray analysis of Myometrial Genes Regulated by NF-κB. Shirin Khanjani, Sheri Lim, Yun S Lee, Mark R Johnson, Phillip R Bennett, Vasso Terzidou. *Parturition Research Group, Institute of Reproductive and Departmental Biology, Imperial College, London, United Kingdom.*

Background

Nuclear factor-kappa B (NF-κB) is a transcription factor family classically associated with inflammation, activated in response to infection and pro-inflammatory cytokines, such as those prevalent during labour. As a cytokine-inducible transcription factor it plays a key role in the expression of a variety of genes involved in inflammatory responses and cell survival. NF-κB DNA binding and transcriptional activity plays an important role in labour associated gene expression in myometrium. In this study we examined the range of genes regulated by NF-κB in myometrium using transient transfections and whole-genome array analysis.

Materials & Methods

Myometrial cells were extracted from myometrial biopsies taken at the time of elective caesarean sections at term. Transient transfections of primary myocytes were performed using expression vectors for NF-κB p65.The amount of DNA was constant and pSG5 was used as a control construct. cDNA was made from myocytes transfected with either NF-κB or pSG5. Affymetrix GeneChip U133 microarray was performed (n=3, each group).

Results

We found that 33 genes were significantly differentially expressed between control and overexpressed NF-κB samples. Twenty eight of these genes were upregulated with NF-κB and 5 were down regulated. Several chemokines and cytokines were identified in the upregulated group. Interleukin 8 demonstrated the highest, 18 fold, induction in the upregulated group, followed by tumor necrosis factor, a-induced protein (TNFAIP3), chemokine ligand 2 (CCL2), chemokine ligand 5 (CCL5), pentaxin related gene (PTX3), interleukin 6 (IL6) and superoxide dismutase (SOD-2). Nine genes were present in the NF-κB group that were absent in the control group. These included chemokine ligand 20 (CCL20), chemokine ligand 11 (CCL11), chemokine ligand 2 (CXCL2) and IL1β. Ingenuity pathway analysis demonstrated that immune response, inflammatory, cell growth and proliferation and cell death were the main pathways involved. Standardisation experiments have been performed, and the microarray results were confirmed with real time RT-PCR in several candidate genes.

Conclusion

Our results provide further support in the role of NF-κB in human labour and suggest its direct link in upregulation of inflammatory genes, cytokines and chemokines, consistent with the imflammatory nature of the biochemistry of labour.

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Nuclear Localization of Secretory Leukocyte Protease Inhibitor in Human Myometrium. Richard R Lee,[1] Aimin Li,[1] Matthew Blitz,[1] Parviz Minoo,[2] Juan C Felix,[1,3] Thomas M Goodwin.[1] *[1]Department of Obstetrics and Gynecology, The Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; [2]Department of Pediatrics, The Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; [3]Department of Pathology, The Keck School of Medicine, University of Southern California, Los Angeles, CA, USA.*

Objectives:

Inflammation is widely accepted to be a key feature of human labor. Secretory leukocyte protease inhibitor (SLPI), an innate immune molecule, has been shown to be an antimicrobial and anti-inflammatory protein. The aims of this study were to verify its expression and localization in human myometrium

Methods:

Specimens were obtained at time of cesarean delivery with or without labor. Expression and localization of SLPI was detected using immunohistochemistry. SLPI expression pattern relative to NF-κB p65 subunit was compared between not in labor and in labor subjects, between different tissue sections as well as in *in vitro* model systems including myometrial explants, uterine smooth muscle cells (USMC) and Ishikawa endometrial adenocarcinoma cells.

Results:

SLPI was predominantly localized to the nuclei of myocytes. The observed nuclear immunoreactivity of myocytes was increased during the labor relative to not in labor, paralleled with p65 nuclear translocation. The nuclear pattern of SLPI is specific to myometrium since SLPI immunostaining was present exclusively in the cytoplasm of all other tissues examined, including amnion, chorion, decidua and endometrium. SLPI staining was also positive in macrophages, indicated by co-localization of SLPI with CD68 positive cells. Treatment with IL-1β or TNF-α induced nuclear translocation of p65 in myometrium explants and USMC, but not in Ishikawa cells.

Conclusions:

In human myometrium, SLPI is predominantly localized in the nuclei of myocytes and in macrophages. The nuclear expression pattern of SLPI is myometrium-specific and increased following the onset of labor and correlated with NF-κB activation. Further understanding of its physiological significance may suggest new strategies aimed at preventing preterm birth.

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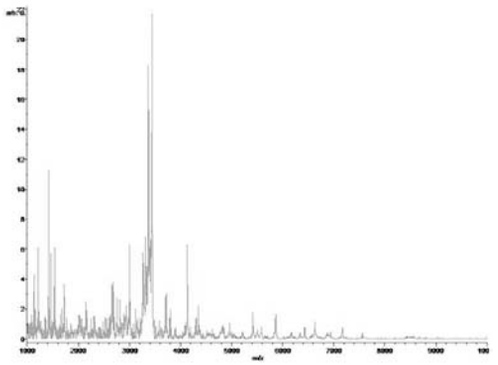
Application of a New Proteomic Technology on Amniotic Fluid in PROM. Sara Consonni,[1] Niccolo Bosso,[2] Marianna Andreani,[1] Agnese Pizzardi,[1] Fulvio Magni,[2] Anna Locatelli.[1] *[1]Obstetrics and Gynaecology, University of Milano-Bicocca, Monza, Italy; [2]Experimental Medicine, University of Milano-Bicocca, Monza, Italy.*

Objective: Mass spectrometry (MS) is the obligatory tool for proteomics studies. Biological samples must be purified before MS analysis due to the matrix complexity. A recent approach combines active surface prepurification with MALDI-TOF (matrix assisted laser desorption) analysis. ClinProt technology provides the prepurification of the sample through the use of magnetic beads (MB) with activated surface. This technique can be carried out by robot in an automated way on a large number of samples. A unique example of the use of MB before MALDI-TOF analysis to determine proteomic profiles of amniotic fluid (AF) is reported for rapid detection of fetal aneuploidies (Wang 2005). The objective of our study was to verify the applicability of ClinProt prepurification before MALDI-TOF analysis on amniotic fluid collected non-invasively in premature rupture of membranes (PROM).

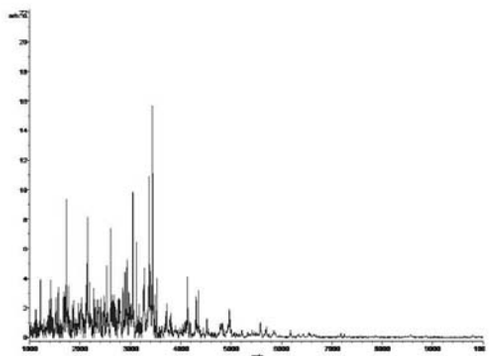
Methods: We sampled AF from vaginal posterior fornix of women with preterm PROM (group 1, n=10) and term PROM (group 2, n=10). Samples were prepared with MB and analyzed with MS MALDI-TOF in order to generate proteomic profiles.

Results: It was possible to generate average proteomic profiles in the two study groups and the observed profiles were different.

Average protein profile of group 1 (preterm PROM)



Average protein profile of group 2 (term PROM)



Conclusions: Samples of AF non invasively collected in PROM can be analyzed by MS MALDI-TOF after preparation with MB. This technique allows to retain part of the eluted sample for characterization of protein peaks of interest. Due to the less laborious characteristics of this method in comparison with techniques based on bidimensional electrophoresis its application can be useful in clinical proteomics.

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Progestins Accentuate the Maternal but Not Fetal Inflammatory Response of Women with Intra-Amniotic Inflammation. Sonya Abdel-Razeq, Irina A Buhimschi, Michael Cackovic, Guoyang Luo, Antonette Dulay, Victor Rosenberg, Mert Bahtiyar, Errol Norwitz, Edmund Funai, Catalin Buhimschi. *Ob./Gyn.& Reprod.Sci., Yale University, New Haven, CT, USA.*

Introduction: Data from animal research suggests that progestins have a marked pro-inflammatory capacity. Recent studies support the administration of 17α-hydroxyprogesterone caproate in women at risk for preterm birth. We sought to determine the impact of progestins during gestation on the extent of maternal and fetal inflammatory responses in pregnant women with intra-amniotic inflammation. **Methods:** Amniotic fluid, placenta and cord blood were obtained from 52 women who delivered preterm (median[range], GA: 27 [18-33] wks). An amniocentesis was done to rule out infection. Women exposed to progestins (n=17) within one week prior to amniocentesis were matched to 35 controls (CRL) by age, parity, history of preterm birth GA, membrane status and interval to delivery. Proteomic profiling of amniotic fluid [Mass restricted (MR) score] identified the presence or absence of intra-amniotic inflammation. An MR score of 3 or 4 confirmed intra-amniotic inflammation. Amniotic fluid and umbilical cord interleukin-6 (IL-6) levels were measured by ELISA. Histological chorioamnionitis was graded based on recognized criteria. **Results:** Overall, women and their fetuses exposed to progestin did not exhibit an increased inflammatory response (Table). However, sub-analysis restricted to women with MR 3 or 4 (n=18) showed that in the context of intra-amniotic inflammation, progestins were associated with significantly elevated amniotic fluid IL-6 levels compared to unexposed women (progestins (n=6): 77 [2-113] vs. CRL (n=12): 12 [0.3-94] ng/mL, p=0.027). These relationships were maintained after correction for steroid and antibiotic exposure. Such significance was not found for amniotic fluid glucose, LDH, WBC count or cord blood IL-6. **Conclusion:** Our results suggest that progestins may amplify the maternal, but not the fetal inflammatory response of women with intra-amniotic inflammation.

Variable	Progestins (n=17)	CRL (n=35)	*P value*
Amniotic fluid			
MR score 3-4	6 (35)	12 (34)	0.533
AF cultures, n (%)	3 (17)	4 (12)	0.670
Glucose, mg/dL	31 [2-46]	22 [2-44]	0.198
LDH, U/L	229 [103-5,580]	224 [104-2,400]	0.838
WBC cells/mm^3	9 [1-1260]	4 [0-5,450]	0.991
Positive Gram stain, (%)	4 (23)	4 (12)	0.413
IL-6, ng/mL	1.8 [0.1-113]	1.2 [0.1-94]	0.656
Cord blood			
IL-6, pg/mL	5.8 [4-2,909]	13.6 [4-3,249]	0.973
Histological chorioamnionitis			
Grade 2-3, n (%)	7 (41)	15 (42)	0.843

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Promegestone (R5020) and Nitroglycerin (NG) Act Synergistically To Inhibit Preterm Labor in Rats. Leili Shi,[1] Shao Q Shi,[2] Kristof Chwalisz,[3] Robert E Garfield.[2] *[1]Neurosciences and Cell Biology, The University of Texas Medical Branch, Galveston, TX, USA; [2]Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, TX, USA; [3]TAP Pharmaceutical Products Inc, Lake Forest, IL, USA.*

Objective: Recently progestins have been shown to reduce the incidence of recurrent preterm labor in women. Progestins have long been known to inhibit preterm labor in some species including rats and are also known to delay term labor. Nitric oxide (NO) donors, including NG, inhibit uterine contractility and have been used as tocolytics. The aim of this study was to examine the inhibitory effects of R5020 on preterm labor in rats induced with a progesterone antagonist (Onapristone, ZK) with and without NG.

Materials and Methods: Charles River S-D timed pregnant rats (n=6/group) were treated with ZK (3 mg/rat, s.c.) alone or vehicle (controls) on day 17 of gestation. Other groups of rats were treated with ZK in combination with various doses of R5020 (0.3, 1 or 2 mg/rat s.c) with and without NG (2 mg s.c. pellet) from days 18 to 19 of gestation. All rats were sacrificed on day 20 of gestation and the number of fetuses and implantation sites were counted to determine the preterm delivery rate. One way ANOVA was used for statistical analysis. $P<0.05$ was considered significantly different.

Results: Rats treated with ZK alone delivered all their fetuses prematurely compared with controls ($P<0.05$) treated with vehicle only (ca. 3% fetuses delivered). NG treatment alone did not affect the delivery rate ($P>0.05$) compared to controls. Similarly ZK + NG did not reduce the preterm delivery rate compared to ZK alone ($P>0.05$). However R5020 dose dependently reduced ($P<0.05$ at all doses) the number of fetuses delivered prematurely in response to ZK and the premature delivery rate was further reduced when treatment included the combination of R5020 plus NG ($P<0.05$).

Conclusions: ZK effectively induces premature delivery. Premature delivery produced by ZK can be effectively reduced with a R5020, a progestin known to bind with high affinity to nuclear progesterone receptors. NG by itself, at the dosage used, does not reduce the prematurity rate caused by ZK. However, R5020 and NG act synergistically to reduce the preterm delivery rate. This study indicates that combinations of a progestin with an NO donor may be an effective treatment for preterm labor and delivery.

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Pharmacokinetic and Pharmacodynamic Analysis of a Novel (Oral) Non-Peptide Oxytocin Antagonist in Non-Pregnant and Pregnant Rhesus Monkeys. PL Grigsby,[1] JP Rasanen,[1,2] DW Sadowsky,[1] M Bertolino,[3] M Carbonatto,[3] E Gillio Tos,[3] S Canali,[3] J Lacy,[4] A Chollet,[4] MJ Novy.[1] *[1]Reprod Sci, Oregon Primate Res Ctr, Beaverton, OR; [2]OB/GYN, Oregon Health Sci Univ, OR, USA; [3]RBM, Merck Serono, Italy; [4]Merck Serono, Switzerland.*

Objective: To investigate the pharmacokinetics (PK) and pharmacodynamics (PD) of AS606521, a novel orally active non-peptide oxytocin (OT) antagonist in rhesus monkeys.

Study Design: Dose finding and PK/PD studies were done in chronically instrumented non-pregnant (n=3) and pregnant (120-150 dGA, term 165d; n=5) monkeys. Maternal and amniotic fluid compartments were serially sampled during dosing and washout. Treatment phases included: OT infusion control, OT infusion + AS606521, and OT rescue therapy. OT infusions (2-64 mU/kg/hr, iv) were given to determine the lowest dose required to produce stable, sub-maximal uterine contractions in each animal. AS606521 was administered p.o. at 5, 10, 20mg/kg and the effects on inhibition of uterine activity (HCA, mmHg.sec/hr) were compared. To verify antagonist reversibility,

OT was administered in a dose-escalating fashion until pre-treatment HCA was restored. Serial assessments of fetal hemodynamics were quantified by Doppler ultrasonography.

Results: Pregnancy resulted in higher clearance (1.40 *vs.* 0.86 L/h/kg) and higher volume of distribution at steady-state (11.8 *vs.* 8.73L/kg). C_{max}, [ng/ml] and T_{max}, [h] did not differ markedly between pregnant and non-pregnant animals (378 *vs*. 389 ng/ml; 2.4 *vs*. 4.5h, respectively). A reduction in bioavailability (AUC_{last} [ng.h/ml]) was observed in pregnant *vs.* non-pregnant monkeys (1639 *vs.* 3417 ng.h/ml), while the t½ was increased from 9.5 to 12.4 h during pregnancy. Onset of action (>50% HCA reduction) with AS606521 (10mg/kg) was 30 mins; duration of maximum effect (85-95% inhibtion) was 3-4h; duration of sub-maximal inhibition (70-85%) was 6 h. The inhibitory effect was reversible after the OT rescue therapy. A proportional dose-response relationship in degree of inhibition and duration of action was demonstrated. No significant alterations in fetal hemodynamics were observed following maternal dosing of AS606521.

Conclusions: Potent competitive inhibition of OT-induced uterine activity by AS606521 was characterized by rapid onset and extended duration of action. Oral dosing of a novel OT antagonist may provide a useful adjunct in the tocolytic management of women with premature uterine contractions.

Support: Merck Serono.

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Expression of Non-Selective Cation Channels in Pregnant Rat Myometrium Is Enhanced by LPS Treatment in Preterm. Satoshi Urabe, Hiroshi Miyoshi, Hisaya Fujiwara, Yoshiki Kudo. *Department of Obstetrics and Gynecology, Hiroshima University, Graduate School of Biomedical Sciences, Hiroshima, Japan.*

Objectives: Infection such as chorioamnionitis is thought to be the cause of premature rupture of the membrane and induce uterine contractions leading to preterm delivery. However, the mechanism for enhancement of uterine contractility is not well understood. We have reported that non-selective cation channels (NSCCs) regulate pacemaker potentials to generate rhythmical contractions and should be targets of magnesium ions used for tocolysis. The purpose of this study is to investigate the changes of the expression of NSCCs during normal pregnancy and the effect of inflammation in preterm.

Methods; ATP receptors (P2X) and transient receptor potential canonical (TrpC) channels were examined as uterine NSCCs. The mRNA was extracted from the rat myometrium of non-pregnant and pregnant rats at days 15, 18, 20 and 22. The expression of each subtype of P2X and TrpC channels was measured by real time RT-PCR (ABI7700) with TaqMan probes (ABI). As an inflammatory model lipopolysaccharide (LPS; 0.2 mg/kg) was injected into intraperitoneal cavity at day 18 and the tissue was sampled after six hours.

Results; P2X4 and P2X7 were determined to be dominant subtypes of P2X channel. The expression of P2X4 was increased by 70% at day 22, compared with day 15 and that of P2X7 was enhanced by 90%. On the other hands, TrpC3 and TrpC4 were detected dominantly. The expression of TrpC3 was increased three times in the late stages of gestation. However, TrpC4 was suppressed by 57%. In the LPS treated rat myometrium COX-2 mRNA expression was measured to be 52 fold higher than that of the control rat, showing inflammatory effects in the myometrium. In this model the expressions of P2X4, P2X7 and TrpC3 were enhanced by 7.4, 18.6 and 24.7 times, but TrpC4 was not changed.

Conclusions: The mRNA expression of P2X4, P2X7 and TrpC3 channels in rat myometrium was increased in the late stages of pregnancy. These channels are suggested to be concerned with onset of labor. In the inflammatory model the expression of these channels was accelerated dramatically and these values were much higher than those in normal pregnancy. This finding supposed inflammation may enhance some types of NSCCs to accelerate uterine contractility and induce preterm delivery.

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Anandamide (AEA), Activates Extracellular Regulated Kinase Signaling in ULTR Human Myometrial Cells. Paul J Brighton, Jonathon M Willets, Justin C Konje. *Endoannabinoid Research Group, Cancer Studies and Molecular Medicine, University of Leicester, Leicester, Leicestershire, United Kingdom.*

Background: The endocannabinoid, Anandamide (AEA) has important roles in reproductive physiology, including embryo development, transport and implantation. Further evidence indicates potential important roles for AEA in myometrial function during labor. AEA exerts its effects through two G protein-coupled receptors, termed cannabinoid receptor 1 (CB1) and CB2. It has direct effects on myometrial smooth muscle cells but the exact nature of the signaling pathway involved remains unclear. mRNA for both CB1 and CB2 have been detected in ULTR human myometrial smooth muscle cells, and hence this provides a good model for studying myometrial cell signaling.

Objective: To examine the effects of AEA on mitogen-activated protein kinases (ERK1/2) signaling in ULTR cells.

Methods: Western blotting was used to measure the phosphospecific (p) (and hence, activated) levels of pERK1 and pERK2 in ULTR cells treated with AEA in the presence and absence of G-protein and other down-stream signaling pathway inhibitors.

Results: Stimulation of ULTR cells with AEA caused an increase in both pERK1 and pERK2 levels from baseline to a maximum 2.80±0.52 and 2.34±0.45 fold increase, respectively (data are mean±sem, n=6). Stimulation was time and concentration-dependent and peaked within 10-15 minutes of stimulation. Maximal pERK1 and pERK2 levels measured after 15min of AEA stimulation occurred at ~30μM with $LogEC_{50}$ values of -5.34±0.44 and -6.16±0.39, respectively (mean±sem, n=4). Inhibition of the $G\alpha_i$ G-protein complex by 24hr pre-treatment of cells with pertussis toxin (1ng ml^{-1}), phosphatidylinositol-3-kinase (PI3K) with LY294002 (100nM) and Src-kinase with PP1 (1mM), abolished AEA-mediated increases in pERK1/2 levels. (All data are significant ($p<0.01$) by one way ANOVA, compared with non-treated cells, n=≥4). In contrast, inhibition of calmodulin-kinase with KN62 (5μM) had no effect on AEA-mediated pERK1/2 levels, suggesting that ERK activation was independent of calmodulin-dependent Ca^{2+}.

Conclusions: These data demonstrate AEA directly stimulates ERK1/2 activation in ULTR cells through a CB receptor-PI3K-Src-dependent pathway. Since ERK1/2 signalling plays important roles in the regulation of gene expression, activation of this signalling pathway by AEA may help to clarify the role of AEA in myometrial activation and labor.

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Oxytocin Mediates ROCK Dependent Phosphorylation of the Regulatory Subunit (PPP1R12A) of Myosin Phosphatase To Increase Myosin Phosphorylation in Human Myometrium. J Lartey,[1] A Lopez Bernal.[2] [1]*School of Surgical and Reproductive Sciences, University of Newcastle, Newcastle, Tyne and Wear, United Kingdom;* [2]*Clinical Sciences South Bristol, Division of Obstetrics and Gynaecology, University of Bristol, Bristol, Avon, United Kingdom.*

Rho GTPases are key regulators of the actin cytoskeleton and stress fibre formation in smooth muscle. In human myometrium, activated RHOA forms a complex with Rho-kinase (ROCK) which phosphorylates and inhibits the regulatory subunit (PPP1R12A) of myosin phosphatase, causing a calcium-independent increase in myosin light chain (MYL) phosphorylation and tension (Ca^{2+}-sensitization)[1]. We have recently suggested that an up regulation of RND2 and RND3 protein expression during gestation is associated with a loss of ROCK mediated phosphorylation of PPP1R12A at Thr 696[2]. Other pathways like the protein kinase C activation of a phospho-inhibitory protein called PPP1R14A (CPI-17) can inhibit myosin phosphatase activity to cause Ca^{2+} independent contraction. We wanted to determine if changes in MYL phosphorylation and contraction caused by contractile agonists like oxytocin and carbachol are associated with changes in PPP1R12A phosphorylation. Freshly harvested myometrial tissue strips were stimulated with oxytocin (10nM) over a 5 minute time course. Whole tissue homogenates from each time course were homogenised and immunoblotted with phosphospecific antibodies for PPP1R12A-Thr 696 and MYL Ser 19/ Thr 18. Densitometry values of phosphorylated bands were normalised to total non phosphorylated protein content.

Oxytocin induced a peak 2-3 fold increase in PPP1R12A and MYL phosphorylation at 1 min ($p<0.01$) which was reduced to a 1-2 fold increase at 5 minutes ($p<0.05$). We conclude that one of the pathways by which oxytocin mediates MYL phosphorylation is through ROCK mediated phosphorylation of PPP1R12A and inhibition of myosin phosphatase.

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Up Regulation of ARFGEF PSCD3 Expression in Labour Represents a Mechanism for Receptor Desensitisation in Human Myometrium. J Lartey,[1] A Lopez Bernal.[2] [1]*School of Surgical and Reproductive Sciences, University of Newcastle, Newcastle, Tyne and Wear, United Kingdom;* [2]*Clinical Sciences South Bristol, Division of Obstetrics and Gynaecology, University of Bristol, Bristol, Avon, United Kingdom.*

ADP ribosylation factors ARF1 and ARF6 are monomeric GTP binding proteins that regulate desensitisation of G protein coupled receptor (GPCRs) like the luteinizing hormone (LH) and oxytocin receptor[1, 2]. ARF GTPases are activated an ARF guanine nucleotide exchange factor ARFGEF called pleckstrin homology, Sec7 and coiled-coil domains 3 (PSCD3) formerly known as ARNO. Activated LHRH binds to β-arrestin, PSCD3 and inactive GDP bound ARF6. PSCD3 catalyses nucleotide exchange on ARF6 allowing the receptor to internalise. PSCD3 binds to membrane bound β-arrestin and frees it bind to GPCRs to induce receptor desensitisation[3].

We postulate that ARF6 and PSCD3 interact with β-arrestin to regulate oxytocin receptor desensitisation in human myometrium. We used immunoblotting and immunohistochemistry to determine the relative expression and localisation of ARF6 and PSCD3 in non pregnant and pregnant not in labour and labouring myometrial tissue homogenates.

We found a marked up regulation of PSCD3 expression in spontaneous labour group relative to non pregnant and not in labour groups ($p<0.05$). ARF6 expression was invariant in all the groups examined. ARF6 and PSCD3 localised to myometrial fibres in tissue sections. The elevated expression of PSCD3 in labouring myometrium may represent a mechanism of receptor desensitisation during parturition.

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Oxytocin and Carbachol Induced Membrane Translocation of RHO GTP Binding Proteins in Human Myometrial Tissue Strips. J Lartey,[1] A Lopez Bernal.[2] [1]*School of Surgical and Reproductive Sciences, University of Newcastle, Newcastle, Tyne and Wear, United Kingdom;* [2]*Clinical Sciences South Bristol, Division of Obstetrics and Gynaecology, University of Bristol, Bristol, Avon, United Kingdom.*

Rho GTPases are small monomeric proteins that regulate acto-myosin interactions in cells[1]. RHO proteins are synthesized in the cytosol and are translocated to the membrane bound structures after prenylation and agonist activation[2]. RHOB an endosomal GTPase regulates myosin phosphorylation in neuronal cells[3]. We have recently reported that the RHO agonist lysophosphatidic acid (LPA) increases GTP bound RHOA and RHOB in cultured human myocytes[4].

To determine the mechanism of RHO protein activation during oxytocin and carbachol induced contraction, freshly prepared myometrial strips in Krebs Henseleit buffer were treated with oxytocin (10 nM) and carbachol (100 μM) under isometric and tension free conditions. Control strips were exposed to buffer only. Treated myometrial strips were solubilised and separated into membrane and cytosolic extracts and equal aliquots were immunoblotted with RHOA and RHOB antibodies.

RHOA translocated to the membrane after oxytocin and carbachol stimulation under both isometric and tension free conditions ($p<0.05$). There were no significant changes in RHOB membrane to cytosol ratios relative to control. Agonist induced contraction in human myometrium is associated with RHOA but not RHOB membrane translocation.

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Transcriptional Regulation of the Protein Kinase A Regulatory Subunit RIIα (RIIα) in the Human Myometrium. Magdalena Karolczak-Bayatti, Stephen C Robson, Nicholas G Europe-Finner. *Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom.*

During pregnancy components of the intracellular cAMP signalling pathway show increased gene expression resulting in the maintenance of myometrial quiescence until term where a substantial decrease in expression of these genes is observed. Protein Kinase A regulatory subunit RIIa (RIIα) is upregulated at both the mRNA and protein levels in the human myometrium during pregnancy. This particular subunit is membrane-bound and by directing phosphorylation to myometrial cytoskeletal proteins may affect contractile machinery thus playing a role in maintaining uterine relaxation.

Acetylation of histones promotes a favourable chromatin environment for transcriptional activity of many genes. This process is largely inhibited by histone deacetylases (HDACs), whereby its activity leads to transcriptional repression. HDACs can be recruited to the promoter region of a gene by other transcription factors such as Sp proteins. Since the RIIα promoter contains three Sp1-4 consensus binding sequences in its proximal part, we investigated whether this gene is a target for transcriptional regulation by HDACs. Using DNA precipitation assays we found that Sp1, 3 and 4 as well as HDAC1 and 2 form complexes with biotin-labelled fragments relating to Sp1-4 elements in the promoter region of RIIα. Additionally, treatment of myometrial primary cell cultures with HDAC inhibitor trichostatin A (TSA), or with the methyltransferase inhibitor 5-AzaC resulted in increased mRNA and proteins levels. Further studies with full and truncated luciferase constructs of the promoter region of the RIIα gene in transiently transfected myometrial cells confirmed that all three Sp1-4 elements are involved in the transcriptional regulation of the gene. This process involves HDACs, as 6h treatment with TSA significantly increased the luciferase signal. Changes in the binding of Sp proteins and HDACs to the promoter after TSA and AzaC treatment were investigated employing Chromatin Immunoprecipitation assays. Alterations in the methylation status of the promoter after treatment were examined by bisulfite modification and DNA sequencing. Together, this study highlights the importance of chromatin modifications in the maintenance of uterine quiescence during pregnancy as well as identifying a potential mechanistic target for drugs that may reduce the incidence of preterm labour.

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Maternal Obesity Does Not Impair Spontaneous or Oxytocin-Induced Contractility of Human Myometrium *In Vitro*. Claire A Higgins,[1] William Martin,[2] Andrew Blanks,[3] Steven Thornton,[3] Jane E Norman,[1] Scott M Nelson.[1] [1]*Reproductive and Maternal Medicine, University of Glasgow, United Kingdom;* [2]*Faculty of Biomedical and Life Science, University of Glasgow, United Kingdom;* [3]*Reproductive Health, University of Warwick, Coventry, United Kingdom.*

Introduction: Maternal obesity is associated with an increased need for operative delivery due to ineffectual progress in labour despite oxytocin (OT) augmentation. It is unclear whether this reflects impaired myometrial contractility, spontaneous or OT-induced, or alternative mechanisms. To clarify this we have examined myometrial activity *in-vitro* and related this to maternal body mass index.

Methods: Lower uterine segment myometrial samples were obtained at term pre-labour elective caesarean section (n=43). Maternal BMI was calculated using early pregnancy weight (12-14 weeks). Myometrial strips were placed in an organ bath of Carbogen gassed Krebs solution and suspended under isometric conditions with a resting tension of 20mN. After establishment of spontaneous contractions, strips were exposed to a single concentration of OT (1nM), with contractions observed for 60 min. OT concentration-response curves, 10^{-12} M-10^{-7} M, were also undertaken (n=21). Amplitude and frequency of contractile activity was recorded using a PowerLab with Chart v3.6 software. Data were analysed using one-way ANOVA, paired t-test and Pearson correlation.

Results: Mean BMI was 26.25±5.19 kg/m^2. Increasing BMI was not associated with a difference in spontaneous myometrial activity; mean amplitude=61.84mN, r=-0.10, p=0.51, mean frequency=7.68 contractions/hr, r=0.25, p=0.10. Exposure to a concentration of 1nM OT produced an immediate increase in activity in all samples (amplitude mean increase=19.74mN, p=<0.01,

frequency mean increase=50.56 contractions/hr p<0.01). % increase in both amplitude and frequency respectively were not related to BMI at immediate (108.7% r=-0.08, p=0.59; 880.8% r=-0.03 p= 0.85), 30min (171.3% r=-0.06, p=0.72; 59.28% r=0.12 p=0.48) or 60min (173.8% r=-0.06, p=0.73; 47.58% r=0.10 p=0.56) timepoints. Analysis of OT concentration-response curves did not show a BMI dependent effect (mean BMI 28.44±6.05 kg/m^2, mean Log EC_{50}=-9.73M, r=0.25, p=0.27).

Conclusion: The association between maternal obesity and operative delivery is not due to impaired spontaneous myometrial activity or responsiveness to OT, and may reflect pelvic adiposity, or a decrease in bioavailabilty of OT with increasing BMI.

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Membrane Progesterone Receptors in the Human Pregnancy Myometrium throughout Pregnancy and with Labor. Amy A Merlino,[1] Huiqing Tan,[2] Li Juan Yi,[2] Brian Mercer,[1] Sam Mesiano.[2] *[1]Reproductive Biology, CASE - MetroHealth Medical Center, Cleveland, OH, USA; [2]Reproductive Biology, CASE - University Hospitals, Cleveland, OH, USA.*

OBJECTIVE: Progesterone maintains pregnancy by promoting myometrial quiescence. Typically progesterone effects are thought to be mediated through the classic genomic pathway. There is evidence, however, that progesterone also acts via a non-genomic pathway by interacting with specific membrane progesterone receptors (mPRs) and in particular progesterone receptor membrane component -1 (PGRMC-1). The role of non-genomic progesterone actions in human pregnancy and parturition is not clearly understood. The goal of this study was to measure the extent of mPR expression in biopsy specimens of human myometrium obtained at cesarean delivery, and to determine whether expression changes with advancing gestation or the onset of labor.

STUDY DESIGN: Lower uterine segment myometrial biopsies were obtained at the time of delivery from consenting women who were at term and not in labor (n=4), preterm and not in labor (n=7) and term and in labor (n=5). Protein extracts were prepared and subjected to polyacrylamide gel electrophoresis and immunoblot analyses for PGRMC-1 and GAPDH. Abundance of PGRMC-1 protein relative to GAPDH was determined by digital densitometry. We also performed immunohistochemistry (IHC) to determine the cellular localization of PGRMC-1 in the human pregnancy myometrium.

RESULTS: PGRMC-1 protein was identified in each biopsy specimen. There was a 2-fold increase in PGRMC-1 protein in term compared with preterm biopsies (relative to GAPDH p<0.04). The relative level of PGRMC-1 protein was not different between biopsy specimens from laboring and non-laboring women at term (compared to GAPDH, p=0.8). PGRMC-1 immunoreactivity was localized to granular cytoplasmic staining.

CONCLUSION: This is the first description of the presence of PGRMC-1 protein in the human myometrium during pregnancy. Its presence suggests that progesterone may influence contractility non-genomically via these receptors. The functional significance of the gestational age associated two fold increase in PGRMC-1 is unclear. Changes in expression of this receptor during pregnancy may be important for the hormonal control of parturition and can be the focus of future studies.

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Progesterone (P4) Inhibits Human Myometrial Contractions during Pregnancy by Action on Membrane Receptors (mPR). Nicole Ruddock,[1] Shao-Qing Shi,[1] Sangeeta Jain,[1] Gary Hankins,[1] Roberto Romero,[2] Robert Garfield.[1] *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA; [2]Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*

Objective: Progestins are used to prevent preterm labor and delivery. Our studies indicate that progesterone (P4), but not 17 alpha hydroxyprogesterone (17P), inhibits human uterine contractions in vitro (Ruddock et. al., abstract SMFM, 2008). The aim was to examine the mechanism of P4 inhibition. **Methods:** Uterine tissues from women (n=55) at term with cesarean section, were suspended in organ chambers and exposed to various agents or solvents. Contractility was registered, stored, analyzed and compared before and after addition of agents or KCl. Tissues were treated with P4 alone (10^{-10} to 10^{-3}M) or P4 bound to bovine serum albumin (BSA/P4, 10^{-6} to 10^{-3}M P4), a progestin with low affinity to mPR (R5020, 10^{-7}-10^{-3}M), or a non-sex steroid (cholesterol, 10^{-5} to 10^{-2}M). Other tissues were pretreated with selective inhibitors of adenylate cyclase (SQ 22536, 10^{-5}M), guanylate cylase (ODQ, 10^{-5}M), phosphodiesterase (rolipram, 10^{-5}M), nitric oxide (NO) synthases (L-NAME, 10^{-4}M) or a nuclear P4 receptor antagonist (mifepristone, MIF, 10^{-5}M), followed by P4. Data were analyzed by ANOVA for statistical differences (P<0.05). **Results:** P4 rapidly (<1 hour), effectively (100%) and dose-dependently inhibits spontaneous and KCl-induced contractility (ED_{50} of $<10^{-5}$M). R5020 has minor (P<0.05) and transient inhibitory effects and cholesterol is not effective (P>0.5). P4 inhibition is not blocked by MIF (P>0.5) or mimicked by BSA/P4 (P>0.05) but is reversible after washing. The P4 inhibitory responses are not suppressed (P>0.05) by SQ, ODQ or L-NAME, but rolipram increases (P<0.05) responses. **Conclusions:** P4, at concentrations equivalent to those in the placenta and uterus, directly suppresses myometrial contractility by nongenomic mechanisms through action on mPR. Thus P4 may maintain pregnancy and is useful in the prevention of prematurity. Inhibition is not seen with steroids that do not bind to the mPR (17P, R5020 or cholesterol). BSA/P4, limited to act on mPR, may not enter the tissue due to size. NO, guanylate cyclase or cGMP are not involved in P4 inhibition. P4 inhibition is due to both cAMP-dependent and -independent mechanisms. Effective treatment of preterm labor may be achieved with P4 plus other agents. Supported by the Perinatalology Research Branch of NIH.

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EP3 Versus EP1 Receptor Antagonism in Human Term Myometrial Contractility. Shankari Arulkumaran,[1] Mandeep S Kandola,[1] Richard J McArthur Wilson,[2] David P Brooks,[3] Phillip R Bennett.[1] *[1]Imperial College Parturition Research Group, Institute of Reproductive and Developmental Biology, London, United Kingdom; [2]GlaxoSmithKline, Stevenage, Hertfordshire, United Kingdom; [3]GlaxoSmithKline, King of Prussia, PA, USA.*

Prostaglandins are produced by arachidonic acid via the cyclooxygenase pathway. Prostaglandins can affect myometrial contractility. Their receptors are divided into a relaxant group (DP, EP2, EP4, IP) and a contractile group (EP1, EP3, FP, TP). We have previously shown an increase in EP3 receptor expression in myometrium at the time of labour (SGI 2007 abstract no.600). In this study, we assesed the effects of EP3 versus EP1 antagonism on myometrial contractility.

Experiments were performed using 5x2 mm human pre-labour, lower uterine segment myometrial strips in a DMT Myograph 800MS in oxygenated Kreb's solution, with ADI Powerlab software. Spontaneous contractions require stretch force. The number of strips attaining spontaneous contractions was greatest at a force of 5-6g. A highly selective EP1 antagonist, ZD-6416 and a highly selective EP3 antagonist, L-798106 were used in this study.

Incubation of strips with ZD-6416, at concentrations up to 1μM for one hour prior to the experiment, led to no significant reduction in the total work done. However, incubation of strips with L-798106 with a concentration of 100nM for one hour prior to the experiment, led to a statistically significant reduction in the total work done after one and a half hours.

Furthermore, when increasing concentrations of PGE_2 (10^{-10} to 10^{-6}) were added to L-798106 (100nM) pre-treated strips, total work done per contraction was comparable to that of non-treated controls. Similar effects were not observed in ZD-6416 (100nM) pre-treated strips.

Since the reduction in contractility caused by L-798106 was greater than that caused by ZD-6416, it is likely that the stimulating effect of PGE_2 acts predominantly via EP3 receptors. Taken together with our previous data showing an increase in EP3 at labour, this data shows that targetting an EP3 receptor may be a useful strategy in managaing pre-term labour.

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Spontaneous Activity and the Complex Action Potential Require the Myometrial Network. Gilles Bru-Mercier, Anatoly Shmygol, Steve Thornton, Andrew M Blanks. *CSRI, Warwick Medical School, University of Warwick, Coventry, United Kingdom.*

Introduction: The process of myometrial activation is characterized *in vivo* as being a switch from contractures to contractions. The biophysical changes underlying this process are thought to be mediated by changes in the expression of genes that govern smooth muscle cell excitability and connectivity. There remains little understanding of this process at a biophysical level. Furthermore, there is no satisfactory explanation of the complex myometrial action potential (based on single cell dynamics) that gives rise to the full phasic contraction at term. **Hypothesis:** The complex myometrial action potential is a product of the myometrial network. **Methods**:Myometrial biopsies were taken from patients undergoing Caesarean Section with informed written consent and local ethics committee approval (REC-05/Q2802/107). 200 μm thick slices were cut using a vibroslicer in Krebs solution (4°C). Each slice was loaded with Ca^{2+}-sensitive Fluo-4 and imaged at 1-5 frame/s on a Zeiss LSM 510 META confocal. To study the role of the intercellular communication on the complex myometrial action potential, we recorded spontaneous activity at 37°C to determine the location, duration and frequency of $[Ca^{2+}]_i$ events. After

a period of equilibration we added the gap junction inhibitor carbenoxolone (200μM) until spontaneous activity was lost. **Results:** Spontaneous contractions demonstrated synchronous (onset<200 msec) rises in intracellular calcium indicative of voltage gated calcium entry and intracellular coupling. Addition of 200 μM carbenoxolone caused a transient increase in $[Ca^{2+}]_i$ event frequency (Spon=0.009+/-0.001Hz vs Carb=0.031+/-0.01Hz, P<0.05 by Students TTest, n=62) whilst reducing the complex action potential to simple spikes (Event duration; Spon=36+/-10 sec vs Carb=2.5+/-0.5 sec, P<0.05, n=62) prior to loss of spontaneous activity. Loss of synchrony was observed between bundles but not within bundles. Independent oscillations were observed in isolated cells that were not blocked by carbenoxolone and were slower in nature than the network events. Spontaneous activity returned after prolonged (<30mins) wash out. **Conclusions:** Spontaneous activity and the complex myometrial action potential require network coupling. The contraction to contracture excitability profile is mimicked by blocking intracellular coupling via gap junctions. Decoupling of inter but not intra-bundle connectivity suggests sub-structure in the network.

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Human Myometrial Expression of a 46kDa Isoform of Estrogen Receptor-Alpha. Robert C Moore,[1,2] David N Hackney,[1,2] Hyagriv N Simhan,[1,2] Steve N Caritis.[1,2] *[1]Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh/Magee-Womens Research Institute, Pittsburgh, PA, USA; [2]Obstetric-Fetal Pharmacology Research Units Network, Bethesda, MD, USA.*

Background: The classic estrogen receptor has two isoforms, alpha (ER-α) and beta (ER-β) both of which have been detected in the human myometrium. Recently a truncated 46kDa isoform of the ER-α has been described in human endothelial and testicular cells. We describe the presence of this 46kDa ER-α isoform in pregnant and immortalized non-pregnant human myometrial cells.

Methods: Myometrial tissue obtained from non-laboring pregnant women undergoing cesarean section at term (n=4) was dissected prior to being finely minced. A portion of the tissue was placed in PBS and sonicated, while the remainder of the tissue was dissociated with collagenase prior to filtration and placement on culture plates. Cells were then cultured in MEM w/10% fetal bovine serum (FBS) until confluence. Cultured cells were then dissociated with 0.05% typsin/EDTA, subsequently re-plated and grown to confluence. Immunohistochemistry directed toward smooth muscle protein was used to verify myometrial phenotype.) Protein was extracted and quantified. Western blot was performed with mouse monoclonal anti-ER-α receptor antibody (Santa Cruz Biotechnology) to the F-10 domain of the human ER-α receptor. An immortalized non-pregnant human myometrial cell line provided by Ann Word, hTERT, was cultured and isolated as described above.

Results: hTERT cells expressed both the truncated (46kDa) and the full length (66kDa) ER-α isoforms. Fresh and cultured myometrial cells from non-labored term pregnant patients also expressed both isoforms of ER-α. Subsequent subcultures of myometrial tissue continued to express the 46kDa ER-α isoform, yet the expression of the 66kDa isoform was lost. A representative blot is below.

Conclusions: We demonstrate, for the first time, the presence of a 46kDa ER-α isoform in cultured and non-cultured pregnant and cultured nonpregnant human myometrial tissue. Discovery of this isoform of ER-α in myometrial tissue could provide insight into the molecular mechanisms involved in parturition.

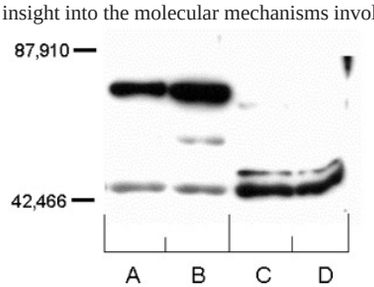

Figure 1: ER-α expression in fresh (A,B) and cultured (C,D) pregnant human myometrial cells

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Prostaglandin $F_{2\alpha}$ Decreases Plasma Membrane Levels of Prostaglandin $F_{2\alpha}$ Receptor Protein in Human Myometrial ULTR Cells in a Protein Kinase C Dependent Manner. Zaragoza Dean, Inge Christiaens, Brin Geoff, David M Olson. *Perinatal Research Centre, University of Alberta, Edmonton, AB, Canada.*

Introduction: The action of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a potent uterotonic stimulant that is associated with labour at term and preterm, is mediated by its receptor, PTGFR. Myometrial PTGFR mRNA levels fall during pregnancy and this likely plays a role in uterine quiescence. However, the mechanisms by which this occurs are poorly understood. We previously reported that $PGF_{2\alpha}$ downregulates FP mRNA expression in cultured human myometrial ULTR cells in a protein kinase C (PKC) dependent manner. In addition to the downregulation of mRNA levels, receptor desensitization may also represent another mechanism of decreasing PTGFR activity because ligand binding to G protein coupled receptors often results in receptor internalization. We therefore **hypothesized** that $PGF_{2\alpha}$ treatment of ULTR cells also results in PKC dependent PTGFR internalization.

Methods: Near confluent cultured human myometrial ULTR cells were treated +/- 10^{-7} M or 10^{-6} M $PGF_{2\alpha}$ for 1, 3, 6 or 24 hr. Cells were fixed with formaldehyde and visualized for localization of PTGFR by immunofluorescence. To examine the potential involvement of PKC in the process, ULTR cells were treated +/- 10^{-6} M $PGF_{2\alpha}$ and +/- 10μM myristoylated PKC inhibitor (20-28) and examined for PTGFR cellular localization as described above.

Results: $PGF_{2\alpha}$ treatment resulted in a dose dependent decrease in PTGFR membrane signal at 1, 3, 6 and 24 h. This decrease was dependent on PKC as cotreatment with the myristoylated PKC inhibitor (20-28) prevented the $PGF_{2\alpha}$ induced decrease in membrane PTGFR at 1 h treatment. There was no visible effect of the PKC inhibitor on PTGFR membrane signal on its own.

Conclusion: We conclude that $PGF_{2\alpha}$ decreases membrane levels of PTGFR protein in human myometrial ULTR cells in a PKC dependent manner. These results suggest that PKC may be required for both the $PGF_{2\alpha}$ induced internalization and desensitization of PTGFR protein and downregulation of PTGFR mRNA in human myometrial ULTR cells. Therefore PKC may play a crucial role in downregulating PTGFR expression and activity and maintaining uterine quiescence during pregnancy.

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RhoGTPase Expression and Function in Hypoxic Uterine Smooth Muscle Cells. Deirdre A O'Shaughnessy,[1] John J Morrison,[2] Terry J Smith.[3] *[1]National Centre for Biomedical Engineering Science, Galway, Ireland; [2]Department of Obstetrics and Gynecology, University College Hospital Galway, Galway, Ireland.*

RhoA is a small GTPase that acts as a molecular switch to control a variety of signalling pathways in smooth muscle, including contractility. It is thought that increases in RhoA-GTP levels facilitates phosphorylation of target proteins such as CPI-17, promoting contractility at pre-term and term labour in humans. However, in situations of acute or chronic hypoxia in the uterus, it is important that myometrial contractility and subsequent labour is not facilitated prematurely. Given the importance of RhoA to a cell's response to hypoxia in other cell types, it was hypothesised that RhoA plays a central role in the mechanism controlling smooth muscle contraction in the uterus too. Following acute hypoxia (0.5% O2) for one to six hours, RhoA mRNA, total protein and activation (RhoA-GTP) levels were analysed, using semi-quantitative PCRs and western blot, and compared to normoxic non-pregnant human uterine smooth muscle control cells. Next, we investigated whether reduced oxygen conditions affected oxytocin induced activation of RhoA, following a two hour treatment of 10 nM oxytocin. Firstly, our results demonstrate that the RhoA itself is significantly activated under low oxygen conditions, resulting in phosphorylation of myosin phosphatase, myosin light chain and cofilin, three proteins known to be central in contraction and actin filament organisation. Secondly, hypoxia significantly reduced the coupling of oxytocin to RhoA activation under the conditions examined. We observed a significantly reduced level of RhoA expression and activation which correlated with an increase in the level of another RhoGTPase protein, RhoE. We propose that RhoA inactivation occurs through a RhoE-mediated mechanism, suggesting a balance in the activity of these two antagonistic RhoGTPases in oxytocin-induced hypoxic human uterine smooth muscle cells. These results provide a possible explanation for the reduced coupling of oxytocin as a stimulant of myometrial contractions during slowly progressing labours.

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Oxytocin-Induced Myometrial Contractions in the Presence of Nifedipine, What Is the Mechanism? Joanna E Gullam, Andrew M Blanks, Steven Thornton, Anatoly Shmygol. *Clinical Sciences Research Institiute, University of Warwick Medical School, Coventry, West Midlands, United Kingdom.*

Oxytocin-induced release of Calcium ions (Ca^{2+}) from sarcoplasmic reticulum (SR) and sensitisation of contractile proteins to Ca^{2+} have been suggested to mediate the oxytocin-induced potentiation of myometrial contractions.

Objective: We investigated the effects of oxytocin in the presence of Nifedipine, a known inhibitor of the L-type calcium channel (LTCC).

Method: Samples of myometrium were obtained from women undergoing term caesarean section with the approval of the Local Ethics Committee. A standard organ bath system (AD Instruments, UK) was employed to analyse contractile activity. Stable spontaneous contractions were recorded for 40-60 minutes before addition of Nifedipine.

Results: In agreement with our previous findings, application of oxytocin to spontaneously active strips produced a two-component effect: a transient tetanus-like contraction, followed by prolonged augmentation of phasic contractions. Nifedipine (1uM) rapidly abolishes spontaneous contractions, subsequent addition of 100nM oxytocin produced an initial, transient rise in force, approxiamately 20% compared to oxytocin alone, followed by high frequency oscillations in >50% of strips. Calcium-free solutions were used to confirm that oscillations were due to Ca^{2+} entry. Disabling the SR store using Thapsigargin (1uM) had no effect on oscillations, confirming the SR not to be involved. The T-type calcium channel blocker, Mibefradil (1uM) showed no inhibition of oscillations. An IP_3 receptor and store-operated calcium channel inhibitor, 2-aminoethyldiphenylborate (2-APB) 50uM also had no effect on oxytocin-induced oscillations. The store-operated calcium channel inhibitor SKF-96365 (10uM) showed partial inhibition of oscillations.

Conclusions: Based on these results, we propose that the most likely mechanism of Ca^{2+} entry producing oxytocin-induced oscillations in the presence of Nifedipine is the Transient Receptor Channel-C (TRPC) channel, known to be present in the human myometrium. Further work needs to be completed to clarify this further.

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Identification of Stim and Orai in Human Myometrium. A New Paradigm in Store-Operated Calcium Signaling. Evonne C Chin-Smith,[1] Mark R Johnson,[2] Rachel M Tribe.[1] *[1]Division of Reproduction and Endocrinology, King's College London, London, United Kingdom; [2]Department of Maternal Fetal Medicine, Imperial College School of Medicine, Chelsea and Wesminster Hospital, London, United Kingdom.*

Background: Recent reports have suggested that two novel proteins, Stim and Orai, are involved in the regulation of store-operated calcium entry. We have previously reported that members of the TRPC family, putative basal and store operated calcium entry channels, are present in human myometrium and regulated by labour associated stimuli IL-1beta and mechanical stretch. Although Stim and Orai isoforms have been reported in other smooth muscle cell types, there are no published reports of Stim and Orai expression in human myometrium. The aim of this study was to identify mRNA expression of Stim1, Stim2, Orai1 and Orai2 in human myometrium. **Methods:** Human myometrial biopsies were obtained from women undergoing elective caesarean section at term (prior to labour) with informed written consent and institutional Ethics Committee approval. Whole myometrial tissue was either snap frozen and stored at -80ºC or used for cell culture. RNA was extracted from whole tissue (n=5), primary cultured myometrial cells (n=4) and passaged (P2) myometrial cells (n=5) and Stim1, Stim2, Orai1 and Orai2 mRNA expression was assesed by quantitative real-time PCR. **Results:** All four genes were expressed in whole myometrial tissue and cells. Stim1 and Stim2 mRNA expression in cultured myometrial smooth muscle cells (primary and passaged) was significantly reduced compared to myometrial tissue expression ($P<0.05$). However, there was no significant difference in either Orai1 or Orai2 expression in whole tissue *versus* cultured myometrial smooth muscle cells. **Conclusion:** To our knowledge this is the first report of Stim1/2 and Orai1/2 mRNA expression in human myometrium. These genes may contribute to the regulation of calcium signalling in human myometrium, but the functional significance of their expression remains to be determined.

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Evaluation of the Effect of Known Mediators on Human Uterine Smooth Muscle Cell Contractility. Joan Fitzgibbon,[1] John J Morrison,[1,2] Terry J Smith,[1] Margaret O'Brien.[1] *[1]National Centre for Biomedical and Engineering Science, National University of Ireland, Galway, Galway, Ireland; [2]Department of Obstetrics and Gynecology, National University of Ireland, Galway, Galway, Ireland.*

Objective: The ability of uterine smooth muscle cells to stimulate collagen contraction has been well established as an *in vitro* model of myometrial contractility. Devost and Zingg (2007) reported that the contractility of human myometrial cell lines layered onto collagen matrices was increased by oxytocin while another group described the stimulation of human uterine smooth muscle cell contractility, cultured within collagen lattices, with endothelin-1 (Dallot et al., 2003). Our study investigated the response of human primary uterine smooth muscle cells cultured within collagen lattices, to various compounds, including the non-specific depolarizing agent potassium chloride (KCL), the inflammatory cytokine TNFα, the ROCK-1 inhibitor Y-27632, oxytocin, and oxytocin plus its clinically used antagonist, atosiban.

Methods: Human primary uterine smooth muscle cells (UTSMC) (Lonza) were maintained in DMEM high glucose media. Cells (150,000 per well) passage 3-8, were embedded in 1.5 mg/ml collagen, in 0.5 ml aliquots, in DMEM-F12 media on 24 well plates (Invitrogen) (Dallot *et al*., 2003). Effects on contraction were studied by monitoring changes in gel area (Alpha Innotech Imager, Image J software (NIH)). Statistical significance was determined by the student t test.

Results: The UTSMCs displayed basal contraction of the collagen gels while the non-contractile cell line HEK293 cells, did not. KCL (20nM) stimulated an 11% increase in contractility (n=3, P=0.0262) while 10 nM TNFα resulted in an 8.4% increase (n=3, P=0.0205), in comparison to unstimulated cells embedded in gel. The ROCK 1 inhibitor Y-27632 (10μM) inhibited contractility, with a 17.5% decrease in collagen gel contractility (n=3, P=0.011). A 13% increase (n=4, P= 0.0146) in UTSMC embedded collagen contractility was observed with 10 nM oxytocin, which was antagonized by atosiban (1 μM) (n=4).

Conclusion: This study highlights the importance of the development and optimisation of a reproducible human *in vitro* myometrial contractility model, to evaluate the effect of various known labor-associated, and also unknown compounds. This should aid in our understanding of the many complex biochemical pathways involved in myometrial contractility at labor, and ultimately contribute to the prevention of preterm labor.

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Changes in Intra-Mural Myometrial Blood Flow during Spontaneous Labour Using 3D Power Doppler Angiography (PDA). NW Jones,[1,2] NJ Raine-Fenning,[2] H Mousa,[1] MJ Taggart,[3] K Jayaprakasan,[2] GJ Bugg.[1] *[1]Nottingham University Hospitals NHS Trust, United Kingdom; [2]Nottingham University, United Kingdom; [3]University of Newcastle upon Tyne, United Kingdom.*

Methods

3D PDA was used to measure the percentage (%) change in the Vascularization Index (VI), the Flow Index (FI) and the Vascularization Flow Index (VFI)[1] in a volume of myometrium at the uterine fundus of 20 nulliparous women at term, in the first stage of uncomplicated spontaneous labour. 3D data sets were obtained during a single cycle of uterine relaxation (R1), contraction and subsequent relaxation (R2). Measurements were made independantly by two authors (NWJ and GJB) using VOCAL® (GE Kretz) and the mean value was used for analysis. The results from each woman are presented as a % change of R1. Data is presented as medians [interquartile range (IQR)] and analysed using non-parametric tests.

Results

The median volume (cm^3) of interest for R1 was 17.9cm^3 (12.6- 24.4cm^3), for the contraction was 17.7(12.3– 21.1cm^3) and for R2 was 16.4 cm^3 (12.4-25.7 cm^3) (p=0. 39).

The contraction caused a significant reduction in VI to 46.7 % (21.4- 67.7%; $p<0.01$), in FI to 86.8% (72.2- 100.7%; $p<0.01$) and in VFI to 43.0% (20.0-62.1%; $p<0.01$) of R1. The VI during R2 was 90.8% (73.3-107.1%), the FI during R2 was 97.5% (86.8-109.4%) and the VFI during R2 was 94.0% (71.4-114.1%) of R1 (Fig.1).

The % change for all three indices between R2 and R1 was not significantly different. However, there was a significant difference between the contraction and R2 ($p< 0.01$).

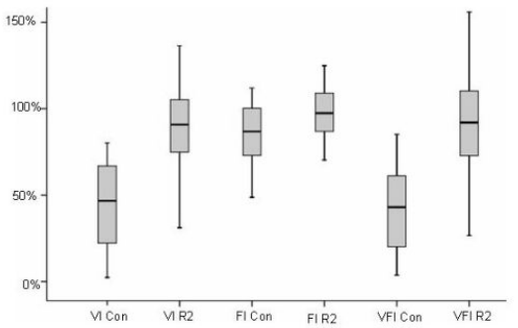
The mean intra-class correlation coefficient and 95% confidence interval (CI) of VI, FI and VFI for the authors (NWJ and GJB) were 0.99 (0.98–0.99), 0.99 (0.98–0.99) and 0.98 (0.96–0.98), indicative of good inter-observer reliability.

Conclusion

3D PDA is a useful and reliable tool in the assessment of changes in intramural myometrial blood flow, which was found to reduce significantly during a contraction but increase again during the following uterine relaxation.

1. Raine-Fenning NJ, et al. The inter-observer reliability of 3D PDA acquisition within the female pelvis. Ultrasound Obstet Gynecol. 2004;23:501-8.

Fig1: The % change of VI, FI and VFI during the contraction and R2 in relation to R1



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The Role of PGF2α on Myometrial Contractility; Studied with a Selective FP Antagonist. Shankari Arulkumaran,[1] Andre Chollet,[2] Phillip R Bennet.[1] *[1]Imperial College Parturition Research Group, Institute of Reproductive and Developmental Biology, Hammersmith Hospital Campus, London, United Kingdom; [2]Merck Serono, Geneva, Switzerland.*

Objectives: Human myometrial strips established in culture will usually begin contracting after one hour. We have previously shown that stretch upregulates prostaglandin synthesis and have therefore hypothesized that spontaneous contractions occur because of stretch-related prostaglandin synthesis.

Methods: Experiments were performed using 5x2mm human pre-labour, lower uterine segment myometrial strips in a DMT Myograph 800MS in oxygenated Kreb's solution, with ADI powerlab software. Spontaneous contractions required stretch force and initial experiments determined that the maximum number of strips attaining spontaneous contractions was greatest at a force of 5-6g. A novel antagonist to PGF2α (FPA) was studied in this model. The FPA has a Ki of 6nM for FP and is 10-100 fold selective for FP compared with other prostanoid receptors.

Results: Addition of PGE_2 and PGF2α after the commencement of spontaneous contractions caused a statistically significant increase in the total work done by the strips. The effect of PGE_2 was being greater than that of PGF2α.

Pre-incubation of the baths with a novel and selective FP antagonist (FPA) with concentrations up to 1μM (10^{-6}) did not affect the total work done by spontaneous contractions compared to non-treated controls. However, increasing concentrations of the FPA (10^{-8} to 10^{-5}) decreased the total work done by 5-fold on strips treated with PGF2α beforehand in comparison the PGF2α - treated strips alone.

Conclusion: These data suggest that stretch and synthesis of prostaglandins is essential for spontaneous contractility in human myometrial strips. Since FPA is able to block PGF2α induced but not spontaneous contractions, it is likely that PGF2α does not play a role in spontaneous myometrial contractility in vitro, although it may do so in vivo. Because other factors may combine to increase contractility, the combination of an FP antagonist with other inhibitors may therefore, be a more effective strategy in reducing pre-term deliveries.

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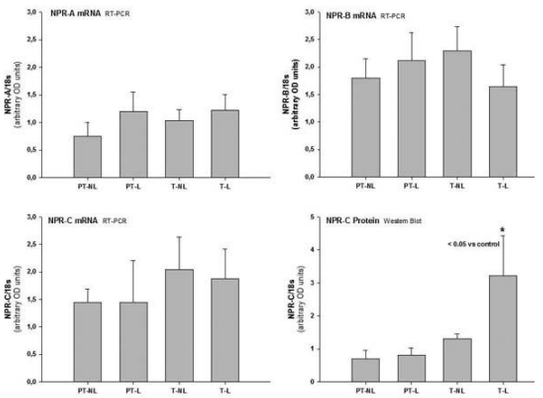
Human Myometrial Natriuretic Peptide Clearance Receptor (NPR-C) Increases during Term Labor. Jorge A Carvajal,[1] Ana M Delpiano,[1] Mauricio A Cuello,[1] Jose A Poblete,[1] Carl P Weiner.[2] *[1]Obstetricia y Ginceologia, Pontificia Universidad Catolica de Chile, Santiago, RM, Chile; [2]Obstetrics and Gynecology, University of Kansas School of Medicine, Kansas City, KS, USA.*

Objectives: Brain Natriuretic Peptide (BNP) is synthesized in fetal membranes and inhibits oxytocin-induced contraction of preterm human myometrium. We hypothesized that BNP may be a paracrine mediator of human myometrial quiescence. We showed BNP content is higher in membranes from preterm pregnancies absent labor and significantly decreased with idiopathic preterm labor. While BNP activates natriuretic peptides receptors A (NPR-A), B (NPR-B), and its clearance receptor (NPR-C), we have shown BNP does not inhibit myometrial contraction via NPR-A or NPR-B. Herein, we test in part the hypothesis that BNP inhibits myometrial contractions by activating NPR-C by quantitating NPR-C in human myometrium at different gestations and labor status.

Methods: Myometrial samples were obtained at the time of cesarean section after informed consent from 4 groups of patients: preterm not in labor (PT-NL), preterm in labor (PT-L), term not in labor (T-NL) and term in labor (T-L). Myometrial samples were obtained from women 30-34 weeks' gestation, and term between 38 and 41 weeks. mRNA for NPR-A, B and C were semi-quantitated by realtime PCR (normalized by 18s mRNA), and NPR-C protein by Western blot.

Results: Natriuretic peptide receptors A, B and C mRNAs were identified in all 4 groups. While there were no differences in mRNA levels among groups, NPR-C protein was increased in samples from term laboring women.

Conclusion: Since BNP inhibits the contraction of human preterm but not term myometrium independent of NPR-A and NPR-B, we have previously speculated that BNP activates another "unknown" receptor. Herein we find that myometrial NPR-C protein but not mRNA increases during human labor at term. The increase in NPR-C at term could compete with the unknown quiescent receptor to functionally reduce the availability of BNP and thus permit or promote myometrial activation/contraction. (Supported by a grant from Chilean Government Fondecyt 1020675).



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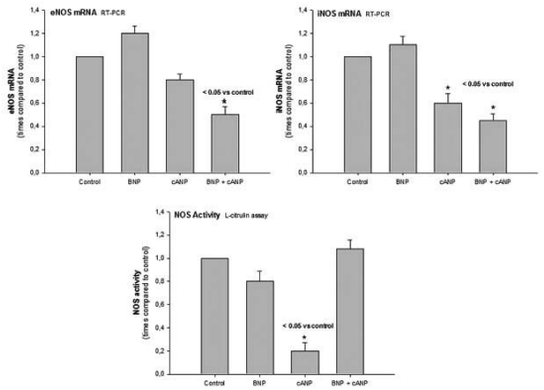
Brain Natriuretic Peptide (BNP) Decreases Expression and Activity of eNOS and iNOS in Primary Human Myometrial Cell Cultures. Jorge A Carvajal,[1] Ana M Delpiano,[1] Carlos Escudero,[1] Paola Casanello,[1] Luis Sobrevia,[1] Mauricio A Cuello,[1] Carl P Weiner.[2] *[1]Obstetricia y Ginecologia, Pontificia Universidad Catolica de Chile, Santiago, RM, Chile; [2]Obstetrics and Gynecology, University of Kansas School of Medicine, Kansas City, KS, USA.*

Objective: BNP is synthesized within human chorion and amnion and inhibits oxytocin-induced contraction of human myometrium. BNP activates guanylate cyclase (GC) natriuretic peptides receptors A (NPR-A) and B (NPR-B). BNP also stimulates the clearance receptor (NPR-C) whose action is not mediated by GC. The intracellular pathway of BNP/NPR-C inhibition is not known. We determined that BNP does not inhibit myometrial contraction via NPR-A or -B. We hypothesized that BNP inhibits myometrium by NPR-C activation. We test aspects of our hypothesis by determining whether BNP/NPR-C pathway inhibits myometrial contractions via myometrial NOS pathway.

Methods: BNP and cANP (specific NPR-C agonist) were added to primary human myometrial cell cultures and eNOS/iNOS activity/expression determined. Cell cultures (n=6) were prepared from myometrial samples of nonlaboring, term pregnant women. After confluence (10d), the cells were incubated (6h) with 100nM BNP and/or cANP. NOS activity was measured (L-citruline assay) and transcription/translation semi quantitated (realtime PCR and Western blotting).

Results: BNP had no effect on either NOS expression or activity. cANP significantly reduced iNOS but not eNOS mRNA level. cANP did not alter the protein level of NOS but significantly reduced NOS activity. Co-incubation with BNP and cANP reduced both mRNA levels ($p < 0.05$) and significantly decreased eNOS, but not iNOS, protein without change in overall NOS activity.

Conclusion: The activation of NPR-C by cANP reduces NOS activity and expression. The same effect was not observed by BNP. The difference may be explained because BNP (but not cANP) activates all natriuretic peptide receptors, and they may have opposite actions on NOS pathway. We conclude unlikely BNP inhibits human myometrial contraction by NPR-C activation via the NOS pathway. (Supported by a grant from Chilean Government Fondecyt 1020675).



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hTERT Rescue Provides an Improved Human Uterine Smooth Muscle Model. Baojun Chang, Leslie Myatt, Xiao-Lan Cui. *Dept. of Ob/Gyn, University of Cincinnati College of Medicine, Cincinnati, OH, USA.*

Introduction: ULTR is a retroviral immortalized human uterine smooth muscle cell line which we use as a model for uterine hypertrophy studies. However, this cell line has limited usage due to a reduced rate of cell division and eventually replicative senescence in culture. One of the mechanisms of human somatic cellular senescence is un-compensated shortening of telomeres, the specialized DNA structures located at the ends of eukaryotic chromosomes. Introduction of human telomere reverse transcriptase (hTERT) has been shown to induce telomerase activity and telomere elongation, and extend life-span of normal human cells.

Objective: To recover ULTR cell division without altering the phenotypic characteristics of smooth muscle cells by introducing hTERT, therefore improving ULTR cell line.

Methods: ULTR cells were transfected with a modified hTERT expression vector at a relatively early stage (passage 28) in the presence of selective antibiotic. ULTR cell growth rate, with (ULTR-hT) or without transfection, was determined by plating cells in multiple plates at a fixed density and counting cell numbers after 7 days. Single cell clones of stably transfected cells were further isolated by plating in 96 well plates. Expression of hTERT, smooth muscle specific genes (SMC-SGs), and target genes was identified by RT-PCR of total RNA extracted from ULTR and ULTR-hT cells.

Results: RT-PCR demonstrated successful introduction of hTERT into ULTR cells. The growth rate of ULTR-hT cells was increased and cell morphology was improved (free of the typical aneupoid appearance). At passage 37, ULTR-hT cells grew 3.1 fold ($p<0.0001$) and 26.9 fold ($p<0.0001$) faster than ULTR cells at passages 28 and 34, respectively. Currently 8 single cell clones have been selected. ULTR-hT cells express a set of SMC-SGs, including α-actin, caldesmon, calponin, myosin heavy chain, SM22, and smoothelin, confirming that the smooth muscle phenotype is preserved. A panel of genes involved in Angiotensin II signalling, including Angiotensin II receptors (AT1/2), Nox family (Nox1, 4, 5 and Duox1), Nox-associated genes (p22phox, p47phox, p67phox, and Rac1/2), was also preserved.

Conclusion: This is the first report of rescuing uterine smooth muscle cell replicative senescence via activation of telomerase. We have established a human uterine smooth muscle cell line which will provide an improved *in vitro* model for studying human myometrium.

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Effect of Protein Kinase C on Spontaneous Contractions and $[Ca^{2+}]_i$ Transients in Pregnant Mice and Human Myometrium. Victor P Fomin,[1] Catherine Kirn-Safran,[1] Andrew Matamoros,[1] Matthew Hoffman.[2] *[1]Biological Sciences, University of Delaware, Newark, DE, USA; [2]Obstetrics and Gynecology, Christiana Care Health Services, Newark, DE, USA.*

At term, myometrium is characterized by spontaneous contractions which vary in the amplitude, frequency and duration depending on specie and hormonal status. Repetitive depolarization of plasma membrane followed by transient elevation in $[Ca^{2+}]_i$ is thought to underlie the contractions. However, the detailed pathways which regulate the spontaneous contractility remain unclear.

Objective: This study was designed to elucidate the effect of protein kinase C (PKC) on the spontaneous contractions and $[Ca^{2+}]_i$ transients in the myometria from term pregnant mice and women.

Methods: The human samples were obtained from women undergoing cesarean section with the approval from the Institutional Review Board Committee while mice myometria were dissected from the 18 days pregnant mice sacrificed according to the Animal Study Protocol. The isometric force and $[Ca^{2+}]_i$ were measured simultaneously in fura-2 loaded myometrial strips using spectrofluorometer equipped with force transducer.

Results: The PKC activator phorbol 12,13-dibutyrate (PDBu) applied at 10^{-7}M first stimulated the amplitude of spontaneous contractions in mice and human myometria followed by their inhibition. Under the PDBu treatment the frequency of the contractions was first increased and then decreased in both species. At the same time, PDBu didn't initially increase the amplitude of $[Ca^{2+}]_i$ transients but attenuated it over time. However, the frequency of the transients was first increased and later decreased upon the PDBu exposure. In addition, PKC activation with PDBu resulted in the elevation of the uterine basal tone without corresponding changes in the basal level of $[Ca^{2+}]_i$ in human myometrium. In mice myometrium, on the contrary, PKC activation didn't result in an increase of the muscle tone and the basal level of $[Ca^{2+}]_i$.

Conclusions: We propose that PKC causes bi-phasic effect on uterine spontaneous contraction in mice and human first to potentiate the amplitudes and the frequencies and later decreases them. The amplitude of $[Ca^{2+}]_i$ was not initially potentiated by PDBu suggestive of the dissociation of the contractile and $[Ca^{2+}]_i$ response. The stimulatory effect of PDBu on the basal muscle tone in human myometrium and the absence of the effect in mouse suggest different mode of PKC action on uterine contraction in human and mice.

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Inhibition of FAK Does Not Affect the Stretch Induced MAPK Cascade in Primary Human Uterine Smooth Muscle Cells. Benjamin Cryar, Suren Sooranna, Mark Tattersall, Philip Bennett, Mark Johnson. *Imperial College, London, United Kingdom.*

Introduction: Mechanical stretch of uterine myocytes is detected through integrins on the cell surface which form part of the focal adhesion complex, this signals through MAPK, ultimately leading to the up-regulation of various pro-labour genes including PGHS-2 and IL-8. On integrin activation FAK is recruited to the focal adhesion complex and activated by autophosphorylation at tyr-397, creating a Src binding site. This promotes further FAK phosphorylation at tyr-576, 577 and 925, enhancing FAK catalytic activity. However FAK can also act as a scaffold protein and its exact role in the expression of pro-labour genes is uncertain. In this study we used inhibitors for the kinase activity of FAK and MEK 1/2 in order to test the affect of FAK kinase activity on expression on PGHS-2 mRNA.

Methods: Primary human myometrial cell cultures were grown from myometrial biopsies taken from women undergoing elective caesarean section. Cells were plated onto 6-well flexible bottom plates coated in type I collagen. Cells were subjected to 11% static stretch for up to 60 minutes. Cells were also incubated with either the Rho kinase inhibitor Y27632 or the MEK 1/2 inhibitor U0126 prior to being stretched. Western blots were performed using antibodies to FAK phospho-397, FAK phospho-925 and ERK 1/2 phospho-202/204, β-actin was used as a loading control. RNA was also extracted and levels of PGHS-2 measured using q-PCR.

Results: Stretch increased levels of phosphorylation at both tyr-397 and tyr-925 with the greatest increases occurring at 30 and 60 minutes. However the increase at tyr-397 appeared to be greater than at tyr-925. Incubation with the Rho kinase inhibitor reduced phosphorylation of FAK-397, however this did not affect phosphorylation of ERK 1/2 or stretch induced up regulation of PGHS-2 mRNA. In contrast incubation with the MEK 1/2 inhibitor reduced ERK 1/2 phosphorylation and expression of PGHS-2 mRNA, whilst also reducing FAK phosphorylation ($n=4$; $p=0.05$).

Conclusions: FAK has previously been shown to be important in activation of the stretch induced MAPK cascade and PGHS-2 expression. However these data suggest that while stretch causes FAK phosphorylation FAK kinase activity is not essential to PGHS-2 expression. This suggests FAK may be acting as a protein scaffold.

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Natural Progesterone but Not Its Metabolite 17α Hydroxyprogesterone Inhibits Spontaneous Contractions of Human Myometrium *In Vitro*. Laurie Anderson,[1] William Martin,[2] Scott M Nelson,[1] Jane E Norman.[1] [1]*Reproductive & Developmental Medicine;* [2]*IBLS, University of Glasgow, United Kingdom.*

Objective

Current evidence suggests that prolonged treatment with progesterone therapy may reduce the incidence of premature delivery[1]. The metabolite 17 alpha hydroxyprogesterone (17αHP) has been shown to reduce the preterm birth rate[2]. Our aim was to examine the effects of both natural progesterone and 17αHP on spontaneous myometrial contractions.

Methods

Myometrial biopsies were taken with informed consent and ethics approval from non-labouring women at elective caesarean section ≥37 weeks gestation. Strips of myometrium 15 mm long ,2 mm wide were cut and suspended under a resting tension of 20 mN in organ baths of Krebs gassed with 95% O^2/ 5% CO^2 within 12 hours of collection. Progesterone or 17αHP were added in a cumulative manner at 20-minute intervals. Changes in amplitude were recorded. Results were compared using ANOVA.

Results

Following equilibration for 2 hours, myometrial strips contracted in a rhythmic manner (amplitude 91.6 ±16.1 mN, n=8 pairs). Progesterone (10nM-30μM) produced a concentration dependent inhibitory effect on myometrial contractions (Fig1), which was greater than that of vehicle ($p<0.05$). Maximum inhibition measured 93.3 ± 2.0% and 67.2 ±14.2% for progesterone and vehicle, respectively. 17αHP exerted an inhibitory effect, this was not significantly different from the vehicle ($p>0.05$).

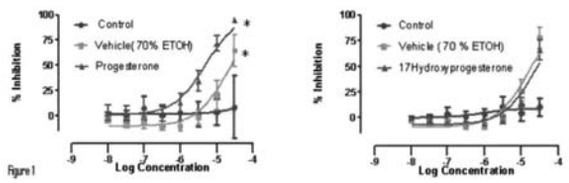

Figure1

Conclusion

Progesterone exerts an inhibitory effect on myometrial contractility *in vitro*. This is apparent within minutes suggesting a nongenomic action. Our data are in agreement with some reports in the literature but conflict with others[3]. This acute inhibition of myometrial contractility may contribute to the mechanism by which progesterone prevents preterm birth. In contrast, we were unable to demonstrate an inhibitory effect of 17αHP on contractility despite its demonstrated ability to reduce the incidence of preterm delivery[4]. Our data suggest that natural progesterone may be a more effective tocolytic agent in the acute setting than 17αHP.

[1] Sanchez Ramos et al 2005
[2] Meis et al 2005
[3] Mesiano S 2007
[4] Elovitz at al 2006.

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Monocyte Chemotactic Protein (MCP) 1 in Human Myometrium. Boonsri Chanrachakul,[1] Domerudee Preechapornprasert,[1] Patama Promsonthi,[1] Wasun Chantratita,[2] Mana Rochanawutanon,[2] Patcharee Karnsombut,[2] Chutatip Srichunrusami,[2] Stephen J Lye.[3] [1]*Obstetrics and Gynecology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand;* [2]*Pathology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand;* [3]*Obstetrics and Gynecology, Samuel Lunenfeld Research Institute, Toronto, Canada.*

Objective: Chemokines has been shown to play an important role in regulating uterine function. Recent evidence demonstrates that monocyte chemotactic protein (MCP) 1 level in amniotic fluid increases during spontaneous labor. The aim of this study was to examine the expression of MCP 1 in human myometrium.

Methods: Myometrial biopsies were taken from nonpregnant women undergoing hysterectomy and term pregnant women undergoing cesarean section followed written consent and local ethics committee approval. Elective cesarean section was performed before the onset of labor while emegency section was done after the onset of labor. Immunolocalization (n = 5 each) was performed on paraffin sections by avidin biotin complex (ABC) technique using monoclonal antibody specific to human MCP 1. Reverse transcription-polymerase chain reaction (n = 10 each) using gene specific primer against MCP 1 and MCP 1 receptor was performed to identify MCP 1 messenger(m) RNA in human myometrium .

Results: Immunohistochemical findings demonstrated MCP 1 in human myometrial cells from nonpregnant, term pregnant women before and after the onset of labor. MCP 1 was labelled on plasma membrane and cytoplasm of myocytes from these three groups of women. Similarly, MCP 1 and MCP 1 receptor mRNA were found in nonpregnant and term pregnant women before and after the onset of labor.

Conclusion: MCP 1 and MCP 1 receptor were expressed in myometrial cells of nonpregnant and term pregnant women with and without labor. Further studies are conducted to examined the role of MCP 1 in human parturition process.

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Women Have Elevated Lipid Concentrations 5-10 Years after Preterm Births Not Complicated by Preeclampsia or Growth Restriction. Janet M Catov,[1] James M Roberts,[1,3] Roberta B Ness.[1,2] [1]*Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA;* [2]*Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA;* [3]*Magee-Womens Research Institute, Pittsburgh, PA, USA.*

Objective: Women with preterm birth (PTB) are at increased risk for cardiovascular disease, but mechanisms relating these conditions are not understood. Women with spontaneous PTB have dyslipidemia early in gestation, but it is unknown if lipid aberrations persist post partum.

Methods: We compared 47 women who delivered preterm (<34 weeks, n=37; 34-<37 weeks, n=10) to 104 women with term births (≥37 weeks). Women with preexisting medical conditions, preeclampsia or growth restriction were excluded. Concentrations of total cholesterol, triglycerides, LDL-C, and HDL-C measured in fasting serum, collected on average 7.4 years (SD 2.3) post partum, were compared using ANOVA. Linear regression was utilized to evaluate lipid concentrations according to preterm status, adjusting for relevant covariates. Logistic regression was used to model the prevalence of hyperlipidemia in women with a prior preterm versus term birth.

Results: Women with a previous preterm vs. term birth had higher concentrations of total cholesterol, and women with PTB <34 weeks had the highest concentrations (<34 weeks, 202.6.1 [SD 42.9]; 34-<37 weeks, 190.1 [35.0]; ≥37 weeks, 180.1 [34.9]; $p<0.01$). Results were similar for LDL-C (<34 weeks, 120.7 [51.6]; 34-<34 weeks, 108.9 [31.1]; ≥37 weeks, 105.5 [30.4]; $p=0.04$). After adjustment for race, age, smoking and BMI, total cholesterol among women with a previous PTB <34 weeks was on average 20.3 mg/dl higher ($p<0.01$) and LDL was 15.3 mg/dl higher ($p=0.01$) compared to women with term births. Women with previous PTB (<37 weeks) were 2.3 (95% CI 0.7, 7.5) times more likely to have cholesterol >240 mg/dl compared to women with term births, after covariate adjustment. Similarly, women with PTB were 3.3 times (95% CI 0.9, 12.5) more likely to have LDL >160 mg/dl. Triglyceride concentrations tended to be higher among women with PTB <34 weeks compared to women with term births, but these results were not significant. There were no differences in HDL-C according to preterm birth status.

Conclusions: Seven years post partum, women with a history of spontaneous PTB had substantially elevated total and LDL cholesterol levels. Lipid aberrations following PTB may be a mechanism linking PTB to later life cardiovascular disease.

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Antenatal Diagnosis of IUGR in a Preeclamptic Population. Jamie Bastek, Sindhu K Srinivas, Michal A Elovitz. *OBGYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

OBJECTIVE: Preeclampsia (PEC) is a known risk factor for IUGR. Classification of PEC becomes severe by ACOG criteria upon antenatal diagnosis of IUGR impacting timing of delivery. Therefore, we sought to assess the accuracy of ultrasound (US) diagnosis of IUGR infants born to preeclamptic mothers and whether other obstetric, demographic or biological factors affect correct antenatal diagnosis.

STUDY DESIGN: 441 women admitted with PEC (gestational hypertension [GHTN], mild PEC [M-PEC], severe pre-eclampsia [S-PEC]) were included in this analysis. Patients without a growth ultrasound at our institution were excluded. Severity of PEC was based on pre-specified maternal criteria and did not include IUGR. IUGR infants (IUGR10) by actual birth weight were compared to normally grown infants. Associations between accurate antenatal diagnosis of IUGR and severity of PEC, gestational age (GA) at the time of ultrasound, maternal age, race, BMI, CHTN, and tobacco use were investigated with chi square analyses. Test characteristics were performed to determine accuracy of sonographic diagnosis of IUGR as a function of both severity of PEC and GA at delivery.

RESULTS: Of the 441 pregnancies, 190 were eligible for analysis. 52 (27.4%) had infants IUGR10. 46% of infants with IUGR10 and 57% of infants with IUGR5 were accurately diagnosed antenatally. There were significant associations between IUGR by birth weight and US diagnosis of IUGR. No significant association between maternal age, race, BMI, CHTN, and tobacco use and the accuracy of antenatal diagnosis was demonstrated. Severity of PEC (P=0.004) and delivery <34 weeks (P=0.0001) were significantly associated with accurate US diagnosis of IUGR. The test characteristics obtained for women with M-PEC compared to S-PEC were significantly different as they were for women delivering before compared to after 34 weeks. (See Table).
CONCLUSION: The accuracy of US diagnosis of IUGR in PEC varies with disease severity and GA at presentation. When incorporating US results into the management of women with PEC, the variability of US as a diagnostic tool should be considered.

Test Characteristics of Ultrasound in Antenatal Diagnosis of IUGR

	N	Prevalence	Sensitivity	Specificity	PPV	NPV
Mild disease* (GHTN + M-PEC)	32	24.1%	14.3%	86.4%	25.0%	76.0%
Severe Disease * (S-PEV + HELLP)	131	31.6%	70.0%	90.8%	77.8%	86.8%
Delivery < 34 wks	41	36.6%	66.7%	96.1%	90.9%	83.3%
Delivery > 34 wks	149	24.0%	37.0%	92.9%	63.6%	81.9%

* Analysis involves only women with growth scan performed within 3 weeks of delivery.

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Fetal Growth Restriction and Development of Fetal Anemia. Kimberly W Hickey,[1] Dennis Amnini,[1] Renee Evans,[2] Sarah H Poggi,[2] Alessandro Ghidini.[2] *[1]Maternal Fetal Medicine, Georgetown University Hospital, Washington, DC, USA; [2]Perinatal Diagnostic Center, Inova Alexandria Hospital, Alexandria, VA, USA.*
Background: Fetal growth restriction (FGR) is a process associated initially with fetal polycythemia and subsequently with anemia. Peak systolic velocity (PSV) at the MCA has been shown to correlate with severity of fetal anemia in a variety of conditions. We followed the natural history of fetal anemia as gathered by MCA-PSV in pregnancies affected by FGR.
Study Design: In a cohort of singleton pregnancies diagnosed with FGR based on estimated fetal weight (EFW) of less than 10th centile or abdominal circumference (AC) less than 5th centile (n=42), demographic, clinical, and sonographic data (including uterine, umbilical, and MCA Doppler indices) were obtained. Evaluation for alternate etiologies of FGR and fetal anemia were undertaken with TORCH titers, evaluation for maternal anemia, and evaluation of fetomaternal hemorrhage. Neonatal outcomes and hematocrits were obtained as indicated at the time of delivery. Neonatal anemia was defined as hematocrit at birth below normative values for gestational age. Statistical analysis included Student's t test and Fisher's exact.
Results: Pregnancies complicated by FGR and neonatal anemia (n=7) had a significantly higher maternal BMI (p=0.005), higher rate of NICU admissions (p=0.031), and major neonatal complications (p=0.031) than those without anemia. Gestational age at birth (34.8+/- 4.0 vs 36.9 +/- 3.0 weeks, P=0.104) and birth weight (1800 +/- 733 g vs 2251 +/- 642, P= 0.109) were not significantly different between the two groups. In cases where MCA-PSV was repeatedly assessed, there is a significant difference between development of anemia and persistence at time of delivery (n=5) vs neonates with anemia during evaluation but resolution by MCA PSV and absence of anemia at the time of delivery (n=21) (p=0.0128).
Conclusions: Pregnancies complicated by FGR and fetal anemia have increased evidence of neonatal morbidity than those not complicated by anemia. PSV in the MCA may be used as an additional indicator of perinatal risk in FGR cases.

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The Ultrasound Evolution of Fetal Abdominal Circumference as Tool for Detecting Macrosomia. Caterina Bocchi, Carlotta Boni, Alberto Imperatore, Michela Torricelli, Gilda Filardi, Filiberto M Severi, Felice Petraglia. *Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Siena, Italy.*
Objective. Macrosomia describes a fetus with a birthweight greater than 4000 g, althought numerous other definitions exist. No clear consensus to predict a macrosomic fetus exists, althought ultrasound is commonly requested by women and clinicians when a large baby is suspected.
Study design. In this prospective study a group of 4075 fetuses was consecutively enrolled. One ultrasound examination was performed to each patient within 6 days from delivery. We considered fetal macrosomia a birthweight ≥ 4000g and big babies a birthweight ≥ 4500g. Cut-off points for identifying the best value of fetal abdominal circumference for fetal macrosomia prediction were chosen by receiving operator characteristics' curve (ROC) analysis. Using the best cut-off indicated by ROC analysis, specificity and sensitivity were calculated.
Results. Mean gestational age at delivery was 38^{+3} weeks (3^{+3} sd). 1015 neonates weighted less than 2500 g, 2818 had a weight range between 2500 and 3999 g and 242 weighted more than 3999 g. The fetal AC measurement was the selected criterium to evaluate the risk of fetal macrosomia. To identify macrosomic fetuses the ROC curve analysis identified a cut off of AC > 350 mm which allowed to select 867 fetuses. Analysing this population we found 3183 true negative cases, 25 false negatives, 650 false positives and 217 true positives, with a sensitivity of 89.6% and a specificity of 83%. To detect big babies the ROC curve analysis identified a cut off of AC > 361 mm (n.452 fetuses), reaching a sensitivity of 100% and a specificity of 90%, without false negative cases and with 413 false positives (39 true positives, 3623 true negatives). The 141 cases that weighted between 4000 and 4499 g, were included in the 413 cases considered false positives by this cut-off.
Conclusions. These results clearly indicated that ultrasounds alone can not be used to manage a pregnancy suspected for fetal macrosomia or big babies. In fact, to avoid shoulder dystocia and all other complications strictly related to macrosomic fetuses, clinicians should perform a large number of useless elective cesarean sections.

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Sex Effects of Maternal Nutrient Restriction (MNR) on Renal Transcriptome Expression in the 0.9 Gestation (G) Fetal Baboon. Laura A Cox,[1] Jeremy Glenn,[1] Natalia E Schlabritz-Loutsevitch,[2] Peter W Nathanielsz,[2] Mark J Nijland.[2] *[1]Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA; [2]Ob/Gyn, CPNR, UTHSCSA, San Antonio, TX, USA.*
BACKGROUND: Adequate maternal nutrition is essential to normal fetal development. We previously reported outcomes of MNR for renal gene expression at 0.5G, including sex-specific effects in males (M), and the importance of the mTOR pathway. We present renal transcriptome analysis (TA) at 0.90G following MNR in the pregnant baboon.
METHODS: We analyzed whole genome expression using Illumina in M and female (F) fetuses of *ad lib* fed (C) and fetuses of MNR dams fed 70% of C from 0.16G to tissue collection at 0.9G.
RESULTS: TA showed 638 genes up- and 1048 down-regulated in CM vs CF (n=3,3; P<0.05). Pathway analysis of differentially expressed genes (PA; z-score ≥ 2.0) showed up-regulation of axon guidance, folate biosynthesis, nitrogen metabolism and down-regulation of steroid biosynthesis, insulin signaling, oxidative phosphorylation, TGF-beta signaling, and ubiquinone biosynthesis pathways in CM vs CF.
Comparison of MNR vs C in F showed 320 genes up- and 354 down-regulated (n=3,3; P<0.05). PA showed up-regulation steroid biosynthesis, fatty acid metabolism, glycolysis/gluconeogenesis, phosphatidylinositol signaling, ketone body metabolism, and ubiquinone biosynthesis pathways and down-regulation of bile acid biosynthesis, cell adhesion molecules, DNA polymerase, notch signaling and TI diabetes mellitus pathways in MNR vs C.
Comparison of MNR vs C in M showed 525 genes up- and 869 down-regulated (n=3,3; P<0.05). PA showed up-regulation of JAK-STAT signaling, autophagy, renin-angiotensin system (RAS), and ubiquinone biosynthesis pathways and down-regulation of apoptosis, basal transcription, folate biosynthesis, nitrogen metabolism, protein export, and SNARE interaction pathways in MNR vs C. Only the ubiquinone biosynthesis pathway up-regulation of MNR vs. C was common to both M and F.
CONCLUSIONS: TA and PA demonstrate sex-specific transcriptome expression in fetal kidneys at 0.9G. In addition, these results show sex-specificity in response to MNR. We have seen similar effects of MNR on gene expression for autophagy, apoptosis, RAS, ubiquinone and cell adhesion pathways at 0.5G, suggesting these pathways may contribute to persistent affects of MNR. Finally, we postulate that stress *in utero* may contribute to sex differences in risk of hypertension in adult life.

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Leptin and Neuropeptied Y Protein Expression Paradoxally Increased in Gestationall Food Restricted Dams. Louiza Belkacemi, Chun-Hung Chen, Andrea Jelks, Michael G Ross, Mina Desai. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*
Objective: Placental insufficiency is associated with marked increase in placental leptin production. This results in a rise in maternal leptin levels that serves as an early index of placental dysfunction. Further increased placental leptin and suppressed neuropeptide Y (NPY) are associated with preeclampsia. Leptin, an anorexigenic hormone and NPY, an orexigenic peptide regulate food intake. Importantly, leptin also serves as a placental and fetal growth factor. We have shown that maternal food restriction (MFR) results in intrauterine growth

restricted newborns that subsequently develop offspring obesity, hypertension and cardiovascular disease. Further, whilst maternal and fetal body weights are reduced at gestational ages E16 and E20, the placental weights remain unchanged in FR pregnancies. Despite the well recognized link between leptin and NPY on central appetite regulation, the role of leptin and NPY in MFR placentas during pregnancy is unknown. Therefore, we sought to determine impact of MFR on placental protein expression of leptin, its receptor (Ob-Rb) and NYP.

Methods: Pregnant rat dams were fed an ad libitum diet (control, n=5) or were 50% food restricted (MFR, n=7) relative to controls beginning at day 10 of gestation. On gestational days E16 and E20, maternal plasma leptin levels (RIA) and placental protein expression of leptin, Ob-Rb and NPY were determined using immunohistochemistry and Western blot analysis. Protein expression was normalized to β-actin and presented as mean of fold change ± SE.

Results: At both E16 and E20, plasma leptin levels were significantly decreased in MFR as compared to control dams (E16: 0.71±0.13 vs. 1.39±0.18 ng/ml; E20: 3.5±0.5 vs. 1.5±0.2 ng/ml; $p<0.05$). Furthermore, placental protein expression of leptin and NPY were significantly increased ($p<0.05$) whereas placental Ob-Rb was significantly reduced ($p<0.01$) in MFR pregnancies as compared to controls.

Conclusion: The increased placental leptin expression in MFR pregnancies may potentially serve as a compensatory mechanism to augment feto-placental growth and maternal-fetal nutrient transfer. Alternatively, it may portray placental insufficiency in MFR pregnancies. The paradoxical increased placental NPY protein expression suggests either immaturity or reduced leptin/NPY signaling pathway.

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Maternal Growth in Pregnant Teenagers Is Not Associated with Reduced Infant Birthweight. Rebecca L Jones,[1] Simon J Wheeler,[2] Lucilla Poston,[2] Cindy Hutchinson,[2] Georgina Bennett,[1] Gemma Wild,[1] Lorna Carruthers,[1] Annette Briley,[2] Robert Oliver,[3] Kirk Siddals,[3] Martin Gibson,[3] Philip N Baker.[1] *[1]Maternal & Fetal Health Research Group, University of Manchester, United Kingdom; [2]Maternal & Fetal Research Unit, King's College London, United Kingdom; [3]Department of Diabetes & Endocrinology, University of Manchester, United Kingdom.*

Background Teenagers are more likely to deliver small-for-gestational age (SGA) infants than adults, even after adjustment for socioeconomic factors[1,2]. Previous studies of mostly black and Hispanic subjects in the USA have suggested that maternal growth may contribute to reduced infant birthweight, due to preferential nutrient partitioning to the mother[3]. The impact of maternal growth on birthweight and nutrient partitioning in pregnant teenagers in the UK has not been examined.

Methods Skeletal growth (change in knee-height from 1st to 3rd trimester), weight gain and skinfold thicknesses were measured in pregnant teenagers (n=369, 45% non-white) in London and Manchester. Key mediators of nutrient partitioning and metabolism: insulin-like growth factor(IGF)-1, IGF binding protein(BP)-1 and leptin, were measured in maternal plasma (28 weeks gestation).

Results Maternal growth (defined as increase in knee-height >2mm/90days) was detected in 36% of pregnant teenagers. This growth was not associated with SGA birth; in fact these mothers were more likely to deliver large-for-gestational age (LGA) infants ($p<0.05$). Maternal weight gain and fat accrual at peripheral and central sites were greater in growers ($p<0.01$). These parameters correlated positively with maternal IGF-1 and leptin but negatively with the IGF inhibitor, IGFBP-1 ($p<0.001$ for all). Subjects delivering SGA infants gained significantly less weight ($p<0.01$) and had lower IGF-1 levels ($p<0.01$) than those delivering non-SGA infants.

Conclusion Maternal growth in teenage pregnancy was not associated with reduced birthweight. Indeed the increased weight gain and fat accrual observed in growing teenagers may protect against SGA birth and promote fetal growth. IGF-1 and leptin promote fetal growth, primarily through effects on maternal metabolism and nutrient partitioning to the fetoplacental unit. These data suggest that higher maternal IGF-1 and leptin in growing teenagers may provide an anabolic drive for both maternal and fetal growth.

[1]Fraser et al 1995 NEJM 332:1113;[2]Chen et al 2007 Int J Epidemiol 36:368;[3]Scholl et al 1994, Am J Clin Nutr, 60:183.

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Evalutation of Oxygen Uptake in IUGR Fetuses. Tatjana Radaelli, Simona Boito, Emanuela Taricco, Veronica Cozzi, Gioia Alvino, Anna Martinelli, Valenina Signorelli, Enrico Iurlaro, Giorgio Pardi, Irene Cetin. *Dept Obstetrics & Gynecology "L.Mangiagalli", IRCCS Policlinico, Mangiagallie Reina Elena, Milan, Italy.*

Background. Umbilical oxygen uptake (O_2 umb uptake) has been estimated in human pregnancies only in acute experiments at the time of caesarean section. The recently developed possibility to measure umbilical blood flow by ultrasound in utero, prompted us to study normal and IUGR pregnancies in order to evaluate fetal oxygen uptake utilizing the Fick Principle, i.e. uptake equals umbilical blood flow times (a-v) differences.

Methods. Thirty-six IUGR pregnancies were studied at the time of elective caesarean section and compared to twenty-one controls (C) (gestational age: C=38.9±0.2 and IUGR=32.0±0.5 wks). An ultrasound examination was performed within 4 hours from the caesarean section in all the recruited patients. Umbilical vein absolute volume flow (Qumb) was measured as the result between umbilical vein area and the time-averaged peak velocity * 0.5. Blood samples from umbilical vein (uv) and artery (ua) were obtained after the delivery and blood gases and acid-base balance were evaluated. Umbilical oxygen uptake was calculated as O_2 umb uptake = Qumb*(uv-ua) O_2Content.

Results. As expected, average fetal and placental weights were significantly different in the studied groups (1287±83 and 254±32 g in IUGR vs 3274±62 and 474±16 g in N). IUGR pregnancies showed a significant reduction in Qumb (98.5±6.6 vs 234.8±12.1 mL/min; $p<0.01$) but no differences in the Qumb/kg of fetal weight. IUGR fetuses showed a significant reduction in O_2Sat, O_2Cont and pO_2 in both uv and ua compared to N. (uv-ua)O_2Content (1.79±0.72 vs 3.3±0.24 mmol/L; $p<0.001$) and O_2 umb uptake/Kg (0.12±0.01 vs 0.24±0.01 mmol/min/Kg; $p<0.01$) were significantly reduced in IUGR.

Conclusions. We here report an evaluation of fetal oxygen uptake that proved surprisingly similar to the values reported in chronically catheterized animals. However, IUGR fetuses showed a significant reduction in both blood and oxygen supply: this latter was reduced more that 50% on a per kg basis. IUGR fetuses therefore utilize less oxygen than normally grown fetuses.

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Circulating Levels of Vitamin D and IL-6 in Pregnancies with IUGR. Calvin J Hobel,[2] Chander P Arora,[1] Adegoke Adeniji,[1] Priya Arora,[1] Susan E Jackman,[1] Olga Miadel,[1] Baldjyan Lilit.[1] *[1]Ob-Gyn, Cedars-Sinai Medical Center, Burns ans Allen Research Institute, Los Angeles, CA, USA; [2]University of California Los Angeles, Los Angeles, CA, USA.*

BACKGROUND: Any condition resulting in under exposure to sunlight, including the use of sun block or poor nutrition may result in insufficiency (37.5-80 nmol/l) or even deficiency of vitamin D (<37.5 nmol/l).Vitamin D regulates placental development and function. Vitamin D deficiency has been linked to increased risk of serious chronic and inflammatory diseases.

OBJECTIVE: To determine if the circulating levels of vitamin D and interleukin -6 (IL-6) in maternal plasma correlates to pregnancies resulting in fetus with Intrauterine growth restriction (IUGR).

HYPOTHESIS: The metabolism of vitamin D initiates the biochemical cascade of events leading to the expression of IL-6 and the inflammatory response in IUGR births.

STUDY DESIGN: In a behavior in pregnancy study, plasma samples at all three time points were analyzed in a cohort of women for 25(OH)D using ELISA. The samples were also analyzed for IL-6 at three stages of pregnancy: T1 (18-20 weeks), T2 (28-30 weeks) and T3 (34-36 weeks). IUGR was defined as birth weight below the tenth percentile for gestational age. None of the IUGR cases had spontaneous preterm birth.

RESULTS: IUGR was diagnosed in 18 of 528 women with available samples from a behavior in pregnancy study (BIPS). Out of these subjects, 22 were selected as matched case controls. Circulating levels of vitamin D (25(OH)D) were significantly lower in IUGR cases at each visit ($p<.001$). The levels indicated deficient (29.4±4.6nmol/l) vitamin D in IUGR group at T1 but sufficient vitamin D levels (83.8±5.8 nmol/l) in controls. Subsequent visits also showed lower levels in the IUGR cases compared to the control group (T2: 19± 3.1 nmol/l vs 45±3.6nmol /l; T3: 22 ± 4.1nmol/l vs 50± 2.9nmol /l). At all three time intervals, significantly ($p<.001$) higher levels of IL-6 were associated with the IUGR cases (415pg/ml, 1329pg/ml and 2526 pg/ml respectively) as compared to the controls (60pg/ml, 330pg/ml and 1240pg/ml respectively).

CONCLUSIONS: Vitamin D deficiency or even insufficiency may be an unrecognized cause of IUGR. It is possible that a primary non-infectious

inflammatory process is activated by vitamin D deficiency. Combined assessment of vitamin D deficiency and IL-6 expression during different stages of pregnancy may facilitate the resognition of the risk of developing IUGR.

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IGF-II and Insulin (Ins) Are Decreased in the Baboon Fetal Pancreas in Response to Global 30% Maternal Nutrient Restriction (MNR). Nathan Drever, Thomas J McDonald, Peter W Nathanielsz, Cun Li. *Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.*

Background: IGF-II is a major growth factor in the developing pancreas and studies in the human fetus demonstrate that the peptide localizes to B cells of the islets (J Endocrinology 165:2). Rodent studies show decreased fetal pancreatic growth and IGF-II and Ins abundance and increased apoptosis with MNR (J Endocrinology 140:10). We previously demonstrated a fall in most components of the placental and fetal baboon liver IGF systems with MNR. Here we have evaluated fetal pancreatic IGF-II and Ins changes in response to MNR. **Methods**: Pregnant baboons were fed *ad lib* (CTR, n=12) or 70% of wt adjusted CTR diet (MNR, n=11) from 0.16 gestation (G) and fetuses were recovered at C-section under general anesthesia at 0.5 (n=9; 5 CTR and 4 MNR) and 0.9G (n=14; 7 CTR and 7 MNR). IGF-II and Ins expression were determined by immunohistochemistry (IHC) and quantified by image analysis for fraction (area immunostained/area of the field x 100%) and density. Data are expressed as mean ± SEM; CTR data are expressed] first; comparison made with two tailed t-test. **Results:** At 0.5G there was no difference in IGF-II or Insulin fraction or density between groups. At 0.9G, IGF-II fraction (2.57 ± 0.42 vs 1.44±0.27,P<0.05) and density ($1.02x10^7$±$1.90 x10^6$ vs $5.19x10^6$±$9.50x10^5$, P<0.05) and Insulin fraction (2.51 ± 0.25 vs 1.58 ± 0.05, P=0.05) were reduced. **Conclusion:** Moderate MNR decreases abundance of fetal pancreatic IGF-II and Ins at 0.9G. in the fetal baboon and supports the extant evidence for impaired pancreatic development with MNR seen in rodents.

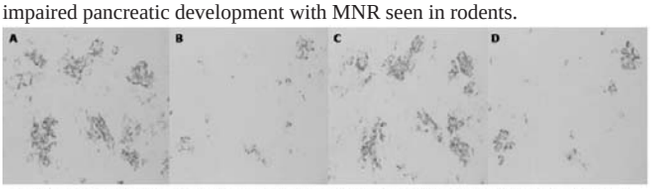

Figure1. IGF-II (A & B) and Insulin (C & D) expression in pancreas taken at 0.9 of gestation, from fetuses of control (CTR; *ad lib* fed ; A & C) or nutrient restricted (fed 70% CTR diet; B & D) baboon mothers. Photomicrographs taken with 20X objective.

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Maintenance of Liver Growth in the Hypoxic Growth Restricted Fetal Sheep: A Role for Intrahepatic GLUT1? Sheridan Gentili, Janna L Morrison, I Caroline McMillen. *Sansom Institute, UniSA, Adelaide, South Australia, Australia.*

Objective: We have previously demonstrated that there is a differential tissue response to chronic placental and fetal growth restriction. Growth of fetal tissues such as the brain and the adrenal are consistently spared in the face of chronic substrate restriction, whilst we have demonstrated that the growth of the fetal liver may be either maintained or reduced. It is unclear whether the growth response of the fetal liver to hypoxia and hypoglycemia are determined by intrahepatic metabolic adaptations.

Hypothesis: We hypothesize that there will be a differential profile of hepatic expression of GLUT1, 11βHSD1, the gluconeogenic and glycolytic enzymes PEPCK and G3PDH and the transcription factors PGC1 and PPARα in animals in which liver growth is maintained or reduced.

Methods: Carunclectomy was performed in 19 non-pregnant ewes to induce placental restriction (PR). Vascular catheters were inserted in 19 PR and 9 control (C) fetuses at 103-117d and arterial blood samples were collected for blood gas analysis. Mean gestation PO_2<17mmHg was defined as hypoxic (H; normoxia, N). Post mortem was performed at 140-145d. Hepatic mRNA expression of GLUT1, 11βHSD1, PEPCK, G3PDH, PGC1 and PPARα was determined using qRT-PCR.

Results: Four experimental groups were defined by fetal PO_2 and liver growth (C-N, PR-N, PR-H and PR-H-Reduced Liver Growth). Fetal weight correlated with mean gestational PO_2 (R^2=0.67, y=0.21x+0.5, P<0.01). Liver weight was significantly lower in a cohort of PR-H fetuses (C, 24.1±1.6; PR-N 22.9±0.9; PR-H 22.0±1.6; PR-H-RLG 15.8±0.3g:Kg; P<0.05). GLUT1 expression was highest in those PR-H fetuses in which liver growth was maintained, whilst the expression of 11βHSD1, PGC1, PPARα and PEPCK was highest in the PR-H fetuses in which liver growth was reduced (P<0.05).

Conclusions: In the PR-H fetuses in which liver growth was reduced, the increase in 11βHSD1 expression may be associated with an increase in hepatic exposure to cortisol and an associated increase in PEPCK, PGC1 and PPARα. Interestingly the compensatory increase in hepatic GLUT1 expression did not occur in fetuses in which liver growth was reduced.

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Predicting the Trajectory of Fetal Growth. Racine N Edwards-Silva,[1] Jeffrey Gornbein,[2] Calvin J Hobel.[3] *[1]Obstetrics & Gynecology, Cedars-Sinai Medical Center, Los Angeles, CA, USA; [2]Biomathematics, David Geffen School of Medicine at University of California, Los Angeles, CA, USA; [3]Obstetrics & Gynecology, Cedars-Sinai Medical Center, David Geffen School of Medicine at University of California, Los Angeles, CA, USA.*

Objective: To evaluate twelve potential predictors of fetal growth trajectory defined as the rate of fetal weight change over time.

Study Design: A longitudinal prospective study of singleton fetal growth trajectory. The twelve potential predictors considered were: fetal gender, gestational age, parity, race, BMI, age, weight at 1st clinical exam, cumulative weight gain at each exam, smoking, and alcohol use. Estimated fetal weight was computed using the Hadlock formula and 4 sonographic fetal biometric parameters at 18-20 weeks, 28-30 weeks, and 34-36 weeks. The rate of change in fetal weight was defined as 60 x (fetal wt at exam j – fetal wt at exam i)(j > i)/ (gestational age at exam j – gestational age at exam i). Bivariate Statistical analysis included the non-parametric Spearman rank correlation and Wilcoxon rank sum test. All factors were assessed multivariately using multiple linear regression.

Results: There were 454 multi-ethnic women included in the study, after 112 were excluded. They underwent a total of 1,277 exams. Fetal gender (p=0.0048), maternal weight at 1st exam (p=0.0055), and cumulative maternal weight gain at exam 3 (p < 0.001) were significant predictors of fetal growth trajectory. In this model, male fetuses had an average rate of fetal weight change of 46.9 grams per 60 days higher than females. The rate of fetal weight change increased by an average of 8.8 grams per 60 days for each 10 lb increase in maternal weight at the 1st exam. The fetal growth rate increased 2.2 grams per 60 days for each 1 lb of cumulative weight gain at the 3rd exam.

Conclusions: In this study, maternal weight at the 1st exam and cumulative maternal weight gain were the significant determinants of fetal growth trajectory. Adequate initial maternal weight and cumulative gestational weight gains probably ensure sufficient nourishment for normal placental growth, uteroplacental blood flow, and fetal nutrient uptake. This supports the emphasis on periconceptual nutritional counseling for all pregnant women. The implication of this study is that similar to fetal programming of adult diseases, there is nutritional programming of fetal growth trajectory.

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Predictors of Low Birthweight in New Mexico: Outcomes from 422,420 Pregnancies over Ten Years. Armida Moreno,[1] Ronald Schrader,[1] Kimberlie Peters,[2] Kara Gwin,[1] Kimberly K Leslie.[1] *[1]University of New Mexico, Albuquerque, NM, USA; [2]Office of New Mexico Vital Records and Health Statistics, Santa Fe, NM, USA.*

Objective: To determine predictors of low birthweight (LBW) in New Mexico (NM), a relatively poor and rural state, where access to prenatal care and medical resources are limited compared to other regions of the US.

Methods: This was a cross-sectional analysis of 422,420 singleton live births in the state of NM from 1995-2005. The medical risk factors tracked were pulmonary, renal, cardiac, diabetes, eclampsia, oligo/polyhydramnios, hypertensive disorders, cervical incompetence, previous preterm delivery, tocolysis and isoimmunization. Birthweight <2500g was defined as LBW. Multiple gestations and congenital anomalies were excluded. The Kotelchuck Index was used as a measure for level of prenatal care described as inadequate, intermediate, adequate, and intensive. Multivariate logistic regression was conducted using SAS 9.1 statistical software.

Results: Statistically significant predictors for LBW in NM are age <15 and >40, single, tobacco/alcohol use, African American race, and the presence of one or more medical risk factors. Statistically significant protective factors for LBW are age 20-34, education surpassing high school, White Hispanic, and Native American race. Counties with high or rising adjusted LBW rates are Grant, Lea, and McKinley. Adjusted LBW rates are decreasing in Roosevelt, Sandoval, Torrance, and Valencia counties. Among patients with medical risk factors, LBW rate had a direct inverse relationship with prenatal care level. High risk patients with intensive care were 25% less likely to have a LBW delivery than similar patients with low levels of care.

Conclusion: LBW rates are elevated in NM residents compared to the US (8.9% vs. 8/1%), with lack of prenatal care being a significant predictor in

high risk patients. Other predictors include the known risk factors of age <15 and >40, single, tobacco/alcohol use, and African American race. Interestingly, Hispanic and Native American patients have a lower LBW rate compared to other groups.

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Moderate Maternal Global Nutrient Restriction (MNR) Decreases Fetal Baboon Liver b1-Adrenergic Receptor (b1-AR): Potential Effects on Insulin Sensitivity and Fat Metabolism. Cun Li,[1] Amrita Kamat,[2,3] Nathan Drever,[1] Thomas J McDonald,[1] Peter W Nathanielsz.[1] *[1]Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; [2]Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; [3]South Texas Veterans Health Care System Audie Muphy Hospital, San Antonio, TX, USA.*

Introduction: Catecholamines released by the sympathetic nervous system and adrenal medulla act via b-ARs to regulate glucose and insulin function in liver, pancreas, adipose and muscle tissue. b-AR knock out mice show increased fat mass and glucose intolerance (Asenslo et al.,Diabetes,2005). MNR animal models have increased sympathetic activity. We, therefore, evaluated effects of MNR on baboon fetal liver b1-AR.

Methods: Baboons were fed as *ad lib* controls (CTR) or 70% of wt adjusted CTR diet (MNR) from 0.16 gestation(G) with fetuses retrieved at C-section under general anesthesia at 0.5 or 0.9G. Protein expression determined by immunohistochemistry for b1-AR in the central liver lobule was quantified by image anaysis and expressed as fraction = area immuno-stained/area of the field x 100%. All data are expressed as mean ± SEM with CTR data presented first. Liver glycogen expression was determined by the periodic accid Schiiff (PAS) method. Comparisions were made with student's t-test with alpha level set at 0.05.

Results: Fetal body and liver wts were not changed by MNR at either age. PAS stained liver glycogen at 0.5G = 197.5 ± 1.7 vs. 162.8 ± 11.7%, p< 0.05 . b1-AR fraction was lower following MNR at 0.5G and at 0.9G (p< 0.05; Fig 1)

Conclusions: MNR decreased b1-AR over 50% at 0.5G and 20% at 0.9G. Decreased fetal liver b1-AR in MNR alters glucose metabolisam and may result in reduced lipolysis predisposing to fatty liver.

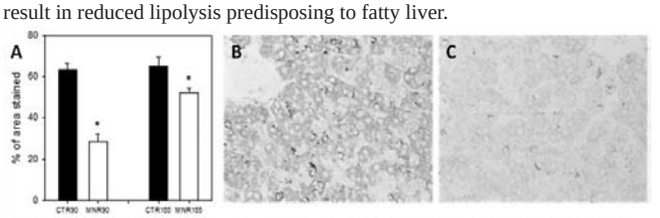

Fig 1. Protein expression of b1-AR livers of baboon fetuses from mothers fed as *ad lib* controls (CTR) or nutrient restricted (MNR; fed 70% CTR diet) from 0.16 of gestation (G) until C-section at 0.5 or 0.9 G. A) summary, and B & C, representative b1-AR expression in sections from CTR and MNR fetuses, respectively. Photomicrographs obtained with 20X objective.

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Adiponectin Is Associated with Low Fetal: Placental Index in Normal Human Pregnancy and in Mothers with Type 1 Diabetes. Scott M Nelson,[1] Dilys F Freeman,[1] Naveed Sattar,[2] Robert S Lindsay.[2] *[1]Reproductive and Maternal Medicine, University of Glasgow, United Kingdom; [2]BHF Glasgow Cardiovascular Research Centre, University of Glasgow, United Kingdom.*

Introduction: Fetal and placental weight are highly correlated. Alteration of the ratio of fetal and placental weight (low feto-placental index: FPI) is associated with increased risk of disease in later life. As fetal adiponectin has been implicated as a predictor of FPI, we hypothesised that adiponectin would predict FPI in both control pregnancies and offspring of mothers with type 1 diabetes (OT1DM), a group reported as having lower adiponectin and FPI, and further examined the relationship of FPI to other hormones associated with fetal and placental growth.

Methods: A prospective study incorporating 122 OT1DM and 46 controls, had concentrations of cord insulin, IGF-I, leptin, adiponectin, CRP, ICAM-1 and hematocrit measured and their relationship to placental weight and feto-placental index (FPI: fetal/placental weight) determined.

Results: Placental weight [control 634.7±133.3g; OT1DM 706.2±187.2g; p=0.019] and gestation adjusted birthweight (p<0.001) were increased in OT1DM, but with no significant difference in FPI [control 5.73±0.9; OT1DM 5.55±1.1; p=0.33]. Placental weight correlated with birthweight [control r=0.56 p<0.001; OT1DM r=0.60 p<0.001] and CRP [control 0.32 p=0.03; OT1DM 0.18, p=0.05], with a relationship to insulin in OT1DM only [r=0.45, p<0.001]. FPI correlated positively with adiponectin [control r=0.43, p=0.004; OT1DM r=0.19, p=0.04], an association which persisted with adjustment for confounders [control CTV:20.3% p=0.002; OT1DM CTV:3.9% p=0.03], however, there was no independent relationship to insulin or inflammatory markers.

Conclusions: A lower feto-placental index is associated with lower cord adiponectin levels in both controls and OT1DM. Whether adiponectin *in-utero* differentially affects fetal versus placental growth, or is a marker of other abnormalities, requires future study.

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Implications for the Type-I Interferon Pathway in Intrauterine Growth Restriction. Megan L Shoemaker,[1] Kathy J Austin,[2] Hana Van Campen,[1] Helle Bielefeldt-Ohmann,[1] Lea Rempel,[2] Natalia P Smirnova,[1] Hyungchul Han,[2] Donald J Montgomery,[4] Russel V Anthony,[1] Alberto Van Olphen,[2] Jeffery A Clapper,[3] Thomas R Hansen.[12] *[1]Dept. of Biomedical Sciences and Dept. of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA; [2]Dept. of Animal Sciences and Dept. of Veterinary Science, University of Wyoming, Laramie, WY, USA; [3]Dept. of Animal and Range Services, South Dakota State University, Brookings, SD, USA; [4]College of Public Health, University of South Florida, Tampa, FL, USA.*

Infection with noncytopathic bovine viral diarrhea virus (ncpBVDV) during early bovine pregnancy (<150d gestation) results in fetal immunotolerance, persistent infection (PI) and intrauterine growth restriction (IUGR). In contrast, infection after the development of adaptive immune competence (>150d gestation or postnatal) results in a transient infection (TI). We have previously reported an IUGR in PI fetuses presenting as decreased body weight and ponderal index. A growth defect can be the result of many factors, including placental insufficiency and nutrient restriction, however it was hypothesized that the IUGR seen in BVDV PI fetuses may be an immunopathological effect caused by the persisting virus. Our two part experimental design examined the relationship between BVDV and its PI host. In Experiment (Exp) 1, blood cell mRNA was collected from PI steers (n=2; confirmed by virus isolation), or uninfected control steers (n=3) and used to identify differentially expressed genes using microarray (Affymetrix) and qtRT-PCR approaches. In Exp2, BVDV naïve pregnant heifers (n=6 per group) were not infected (control) or infected with ncpBVDV on d.75 or d.175 of pregnancy creating PI and TI fetuses, respectively. Fetuses were collected by c-section on d.190; infection was confirmed by ELISA and qtRT-PCR. Histology of placental tissue revealed no placentitis or pathology, and glucose and lactate levels in fetal serum were normal. Microarray analysis revealed 294 genes that were differentially regulated in PI vs. controls (p<0.05, >1.5 fold). qtRT-PCR of steer and fetal blood revealed a significant upregulation of activators and products of the antiviral type-I interferon (IFN-I) pathway. As IFN-I can act as a growth-suppressive cytokine, a long-term upregulation may contribute to the IUGR seen in persistent BVDV infection and in other viral infections observed during pregnancy. NRICG 2006-03907 from the CSREES.

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Does a Short Cervix Increase the Risk for a Small for Gestational Age Neonate? Vivian Romero,[1] Sonia Hassan,[1] Clara Ward,[1] Juan Pedro Kusanovic,[2] Lorraine Nikita,[1] Shali Mazaki-Tovi,[1] Natalia Camacho,[1] Pooja Mittal,[1] Roberto Romero.[2] *[1]Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA; [2]Perinatology Research Branch, NICHD, NIH, DHHS, Bethesda, MD, USA.*

OBJECTIVE: Preterm delivery is associated with a higher frequency of SGA. Patients with a short cervix are at an increased risk for spontaneous preterm delivery. Therefore, it is possible that women with a short cervix during pregnancy are also at risk to deliver an SGA neonate. This study was conducted to address this question.

STUDY DESIGN: Patients >14 weeks of gestation were prospectively enrolled into an observational study (08/2002 to 06/2007). Transvaginal sonographic examinations were performed every 2 weeks until delivery. The shortest cervical length between 14-32 weeks was used for analysis. SGA was defined as less than the tenth percentile of birth weight.

RESULTS: 303 asymptomatic patients were studied. 19.8% of patients delivered an SGA neonate (60/303). The median cervical length was 21 mm (0 to 55mm); 184 patients had a cervical length <25mm. Of these, 17% (32/184) delivered an SGA neonate. Similarly, 17% (17/98) patients with a cervical length <15mm had an SGA neonate. No relationship was found between a short cervix and SGA (short cervix was defined as either < 25mm and <15 mm). The frequency of SGA was not significantly different between women with a short cervix and those with a long cervix [17% (32/184) vs. 24% (28/119); p=0.34].

CONCLUSION: A sonographic short cervix is a risk factor for preterm delivery, but not for SGA. This information is of value in patient counseling.

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Periconceptional Folic Acid Supplementation Significantly Affects Intrauterine Fetal Growth. Sarah Timmermans,[1,2] Vincent WV Jaddoe,[2,3,4] Albert Hofman,[3] Regine PM Steegers-Theunissen,[1,3,4,6] Eric AP Steegers.[1]
[1]Department of Obstetrics and Gynecology/Division of Obstetrics and Prenatale Medicine, Erasmus Medical Centre, Rotterdam, Netherlands; [2]The Generation R Study Group, Erasmus Medical Centre, Rotterdam, Netherlands; [3]Department of Epidemiology, Erasmus Medical Centre, Rotterdam, Netherlands; [4]Department of Pediatrics, Erasmus Medical Centre, Rotterdam, Netherlands; [5]Department of Clinical Genetics, Erasmus Medical Centre, Rotterdam, Netherlands.

Background Folate is an essential micronutrient for cellular growth. Recommendations on periconceptional folic acid use are mainly focussed on prevention of neural tube defects, despite growing evidence that folic acid use may have positive effects on birth weight.

Objective To examine associations between folic acid use, intrauterine fetal growth and birth weight.

Design The study was embedded in the Generation R Study in Rotterdam, the Netherlands, a population-based prospective cohort study from early pregnancy onwards.

Methods Information on folic acid use was obtained by questionnaires and categorized into three groups: 1) preconception start of folic acid use; 2) start of folic acid use in first ten weeks of gestation; 3) no folic acid use at all. Fetal growth measurements included head circumference, abdominal circumference and femur length measured in mid- and late pregnancy, i.e., gestational age 18-25 and >25 weeks, respectively, and birth weight. Fetal weight in mid- and late pregnancy was estimated using the Haddock method.

Results Data from 6,719 pregnant women were available. Overall, folic acid use was positively associated with fetal growth. Preconceptional folic acid use resulted in an increased growth of 6 grams (95%CI 2.92-9.01, p<0.001) per week from late pregnancy to birth, compared to no folic acid use. Similarly, start of folic acid use in the first ten weeks of gestation resulted in an increased growth of 5 grams (95%CI 2.19-7.88, p=0.001) per week from late pregnancy to birth. Both preconceptional folic acid use and folic acid use started in the first ten weeks of gestation resulted in higher birth weights of 69 grams (95%CI 39-99) and 51 grams (95%CI 23-80), respectively, compared to no folic acid use. A tendency was found for an increased risk of birth weight less than 2500 grams when folic acid was not started preconceptionally (OR 1.5, 95%CI 0.9-2.4).

Conclusion Periconceptional folic acid use is significantly associated with increased fetal growth resulting in a higher birth weight.

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Ductus Venosus Isovolumetric Relaxation in Severely Premature Growth-Restricted Fetuses. Jason L Picconi, Katherine Drennan, Farhan Hanif, Michael Kruger, Giancarlo Mari. *Obstetrics and Gynecology, Wayne State University/DMC, Detroit, MI, USA.*

Objective: Ductus venosus (DV) Doppler waveforms are characterized by two periods in which blood velocity decreases. The first represents the isovolumetric relaxation (IR) at the end of ventricular systole and the second represents atrial contraction at the end of ventricular diastole (a). Ductus venosus reversed flow (DVRF) occurring at the time of the a-wave is considered a risk factor for intrauterine fetal demise (IUFD). We have previously reported that absent or reversed a-wave flow can be present for weeks before IUFD occurs or delivery is performed for non-reassuring fetal testing. The guiding hypothesis for this study is that decreased flow at the time of IR in combination with absent or reversed a-wave flow allows a more accurate prediction of fetal outcome than a-wave absent or reversed flow alone.

Material and Methods: Ductus venosus Doppler was serially studied in 17 severely premature IUGR fetuses (estimated fetal weight < 10th percentile and umbilical artery pulsatility index > 95th percentile) from diagnosis until demise or delivery. Ductus venosus waveforms were assessed quantitatively for peak systolic velocity (PSV), isovolumetric relaxation velocity (IRV), and end diastolic velocity (EDV). The PSV/IRV + EDV were compared to fetal and neonatal outcome. A Kruskal-Wallis one way ANOVA, Mann Whitney U post-hoc test, and a ROC, were used for statistical analysis. A P < .05 was considered statistically significant.

Results: All fetuses were delivered at < 32 weeks. Six cases resulted in IUFD, five cases resulted in neonatal demise (ND), and six cases resulted in neonatal survival (NS) at the time of discharge from the hospital. The PSV/IRV+EDV correlated better than a-wave reversal of flow with perinatal outcome. A PSV/IRV+EDV score less than -1.8 resulted in IUFD, whereas a score greater than -1.8 resulted in live birth. Live births segregated based on estimated gestational age, where those fetuses at less than 28 weeks resulted in ND and those fetuses at or greater than 28 weeks resulted in NS. All results were statistically significant.

Conclusions: The isovolumetric relaxation velocity is a novel Doppler parameter in the assessment of severely premature IUGR fetuses. These data indicate that assessment of IRV should be considered part of the evaluation of severely IUGR fetuses.

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Is Umbilical Cord Collagen Gene Expression Altered by Experimental Fetal Growth Restriction? Wendy Kinzler, Lillian Kaminsky, Oskar Kizhner, Cande Ananth, John Smulian, Morgan Peltier. *Obstetrics, Gynecology and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA.*

Background: Fetal growth restriction has been linked to an increased incidence of chronic hypertension, which may be the result of extracellular matrix changes (ECM) within the vascular tree. In previous studies, ECM changes were observed in the umbilical arteries of preterm growth restricted infants.

Objective: To determine if there are alterations in collagen subtypes within the umbilical cords from growth restricted fetal rat pups after a period of maternal nutrient restriction.

Methods: Timed pregnant Sprague-Dawley rats were fed either a 50% food restricted diet (MFR; n=5) or were fed ad libidum (control; n=5) from d10 until d21 of gestation. Litter size, fetal weights and placental weights were then noted and umbilical cords from 3 randomly selected pups in each litter were snap frozen. Gene expression for collagens I, III, XIV and decorin was evaluated by real-time RT-PCR with normalization to the GADPH housekeeping gene. Data were analyzed from fitting general linear regression models with estimation based on the quasi-likelihood estimation (generalized estimating equations) to account for clustering of responses within litters. Data are shown as cycles to amplification (Ct), which is inversely proportional to mRNA levels.

Results: No difference in median litter size was detected. However, fetal and placental weights in the MFR group were significantly less than those from control dams. No significant differences in umbilical cord gene expression for collagens I, III, XIV or decorin were detected between MFR and control pups.

Conclusions: Maternal food restriction does not result in any detectable alterations in collagen or decorin expression within the intact umbilical cord, despite causing significant reductions in fetal growth.

	MFR (n=5)	Control (n=5)	P-value
Litter size (median, range)	11 (8,15)	12 (9,15)	0.59
Fetal weight (mean ± SD) g	3.29 ± 0.12	4.0 ± 0.1	<0.001
Placental weight (mean ± SD) g	0.73 ± 0.05	0.90 ± 0.03	0.007
Collagen I (mean ± SEM) Ct	17.9 ± 0.13	18.1 ± 0.25	0.59
Collagen III (mean ± SEM) Ct	28.0 ± 0.22	28.1 ± 0.27	0.81
Collagen XIV (mean ± SEM) Ct	17.2 ± 0.16	17.4 ± 0.28	0.50
Decorin (mean ± SEM) Ct	18.7 ± 0.18	19.2 ± 0.32	0.17

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The Effect of Hypoxia on NGF Expression in the Aorta and Femoral Artery in the Growth Restricted Sheep Fetus. Andrew D Snell,[1,2] I Caroline McMillen,[1] Sheridan Gentili,[1] Janna L Morrison.[1] *[1]Sansom Institute, UniSA, Adelaide, South Australia, Australia; [2]Discipline of Physiolgy, Adelaide University, Adelaide, South Australia, Australia.*

Objective: Intrauterine growth restriction is associated with an increased risk of developing cardiovascular disease during adult life. We have previously demonstrated that the restriction of placental and fetal growth results in fetal hypoxia and fetal brain sparing suggesting a redistribution of cardiac output. Furthermore, placentally restricted (PR) hypoxic fetuses are more dependent on their sympathetic nervous system for the maintenance of blood pressure during late gestation. Nerve growth factor (NGF) plays a significant role in sympathetic innervation.

Hypothesis: We hypothesize that the expression of NGF will be higher in the aorta and femoral artery of the PR hypoxic compared to the control fetus.

Method: Carunclectomy was performed in 6 non-pregnant ewes to induce PR. Vascular catheters were inserted in 6 PR and 4 control (C) fetuses at 103-117d and arterial blood samples were collected for blood gas analysis. All PR fetuses

were chronically hypoxic (mean gestational arterial PO_2<17mmHg). Post mortem was performed at 139-141d. NGF mRNA expression was determined by qRT-PCR.

RESULTS: Fetal weight (C, 4.3± 0.3kg; PR, 2.5± 0.3kg) and mean gestational arterial PO_2 (C, 20.6± 1.9; PR, 12.7 ±0.8mmHg) were significantly lower in PR fetuses (P<0.01). There was no difference in the expression of NGF in the aorta (C, $0.0001\pm2.8x10^{-5}$; PR, $0.0001\pm1.3x10^{-5}$) or femoral artery (C, 0.001±0.0002; PR, 0.001±0.0002) between the normoxic control and hypoxic PR fetal sheep.

CONCLUSIONS: These data suggest that NGF expression at 139-141d gestation is not higher in the vasculature of the hypoxic fetus at a time when the maintenance of arterial blood pressure is more dependent on the sympathetic nervous system in the IUGR fetus.

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Moderate Maternal Nutrient Restriction Reduces Brain Glucose Transporter Protein 3 (GLUT3) Expression in Fetal Baboon at 0.9 Gestation. Iwa Antonow-Schlorke,[1] Natalie Seidel,[1] Natalia E Schlabritz-Loutsevitch,[2,3] Tom J McDonald,[2] Otto W Witte,[1] Matthias Schwab,[1] Peter W Nathanielsz.[2,3] *[1]Department of Neurology, Friedrich Schiller University Jena, Jena, Germany; [2]Center for Pregnancy and Newborn Research and University of Texas Health Sciences Center; [3]Southwest Foundation for Biomedical Research, San Antonio, TX, USA.*

GLUT3 is the predominant neuronal glucose transporter protein expressed in mature neurons. Maturation of GLUT3 expression is directly related to rates of cerebral glucose utilization associated with functional activity (Vannucci and Simpson, AJP-Endo 2003; Nagamatsu et al., FEBS Letters 1994; Vannucci, J Neurochem 1994).

Aim: To estimate the effect of chronic moderate maternal nutrient restriction on neuronal GLUT3 expression in the fetal primate brain.

Methods: From 0.16 until 0.90 gestation pregnant baboons were either fed *ad libitum* (controls, n=6) or 70% of weight adjusted *ad libitum* diet (n=6). After delivery by Cesarean section and euthanasia under general anesthesia, fetal brain samples were fixed with 4% paraformaldehyde and embedded in wax. Frontal brain sections including the neocortex and the striatum were stained against GLUT3 using the ABC-technique. Specific labeling was semi-quantified in the dorsolateral striatum and the cerebral cortex including the inner neocortical layers LV,VI and the outer neocortical layers LII-IV, using a computer-assisted image analysis program (Scion Image 6.21, NIH, USA).

Results: Maternal nutrient restriction reduced GLUT3 expression by 24.1%, 19.7% and 28.2% in the striatum, the inner and outer neocortical layers (p<0.01), respectively. In addition, number of GLUT3 immunoreactive neurons dropped by 25.3% within the outer neocortical layers compared to controls (p<0.05).

Conclusions: Reduction in cerebral GLUT3 expression suggests an alteration in metabolic state of the fetal brain and, probably, cerebral function by chronic moderate maternal nutrient restriction. Lower number of GLUT3 expressing neurons in the outer neocortical layers indicates delayed cortical maturation.

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Overexpression of Soluble Vascular Endothelial Growth Factor Receptor-1 (sFlt-1) in Isolated Placental Vascular Disease without Pre-Eclampsia, Suggests a Placental Disease Origin. Thushari I Alahakoon, Brian J Trudinger, WeiYi Zhang. *Obstetrics and Gynecology, University of Sydeny at Westmead Hospital, Westmead, NSW, Australia.*

Introduction

Elevated sFlt-1 levels have been shown to be a feature of pre-eclampsia and is considered to play a significant role in the pathogenesis of the condition. The role of angiogenic factors in fetal growth restriction has not been as well established. This study evaluated the levels of circulating vascular endothelial growth factor (VEGF) and its soluble receptor sFlt-1, in normal pregnancies and isolated placental vascular disease without evidence of pre-eclampsia.

Method

42 maternal peripheral venous samples were collected antenatally from two groups of pregnant women between 25-40 weeks of gestation. Group A: uncomplicated normal pregnancies (n = 20) and group B: pregnancies complicated by isolated placental vascular disease(n = 22) as defined by birth weight less than 10th centile for gestation and umbilical artery Doppler S:D ratio above the 95th centile for gestation, with no evidence of maternal pre-eclampsia or pre-existing hypertension. Plasma VEGF and sFlt-1 levels were measured using standard ELIZA techniques. Comparison between groups were performed by using one way analysis of variance.

Results

The maternal plasma sFlt-1 levels (Figure1) in group A: normal pregnancies increased with gestation (p < 0.001). The sFlt-1 levels in group B: isolated placental vascular disease were significantly higher throughout all gestations (p = 0.002) and did not show a significant variation with gestation (p = 0.47). The plasma VEGF levels were below the detectable levels of the assay in all samples except 3 normal pregnancies.

Discussion

The significantly increased maternal plasma sFlt-1 levels in established isolated placental vascular disease without evidence of pre-eclampsia suggest a disease of placental origin. The dysregulation of angiogenic factors may be part of a repair and regeneration process in the placenta.

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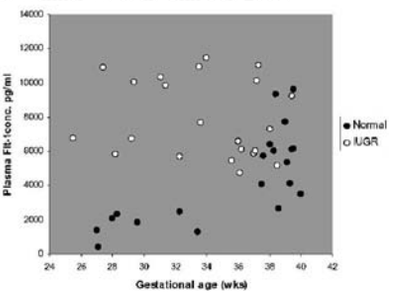

Figure 1- Maternal plasma Flt-1 concentration.

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Effect of Inhibition of 5α Reduced Steroid Synthesis on Apoptotic Brain Cell Death and Neurosteroidogenic Enzyme Expression in the Fetal and Neonatal Guinea Pig. Meredith A Kelleher,[1] Hannah K Palliser,[1] Della M Yates,[1] Robert KP Sullivan,[3] David W Walker,[2] Jonathan J Hirst.[3] *[1]Mothers & Babies Research Centre, University of Newcastle, Newcastle, NSW, Australia; [2]Physiology, Monash University, Clayton, VIC, Australia; [3]School of Biomedical Sciences, University of Newcastle, Newcastle, NSW, Australia.*

Objectives: Production of 5α-reduced neurosteroids is critical for reducing fetal vulnerability to stressors in pregnancy and inhibition of 5α-reductases (5αR) increase acute hypoxia-induced apoptosis in the fetal brain (Yawno *et al* 2007 Neurosci 146:1726). We have developed a model of placental insufficiency that results in fetal growth restriction (GR) in the guinea pig (Palliser *et al* 2007 Repro Sci 14 #381). The aim of the present study was to determine the effects of suppression of 5α-reduced steroid synthesis on apoptosis in vulnerable regions of the fetal brain and on neurosteroid synthetic enzymes in pregnancies compromised by chronic placental insufficiency.

Methods: Placental insufficiency was induced in guinea pig dams by surgical ablation of uterine artery branches at mid gestation (term 68d). Sham operated or GR dams received finasteride (a 5αR inhibitor; 25mg/kg/day) during late gestation (55d until term). Activated caspase-3, a marker of apoptotic cell death, was measured by immunohistochemistry and steroidogenic enzymes, 5αR1 and cytochrome P450 side chain cleavage (P450scc) were measured by real time PCR and western blotting in fetuses (65d) and neonates 24h after birth.

Results: Placental insufficiency significantly reduced fetal body and organ weight by 30% whilst sparing brain weight. The number of activated caspase-3 positive cells was significantly increased in the fetal hippocampus of GR fetuses and further increased in the cortex of GR fetuses receiving finasteride. The neonatal brain also exhibited changes in caspase-3 activation following GR and finasteride treatment. The fetal adrenal and the placenta responded to the compromise with increased expression of P450scc mRNA and 5αR1 protein, respectively.

Conclusion: The combination of placental insufficiency and suppressed neurosteroid system leads to markedly increased apoptotic cell death in the fetal brain which continues to affect the neonatal brain. The fetus responds to these conditions by increasing steroid synthetic enzyme expression in the placenta and adrenal glands suggestive of a possible neuroprotective feedback process.

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Effect of Smoking on the Human Fetal and Neonatal Cortical Activity. Rathinaswamy B Govindan, Pam Murphy, Hubert Preissl, Curtis L Lowery, Hari Eswaran. *Department of Obstetrics and Gynecology, University of Arkansas for Medical Sciences, Little Rock, AR, USA.*

Objective: To study the effect of smoking by mothers on the fetal and neonatal brain using non-invasive magnetoencephalography technique (MEG).

Materials and Methods: Using fetal magnetoencephalography, cortical auditory evoked responses (AER) were measured from 21 fetuses ranging from 27 to 39 weeks gestational age for a total of 69 recordings. 29 measurements were taken from 10 mothers with a history of smoking (SM) and 40 from 11 mothers with no smoking history (NS). After delivery, five SM and five NS newborns had MEG AER measurements twice for a total of 20 recordings. AER was quantified by cross-correlation analysis and its significance was assessed by boot-strap technique.

Results: AERs were detectable in 35 out of 40 fetal recordings in the NS group and 23 of 29 in the SM group. The neonatal response rate was 90% for each group. The latencies were divided into three components: C1 (100-300 ms), C2 (301-600 ms) and C3 (601-900 ms). In both fetuses and neonates as well, there was a statistically significant difference ($p<0.05$) between the two groups in the C2-component and the SM group showed faster AER compared to the LR group.

Conclusion: MEG technique provides a non-invasive approach to study the effects of smoking on developing fetal and neonatal brain. The observed decrease in the latency of fetuses and neonates of the smoking mothers could indicate a hypersensitive cortical response to auditory tone.

Table: Comparison of latencies of low-risk fetuses and fetuses of smokers in three different components namely C1 (100-300 ms), C2 (301-600 ms) and C3 (601-900 ms).

Category	C1 (100-300 ms)	C2 (301-600 ms)	C3 (601-900 ms)
NS mothers (fetus)	199.68	475.31	736.61
SM mothers (fetus)	205.44	432.64	732.53
p-value	0.8	0.005*	0.89
NS mothers (neonate)	237.52	391.31	-
SM mothers (neonate)	218.84	353.54	-
p-value	0.12	0.03*	-

*significant at 0.05 level

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Adenosine Modulates Glucose, Lactate, and Insulin Concentrations in Fetal Sheep. Takatsugu Maeda, Brian J Koos. *Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.*

Adenosine (ADO) modulates metabolism in adult mammals through multiple mechanisms that involve ADO A1 and A2A receptors. OBJECTIVE: This study was designed to test the hypothesis that ADO A1 and A2A receptors participate in fetal metabolic homeostasis. METHODS: Experiments were performed in chronically catheterized fetal sheep (>0.8 term). Intravascular infusion for 1 h of DPCPX (A1 receptor antagonist) or ZM241385 (ZM, A2A receptor antagonist) was performed alone or in concert with ADO administration. The highly selective ADO receptor antagonists were also infused in fetuses in which hypoxia was induced for 1 h by having the ewe breathe a hypoxic gas mixture (FIO2 = 0.10). Data were analyzed by two-way repeated measures of ANOVA. RESULTS: Blockade of ADO A1 receptors (n=8) increased significantly ($p < 0.05$) fetal concentrations of glucose [control (C): 15.6 ± 1.0 (SE)]; experiment (E): 17.38 ± 1.1 mg/dl] and lactate (C: 16.5 ± 2.4; E: 22.5 ± 4 mg/dl) without significantly altering insulin levels and arterial blood gases or pH. Antagonism of ADO A2A receptors (n=6) did not affect plasma levels of glucose, lactate, or insulin. Intravenous infusion of ADO (n=11), which did not alter PaO2 or PaCO2, increased concentrations of glucose (C: 18.0 ± 0.8; E: 22.6± 1.3 mg/dl) and lactate (C: 12.2 ± 1.0; E: 20.4 ± 1.6). ZM (n= 7), but not DPCPX (n=9), abolished ADO-induced rise in glucose and lactate concentrations. Isocapnic hypoxia (PaO2 ~13 Torr), which increases (2-3 fold) fetal plasma ADO levels to those similar to ADO infusion, decreased arterial pH (C: 7.331 ± 0.016; E: 7.140 ± 0.032), and increased fetal levels of glucose (C: 16.2 ± 1.3; E: 33.7 ± 7.8 mg/dl) and lactate (C: 13.3 ± 1.4; E: 97.3 ± 7.7 mg/dl) without altering changing insulin concentrations. These effects of hypoxia were not altered by DPCPX or ZM. CONCLUSIONS: 1) A1 receptors modulate plasma levels of glucose and lactate in normoxic fetuses; 2) A2A receptors mediate the ADO-induced rise in plasma glucose and lactate; and 3) A1 and A2A receptors are not significant modulators of hypoxia-induced changes in plasma levels of glucose and lactate. Supported by USPHS HD-18478.

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Changes in the Relationship between Leptin, Resistin and Adiponectin Levels in Fetal and Early Neonatal Life. Giovanna Corona,[1] Emanuela Marinoni,[1] Francesca Ciardo,[1] Massimo Moscarini,[1] Alfredo Patella,[2] Romolo Di Iorio.[1] *[1]Dept. Gynecology, Perinatology and Child Health, University "Sapienza", Rome, Italy; [2]Dept. OB/GYN, University of Ferrara, Ferrara, Italy.*

Objective: To investigate the dynamic changes of the relationship between leptin, adiponectin and resistin in maternal and fetal circulation during pregnancy and in the early post-natal period.

Materials and methods: Thirty pregnant women with uncomplicated singleton pregnancy delivered at term. Maternal and fetal/neonatal venous blood samples were obtained at delivery and at 72 hours from birth. Leptin, adiponectin and resistin were measured by specific ELISA assays. Neonatal anthropometric measurements, glucose metabolism and lipid profile, blood pressure information were obtained. Statistical analysis was performed by ANOVA followed by Student t test or Duncan's test whenever appropriate. Correlations were calculated by using the Pearson coefficient.

Results: Adipokines concentration at birth and at 72h from birth was showed in table. Multivariate regression analysis showed that fetal leptin levels were positively associated with female gender and adiponectin levels, but not with anthropometric characteristics. Fetal leptin and adiponectin levels were not correlated with maternal concentration, whereas fetal and maternal resistin levels were. Fetal and maternal resistin concentration was positively associated with gestational age and birth weight and fetal resistin levels correlated negatively with lipid profile. After birth leptin concentration in maternal and neonatal circulation decreased dramatically and the correlation between leptin and adiponectin levels was lost. A positive correlation between resistin and leptin concentration in neonatal circulation was found at 72h from birth.

Conclusions: In contrast to adult life a positive correlation between leptin and adiponectin is present in fetal life. Placental secretion of leptin, but not of adiponectin and resistin, contributes significantly to maternal and fetal circulating levels. Significant changes in the relationship among adipokines occurred immediately after birth and may affect growth and development in early post-natal period.

adipokines concentration in maternal and fetal circulation

	mother		newborn	
	at birth	72h	at birth	72h
leptin (ng/ml)	15.7±2.2	6.1±1.2*	6.8±1.6	1.9±0.3*
resistin (ng/ml)	6.8±0.8	7.1±1.4	12.3±2.5	15.6±3.5
adiponectin (µg/ml)	15.0±2.1	11.2±1.2	31.5±3.8	40.3±4.9

*p<0.01

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Impact of Maternally Administered Endothelin Receptor Antagonism on Neonatal Oxygen Saturation in the Rat. Larry G Thaete,[1,2] Sylvia Synowiec,[1] Saira Khan,[1] Mark G Neerhof.[1,2] *[1]Obstetrics & Gynecology, Evanston Northwestern Healthcare, Evanston, IL, USA; [2]Obstetrics & Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.*

Background: Endothelin receptor A (ET_A) antagonism has been shown to normalize placental perfusion and fetal growth in several rat models of fetal growth restriction. However, direct administration of ET_A antagonists to newborn rats within 3 hours of delivery has been consistently associated with neonatal demise due to failure of the ductus arteriosus to close, raising concerns about the safety of their use late in pregnancy. Perinatal exposure to ET_A antagonists (maternal administration in late gestation) and its impact on rat pup survival and oxygen saturation has not been investigated.

Objective: To determine the impact of a maternally administered ET_A antagonist on oxygen saturation in newborn and 7-day-old rat pups.

Methods: Timed pregnant Sprague-Dawley rats were treated with FR139317 (12 mg/kg/day; ET_A antagonist) or 4.2% $NaHCO_3$ vehicle, by subcutaneous osmotic pump connected to an intravenous catheter, from gestational day 14 (term=22 days) through parturition. All five pregnant rats in each group delivered spontaneously and nursed their pups through postpartum day 7. Oxygen saturation of each rat pup was measured by pulse oximeter on postpartum days 1 and 7. Results are presented as means ± SE.

Results:

Oxygen Saturation (%O2) in Neonatal Rats following Maternal ET_A Antagonism

Group	Newborn	7-day Neonate
Vehicle	92.6 ± 0.9	94.4 ± 0.8
ETA Antagonist	94.7 ± 0.8	91.6 ± 1.1

There were no statistically significant differences between the treatment groups.

Conclusion: Maternal administration of an ET_A antagonist from gestational day 14 through parturition, at a dose sufficient to ameliorate fetal growth restriction, has no adverse impact on oxygen saturation in neonatal rat pups.

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Maternal Treatment with Allopurinol Diminishes Fetal Cardiac Oxidative Stress Following Repeated Episodes of Ischemia-Reperfusion in Sheep. Helen L Torrance,[1] Jan B Derks,[1] Martijn A Oudijk,[1] Avnesh S Thakor,[2] Tereza Cindrova-Davies,[2] Frank van Bel,[1] Gerard HA Visser,[1] Graham J Burton,[2] Dino A Giussani.[2] *[1]Perinatal Center, University Medical Center Utrecht, Netherlands; [2]Department of Physiology, Development & Neuroscience, University of Cambridge, United Kingdom.*

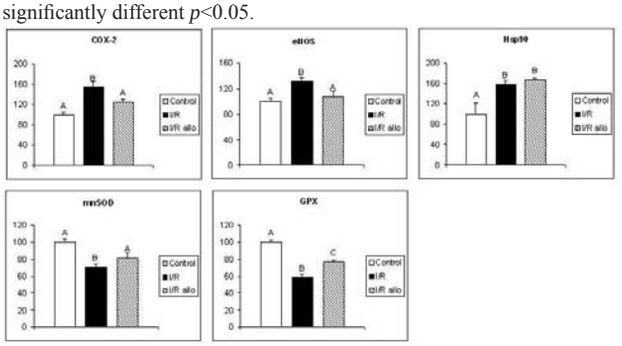
Introduction: The management of perinatal asphyxia remains a major concerns in obstetrics. Umbilical cord compressions (UCC) induce fetal asphyxia and ischaemia-reperfusion (I/R). I/R increases reactive oxygen species, for instance via activation of the xanthine oxidase (XO) pathway, which may promote oxidative stress in the fetal circulation. While treatment with allopurinol of asphyxic human neonates reduced free radicals and improved cardiovascular status, treatment started postnatally was deemed too late to prevent oxidative damage (Benders *et al.* Arch Dis Child **91**:163, 2006). Consequently, in complicated pregnancy, recommendations to treat the fetus via the mother, rather than the neonate, with allopurinol are being entertained today. We investigated the effects of maternal allopurinol treatment on indices of oxidative stress in the fetal heart following repeated UCC in sheep.

Methods: At 0.8 of gestation,10 surgically instrumented sheep fetuses were submitted to I/R (5 x 10 min repeated UCC) under maternal allopurinol (n=5) or saline vehicle (n=5) infusion. Fetal hearts were collected 48h after I/R and snap frozen for measurement of (anti)oxidant proteins by Western blot. Hearts from 5 uninstrumented fetal sheep at 0.8 gestation served as controls. Statistical comparisons were made using one-way ANOVA.

Results: I/R episodes led to increased expression of COX-2, eNOS, Hsp90 and decreased expression of SOD and gluthatione peroxidase (GPX) in the fetal heart, findings consistent with cardiac oxidative stress. Maternal treatment with allopurinol ameliorated these effects (Fig. 1).

Conclusion: Repeated UCC promotes oxidative stress in the fetal heart. Maternal treatment with allopurinol offers potential therapeutic treatment against this effect.

Mean ± SEM expressed as a percentage of control. Different letters are significantly different $p<0.05$.



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Fetal Adaptation to Chronic Hypoxemia (HPX) Alters the Proteome of the Fetal Brain in a 'Dose' Dependent Fashion. Y Dong,[1] W Hou,[1] A Artigues,[2] M Villar,[2] CP Weiner.[1] *[1]Obstetrics and Gynecology, University of Kansas School of Medicine, Kansas City, KS, USA; [2]Biochemistry and Molecular Biology, University of Kansas School of Medicine, Kansas City, KS, USA.*

OBJECTIVES: Hypoxic-ischemic fetal brain injury (HIE) is a major cause of neonatal death and morbidity. Evidence suggests that the brain cell injury associated with chronic HPX (in contrast to an acute ischemic reperfusion injury) is a complex process reflecting a series of adaptive intracellular events that are duration and gestational age dependent. We applied advanced proteomic tools as a next step toward ascertaining a more complete understanding of the impact of HPX on the fetal brain proteome.

METHODS: Time-mated guinea pigs were housed in a chamber beginning on day 50 for 14d, breathing either room air (NMX), or 10.5% or 12% O_2 (12% O_2 HPX or 10.5% O_2 HPX). On day 65 (term), the fetal brains were removed and preserved for study. Total protein was extracted, and the proteome first characterized by 2D gel electrophoresis. The density of the resulting protein spots were acquired and analyzed using the GS800 Densitometer and PDQuest software. Identified spots of interest were trypsin digested and subject to MALDI mass spectrometry using the 4700 Proteomics Analyzer Mass Spectrometer for peptide mapping or sequencing. The HPX-induced protein spots were identified based on a minimum of a 2X change from NMX.

RESULTS: HPX had a clear effect on the fetal brain proteome. Superoxide dismutase (SOD), heat shock protein 70 (HSP70), γactin (ACTG1), interleukin-1 (IL-1), and glutamine synthetase (GS) were each up regulated, while cofilin-1, brain-type creatine kinase (BB-CK), and β-1 GTP binding protein were each down regulated. All protein changes were proportional to the HPX (12% O_2 HPX vs NMX, 12% O_2 HPX vs 10.5% O_2 HPX, $p<0.05$).

CONCLUSIONS: This initial application of proteomic techniques confirms that fetal brain damage secondary to chronic HPX (in contrast to acute HPX) is a complex processes characterized by fetal adaptations mediated by multiple protein activations and inactivations. While SOD, IL-1, and GS have each been previously investigated, the potential roles of ACTG1, BB-CK, beta-1 GTP binding protein, and cofilin-1 in the fetal response to HPX and the associated brain damage are unclear and represent strong candidates for future investigation.

ACKNOWLEGEMENT: This study was supported by grants from the PHS (R01 HL049041-12, cpw) and CDC grant (U7DP00187-03, cpw).

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Intermittent Umbilical Cord Occlusions Alter Developmental Deposition of Elastin in the Carotid Artery. Jennifer A Thompson,[1] Sarah A Folliott,[1] Brian S Richardson,[2] Robert Gagnon.[2] *[1]Physiology, The University of Western Ontario, London, ON, Canada; [2]Obstetrics and Gynecology, The University of Western Ontario, London, ON, Canada.*

Intermittent umbilical cord occlusion occurs in 5% of human pregnancies.Given that elastogenesis within the vascular wall is in part mediated by hemodynamic conditions during development, the blood pressure response to acute hypoxic insults such as cord occlusion may alter arterial composition. Elastin content of a central and peripheral artery and blood pressure responses in fetal sheep exposed to varying degrees of cord occlusion were determined.

Methods: Over a 4 day period, near term fetal sheep received total umbilical cord occlusion (UCO) lasting 1 min/ hour (mild group; n=5), 2 min/hour (moderate group; n=4), 3 min/hour (severe group; n = 6) or no occlusion (control group; n=7). Fetal arterial blood samples were drawn 5 min prior to and at the end of cord occlusions. Mean arterial pressure (MAP) was monitored continuously. The carotid and superior mesenteric arteries were excised and a colorimetric assay (BioColor) performed for determination of elastin content. Results are presented as mean ± SEM.

Results: Umbilical cord occlusions produced decreases in fetal arterial oxygen pressure and oxygen saturation that were progressively more pronounced across mild, moderate and severe UCO groups ($p < .01$). Lactate concentration rose during occlusions in the moderate and severe groups, but not the mild group ($p < .01$).Elastin content of the superior mesenteric artery did not differ between the 3 experimental groups and the control group.

Elastin Content of the carotid artery and MAP response

	Elastin content (μg/mg tissue)	Max Δ in MAP	Duration of rise in MAP (min)
Control	5.7 ± 0.4	4.0 ± 1.7	-
Mild	7.0 ± 0.7	21.06 ± 2.5 **	10.39 ± 0.6
Moderate	7.4 ± 0.7	32.4 ± 1.6 ** †	14.3 ± 0.5 †
Severe	9.5 ± 1.0 *	34.5 ± 0.6 ** ††	17.4 ± 1.2 ††

* $p < .05$; ** $p < .01$: Experimental groups compared to control† $p < .05$; †† $p < .01$: Compared to mild group

The max Δ in MAP was positively correlated with elastin content ($R^2 = .56$, $p < .05$).

Conclusions: The transient rise in blood pressure and preferential blood flow to the brain that occur in response to acute hypoxemia during severe intermittent umbilical cord occlusion induce an increase in elastin synthesis in the carotid artery.This may give rise to adaptive programming of postnatal central arterial compliance.

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Electrocortical Activity during Repetitive Umbilical Cord Occlusions (UCO) with Worsening Acidemia in the Ovine Fetus near Term. Martin G Frasch,[1] Roy Mansano,[2] Michael G Ross,[2] Robert Gagnon,[1] Bryan S Richardson.[1] *[1]ObGyn, CHRI, Univ of Western Ontario, London, Canada; [2]ObGyn, Harbor-UCLA Med Ctr, Torrance, CA.*

Objective: Uterine contractions during labour can restrict umbilical blood flow compromising fetal oxygenation and leading to adverse neonatal outcome including newborn encephalopathy/subsequent cerebral palsy. While electronic fetal heart rate (FHR) monitoring is widely used for assessment during labour,

abnormal FHR patterns as used clinically have a poor positive predictive value for concerning/significant acidosis at birth. There is limited study of fetal electrocortical activity (ECOG) in animal models with induced hypoxia/acidemia as might be seen during labour. Thus, we aimed to induce repetitive UCOs in fetal sheep leading to worsening acidemia to determine the predictive value of ECOG activity for fetal compromise.

Methods: Near-term fetal sheep (N=10) underwent chronic preparation with artery catheters, ECG/ECOG electrodes and placement of an inflatable umbilical cord occluder. Following a baseline recording period, fetuses underwent a series of mild (1min every 5min), moderate (1min every 3min) and severe (1min every 2min) UCOs with each series lasting 1h or until fetal arterial pH decreased to <7.0. Fetal arterial blood samples for blood gases and pH were taken at selected time points during the baseline, UCOs, and recovery periods. Arterial blood pressure (ABP) and FHR were continuously monitored and spectral edge frequency (SEF) was calculated from ECOG. Correlation of SEF with FHR change was calculated for time intervals using SEF maxima and FHR minima during UCO series. Data are presented as means±SEM.

Results: Repetitive UCOs led to development of a marked acidosis (pH 7.36±0.03 to 6.91±0.12, $p<0.001$). 52±13 min prior to fetal pH drop <7.0, the SEF of ECOG began to increase abruptly during each FHR deceleration from 3±1 Hz up to 23±2 Hz ($p<0.001$) and was correlated to both FHR change and a decrease in fetal ABP during each FHR deceleration at this time ($p<0.001$).

Conclusion: Our findings suggest that in the animal model studied (1) fetal ECOG activity is impaired with progressive acidemia accompanied by FHR decelerations and pathological ABP decreases; (2) there is a consistent temporal relationship between the occurrence of ECOG alterations and the subsequent critical drop of pH <7.0. These findings could contribute to improvement in the clinical ability to predict fetal compromise during labour using ECOG/FHR monitoring.

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17β-Estradiol and Progesterone Mediate Dilation of the Mouse Fetal Ductus Arteriosus Via Nitric Oxide, Prostaglandin, and Non-NO, Non-PG Pathways. Chelsea Tolentino, Stan Poole, Naoko Brown, BC Paria, Jeff Reese. *Dept of Pediatrics, Vanderbilt University Medical Center, Nashville, TN.*

Background: The ductus arteriosus (DA) plays a pivotal role in fetal development and circulation. During gestation, patency of the fetal DA is maintained by nitric oxide (NO) and prostaglandins (PG). NO and PGs are downstream effectors of estrogen (E2) and progesterone (P4). However, the roles of E2 and P4 in DA regulation have not been studied.

Objective: We hypothesized that: E2 and P4 have opposite effects in the DA; that rising E2 and falling P4 levels help trigger DA closure via specific hormone receptors; and that NO and/or PGs mediate DA responses to E2 and P4.

Methods: Expression of ERα, ERβ, PRA, PRB, and the putative membrane receptors MPR-α, -β, -γ, PGRMC-1, -2, and SERBP-1 was examined by RT-PCR and qPCR on days 15, 17, 19 (term), and P1. The effects of E2 and P4 (10^{-9}-10^{-4} M) on the d19 fetal DA were examined in a cannulated microvessel myography system. E2 and P4 effects were also studied in the presence of receptor antagonists (ICI 182780, RU486) and NO and PG inhibitors (L-NAME, indomethacin).

Results: ER and PR receptors were expressed at low levels; PGRMC-2 expression was stronger than other membrane PRs, and increased with advancing gestation. Under fetal O2 conditions, E2 induced rapid, concentration-dependent dilation at 10^{-6}-10^{-4} M (n=11); P4 induced progressive vasodilation at all doses (n=10). E2 and P4-induced dilation occurred within 60-180 seconds of exposure. While E2-dilated DAs did not respond to ICI, ICI-pretreated DAs failed to dilate to the same dose of E2 (n=10) suggesting antagonism of E2 effects. The vasodilatory effects of E2 were partially inhibited by pretreatment with L-NAME. P4-dilated DAs did not respond to RU486, whereas RU486-pretreated DAs showed a small, significant response to P4 (n=10), suggesting partial antagonism or signaling via alternative, RU486-independent pathways. Pre-treatment with Indocin did not block the vasodilatory effects of P4.

Conclusions: Contrary to our expectation, both E2 and P4 have dilating effects on the fetal DA, via NO, PGs and non-NO, non-PG pathways. Expression studies and the rapid response of *ex vivo* DAs are consistent with non-genomic actions via membrane receptors. Hormone shifts in parturition may have long-term effects on DA preparation for postnatal closure, but strategies to maintain fetal DA patency or treat newborns with PDA will require better understanding of this process.

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Fetal Cerebral Blood Flow, Electrocorticographic Activity, and Oxygenation: Responses to Acute Hypoxia. Stephen J Lee, Takuji Tomimatsu, Jorge Pereya-Pena, Douglas P Hatran, Jonathon M Ross, Grant A McAuley, Lawrence D Longo. *Center for Perinatal Biology, Depts. of Physiology and Obstetrics and Gynecology, Loma Linda University, School of Medicine, Loma Linda, CA, USA.*

Background. For the fetus, arterial blood gases are critical in the regulation of cerebral blood flow (CBF) and cerebral oxygenation. However, the relation of CBF, cortical tissue PO_2 (tPO_2), and electrocorticographic (ECoG) activity to arterial O_2 tension (PaO_2) are not well defined. In an effort to elucidate these interrelations, we tested the null hypothesis that in the near-term fetus, acute hypoxic-associated cerebral oxygenation and related variables are not closely associated with ECoG state.

Methods. By use of a laser Doppler flowmeter with a fluorescent tissue O_2 probe, and with fluorescent-labeled microspheres, and with ECoG electrodes, in near-term fetal sheep (n = 8) we measured laser Doppler CBF (LD-CBF), tPO_2, ECoG (Root Mean Square (RMS) Voltage with High Voltage Low Frequency, HVLF versus Low Voltage High Frequency, LVHF) and spectral edge frequency-90% (SEF_{90}) in response to 40 min moderate isocapnic hypoxia.

Results. LD-CBF, cerebral O_2 delivery, tPO_2, and several other variables correlated highly with ECoG state. In the normoxic control fetus, in association with a shift from HVLF to LVHF ECoG activity, tPO_2 decreased briefly to 7±1 from a control value of 10±1 Torr; however, as LD-CBF increased 18±5%, and SEF_{90} increased to14±2 from 7±1%, tPO_2 returned to near normal value. With acute hypoxia (PaO_2 = 12±1 Torr) when in the LVHF state LD-CBF increased only 23±14%, as opposed to a 52±10% increase when in HVLF ECoG state. With this degree of hypoxia, tPO_2 decreased to 3±1 Torr, SEF_{90} remained at 8±2%, and cerebral metabolic rate for O_2 ($CMRO_2$) decreased 32±5% ($p<0.05$).

Conclusions. For the near-term fetus, normoxia with changes in ECoG state was associated with brief periods of decrease in tPO_2, which were restored quickly by increased LD-CBF. In contrast, acute hypoxia was associated with a significant depression of cortical tPO_2, $CMRO_2$, and ECoG state, with increased LD-CBF failing to restore cortical tPO_2. Thus, we reject the null hypothesis that in such fetuses, hypoxia demonstrates no compromise in cerebral oxygenation. (Supported by USPHS HD-03807).

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Long Term Hypoxia Enhances Pituitary Responsiveness to Corticotrophic Releasing Hormone and Arginine Vasopressin in the Ovine Fetus. Charles A Ducsay,[1] Kanchan M Kaushal,[1] Malgorzata Mlynarczyk,[1] Kimberly Hyatt,[2] Dean A Myers.[2] *[1]Ctr. for Perinatal Biol., Loma Linda Univ., Loma Linda, CA; [2]Ob/Gyn, Univ. Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK.*

Background: Long term hypoxia (LTH) profoundly affects the hypothalamic-pituitary-adrenal axis of the fetal sheep. We previously showed that LTH causes augmented corticotrope function. The present study was designed to test the hypothesis that LTH enhances sensitivity to the ACTH secretagogues; cortiocotrophic releasing hormone (CRH) and arginine vasopressin (AVP) resulting in increased anterior pituitary corticotrope secretion of ACTH.

Methods: Pregnant ewes were maintained at high altitude (3,820 m) from day ~ 40 to 130-131 of gestation (dG), when they were returned to the lab and a maternal tracheal catheter was implanted. Maternal PO_2 was maintained at a level comparable to that observed at altitude (~60 mmHg) by nitrogen infusion. On 132 dG, LTH (n = 4) and age-matched, normoxic control (n = 4) fetuses were implantated with vascular catheters. Each fetus received a 15 min infusion of either saline vehicle, 100 ng/kg of ovine CRH or 20 ng/kg of AVP (estimated body weight/min) in a randomized order over 3 consecutive days (137-139dG). Blood samples were collected at 0 min (baseline prior to infusion), 15, 30, 60 and 90 min following the start of the infusion and analyzed for ACTH, as well as the ACTH precursors pro-opiomelanocortin and the major processing intermediate 22 kDa proACTH.

Results: Vehicle had no effect on any of the measured parameters. With CRH infusion, ACTH (pg/ml) increased in both groups over the course of the study. However, peak concentrations (at 90 min) were significantly higher in the LTH group compared to control (240±17 vs. 110±14, respectively; $p<0.01$). ACTH precursor secretion (pM) was greater in LTH fetuses compared to controls during the experiment ($p<0.01$). In response to AVP, peak ACTH concentrations were also higher in the LTH fetuses compared to control (209±51 vs. 67±20 respectively; $p<0.01$), however peak levels were reached at between 15 and 30 min after start of infusion with levels in both groups returning to pre-infusion values. A similar pattern was observed with precursor levels (102.1±27.9 vs. 66.1±10.2, $p<0.05$, LTH vs. control).

Conclusions: LTH significantly increases pituitary sensitivity to both CRH and AVP. This enhanced sensitivity may be mechanism of our previously observed enhanced corticotrope function. (Supported my NIH grants HD33147 and HD31226).

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Do Repetitive Umbilical Cord Occlusions (UCO) with Worsening Acidemia Induce the Bezold-Jarisch Reflex (BJR) in the Ovine Fetus near Term? Martin G Frasch,[1] Roy Mansano,[2] Michael G Ross,[2] Robert Gagnon,[1] Bryan S Richardson.[1] *[1]Ob/Gyn, CHRI, University of Western Ontario, London, Canada; [2]Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA.*

Objective: Repetitive UCO leading to worsening fetal acidosis with fetal heart rate (FHR) decelerations (FHR_{dec}) are accompanied by pathological decreases of fetal arterial blood pressure (BP). We hypothesized this BP change may be caused by the BJR, a vagally mediated reflex with bradycardia and hypotension to reduce cardiac workload, via stimulation of cardiac chemoreceptors during systemic acidemia.

Methods: Ten near-term fetal sheep (125 ± 2dGA) underwent chronic preparation with brachial artery catheters and placement of an inflatable umbilical cord occluder. After a control period, fetuses underwent a series of mild (1 min every 5 min), moderate (1 min every 3 min) and severe (1 min every 2 min) UCO each lasting 1 h or until pH decreased to < 7.0. Fetal arterial blood samples were taken at selected time points during the control and UCO periods. BP and FHR were continuously monitored. Individual FHR nadir during each FHR_{dec} and accompanying BP change (ΔBP = [BP at the time of a FHR nadir] - [BP at baseline preceding a UCO series]) were determined during all UCO. Data are presented as means±SEM.

Results: Control period pH (7.36 ± 0.03), FHR (163 ± 5 bpm) and BP (45 ± 4 mmHg) were within the physiological range. Average depth of FHR_{dec} for all UCO was 93 ± 11 bpm and increased with higher lactate concentrations ($R = 0.40$, $p < 0.05$) and lower pH ($R = 0.34$, $p = 0.08$). ΔBP during all UCO demonstrated an initial hypertensive response to FHR_{dec}, which decreased with lower pH ($R = 0.49$, $p < 0.05$). ΔBP increased 11 ± 2 mmHg ($p < 0.05$) during mild UCO (pH to 7.32 ± 0.03), 4 ± 3 mmHg during moderate UCO (pH to 7.19 ± 0.04, $p < 0.05$) and 2 ± 4 mmHg during severe UCO (pH to 6.91 ± 0.12, $p < 0.05$).

Conclusion: These results suggest that BJR, a short-acting, pH dependent depressor reflex, blunts the physiologic hypertensive response to cord occlusion insults leading to worsening acidemia as might be seen in the human fetus during labour with repetitive variable FHR_{dec}. The failure to increase BP may prevent optimal blood flow distribution responses necessary for preservation of vital organs.

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Role of NOS and PDE5 in Placental Dysfunction Following Fetal Bypass. Mitali Basu,[1] R Scott Baker,[1] Christopher T Lam,[1] Kenneth E Clark,[2] Pirooz Eghtesady.[1] *[1]Cardiothoracic Surgery, Cincinnati Children's Hospital, Cincinnati, OH, USA; [2]Ob/Gyn, University of Cincinnati, Cincinnati, OH, USA.*

Introduction

Rising placental vascular resistance following fetal cardiopulmonary bypass (bypass) remains the Achilles' heel of fetal cardiac surgery. We have previously shown in real-time that nitric oxide (NO) production rises during bypass and falls post-bypass, while cGMP levels rise throughout. Using immunohistochemical and Western analysis of placenta, we examined the involvement NO pathway components in this placental vascular pathophysiology.

Methods

Ovine fetuses at 100-110 days gestation were placed on bypass for 30 minutes and followed post-bypass for 2 hours. Placental samples were collected immediately prior to bypass and at 30 and 120 min post-bypass (n=7) and compared to a group of similarly instrumented controls, (n=6). Placental eNOS, iNOS and PDE5 protein expression was measured using standard methods and relative expression normalized to beta-actin. Statistical analysis utilized Students t-test, and ANOVA for trend and group-wise analysis, (significance at p=0.05).

Results

Pre-bypass protein expression did not differ between groups. PDE5 protein levels and phosphorylated PDE-5 expression were both elevated 30 min post bypass, and reduced 120 min post bypass compared to sham, (p=0.01). eNOS levels in the bypass group increased linearly from pre-bypass to 120 min post-bypass (p<0.01), and were also elevated compared with shams (p<0.01), while shams had declined significantly by 120 min post bypass, (p<0.01). Similarly, phosphorylated eNOS expression in the bypass group increased linearly from pre-bypass to 120 min post-bypass, (p=0.058), while shams trended towards decline by 120 min post-bypass. Simultaneously, placental iNOS expression remained stable within groups, but was lower in the bypass group at 30 and 120 min post-bypass, (p<0.04). The preceding data correlated with observed immunohistological changes in the same placental cotyledons.

Conclusion

Fetal bypass leads to significant increases in placental protein levels of PDE5, phosphorylated PDE-5, eNOS and ostensibly phosphorylated eNOS. Increased PDE5 expression may be a response to increased NO and the generated cGMP. This data suggests a compensatory upregulation of PDE5 and eNOS that eventually fails, leading to increasing placental vascular resistance and subsequent lethal placental dysfunction.

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Chronic Mild Hypoxemia during Late Gestation Decreases Baroreceptor Gain in Fetal Sheep. Victor Pulgar,[1] Debra Diz,[2] Jason K Hong,[2] Jorge P Figueroa.[1] *[1]Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA; [2]Surgical Sciences, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

Fetal hypoxia constitutes an important determinant of developmental outcome. Angiotensin receptors (rAt-II) in neuronal nuclei of the brainstem have been implicated in integration of baroreceptor responses. In near term fetal sheep, we have previously shown that 5 days of mild chronic hypoxemia increases heart rate (HR) and blood pressure (BP). The aim of the present study was to investigate the effects of chronic mild hypoxemia on the expression of rAt-II in the medulla oblongata and on baroreflex control of the circulation.

METHODS: At 125 days of gestational age, pregnant sheep were submitted to 5 days of hypoxemia (75% of fetal arterial PO_2; at day 5 Fetus 14±1 vs 20±0.5; Mother 67±5 vs 116±3 mmHg). HR and arterial BP were continuously recorded from mother and fetus in control (n=10) and hypoxemic (n=10) animals. Baroreflex sensitivity (BRS) as well as HR and diastolic BP variability were analyzed (Nevrokard Software). Brainstem was collected and rAt-II expression in the nucleus tractus solitarius (nTS) and dorsal motor nucleus of the vagus (dmnx) was measured by autoradiography using ^{125}I-Sarthran labeling. Data are shown as mean±SEM and were analyzed by t-test.

RESULTS: Hypoxemia induced a greater total binding of ^{125}I-Sarthran in nTS and dmnx resulting from an increased expression of AT1 receptors. In the time-domain analysis of BRS hypoxemia induces lower baroreflex sensitivity in the fetus (BRS in ms/mmHg; 8.5±0.6 vs. 11.5±1.2, p<0.05). In the frequency domain, hypoxemia changes the relative power of low frequencies (LF: 0.04-0.15 Hz) and high frequencies (HF: 0.15-0.4 Hz) of RRI and DBP with an increased value of the LF/HF ratio (RRI LF/HF 3.3±0.4 vs 2±0.5, p<0.05; DBP LF/HF 2.7±0.3 vs 1.8±0.2, p<0.05). Also the alpha index for baroreflex sensitivity is decreased in hypoxemic animals (alpha LF 9.2±1 *vs* 15.3±2.6, p<0.05; alpha HF 8.5±1.4 *vs* 19.8±5.3, p<0.05). No differences were observed in maternal variables.

CONCLUSIONS: Our results suggest a link between a prenatal insult, alterations in CNS receptors and functional alterations of baroreflex responses. A higher sympathetic outflow, suggested by a greater LF/HF ratio, and impaired reflex gain, both possibly mediated by increases in nTS rAT1, may have potential long-term consequences for the development of hypertension. HD37885;HD047584;HL51952.

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Rheological Study of Fetal Descending Aorta in Real-Time Using Multigate Spectral Doppler Analysis. Gabriele Urban,[1] Patrizia Vergani,[1] Stefano Ricci,[2] Piero Tortoli,[2] Michael Paidas.[3] *[1]Obstetrics and Gynecology, University of Milan Bicocca, Monza, Italy; [2]Department of Electronics and Telecomunications, University of Florence, Florence, Italy; [3]Obstetrics Gynecology and Reproductive Sciences, Yale University, New Haven, Italy.*

OBJECTIVE : We evaluated velocity profiles, relative wall distension rate (rWDR), and wall shear rate (WSR) of fetal descending aorta (FDA) in uncomplicated singleton gestations. **STUDY DESIGN:** Ninety seven uncomplicated singleton fetuses were studied throughout gestation using Multigate Spectral Doppler Analysis (MSDA) working with GASP software. This consists of a personal computer (PC) add-on board including a single high-speed digital signal processor. The analysis of echo-signals backscattered from 256 range cells located along the axis of the interrogating ultrasound (US) beam. Post-processing was accomplished using GASP software. Statistical analysis consisted of Spearman correlation and chi-square test. **RESULTS:** Velocity profiles, wall distension, wall shear rate were obtained from fetal

descending aorta throughout gestation establishing gestational age specific norms. WDR[%] is highly correlated with gestational age in appropriate growth fetuses (2.5±1.6 , rs=0.51 p<0.01) with linear regression with standardized coefficient of 0.4 (p<0.01). In contrast, WSR (421±97) is unchanged during the first , second and third trimesters (P=0.8).**CONCLUSION:** We speculate that the relative WDR changes observed during gestation, in normally grown fetuses, may be secondary to adaptive vascular and autonomic responses and the evolving composition of the vessel wall, particularly with respect to elastin. Conversely, the mean WSR for the study group was independent and constant throughout the gestation. These findings suggest that there is an increase in the diameter of the fetal aorta, which provides adaptation to the progressive flow demands, while preserving other key hemodynamic parameters. In this study, we have established normative values of rWDR and WSR, which are new rheological parameters that may be useful in distinguishing normal and pathologic hemodynamic states.

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Serum Collected during and after Fetal Sheep Cardiopulmonary Bypass Stimulates Nitric Oxide and Endothelin-1 Production by Umbilical Vein Endothelial Cells. Hani Sideek,[1] Pirooz Eghtesady,[1,2] Kenneth E Clark,[3] Connie J Wagner,[1] Jodie Y Duffy.[1,2] *[1]Pediatric Cardiothoracic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [2]Department of Surgery, University of Cincinnati, Cincinnati, OH, USA; [3]Department of Obstetrics and Genecology, University of Cincinnati, Cincinnati, OH, USA.*

Objective: Placental dysfunction is a key barrier to successful fetal cardiopulmonary bypass (CPB) for repair of congenital heart defects *in utero*. Endothelial cells regulate vascular tone during fetal CPB through interactions of vasodilation by nitric oxide (NO) and endothelin-1 (ET-1)-mediated vasoconstriction. The objective was to determine the time during fetal CPB when endothelial cell-mediated changes occur.

Methods: Human umbilical vein endothelial cells (HUVEC) were cultured in media containing 10% serum collected from ovine fetuses (n=3) that underwent 30 min of CPB, then were maintained for 120 min. Serum was collected before CPB, from pump prime before initiation of CPB, 30 min on CPB, or 30 and 120 min after fetal CPB. Control cells were cultured in normal fetal serum. Cells were harvested 24 and 48 hr after addition of fetal serum. NO production was measured in real time with an electrochemical detection system (inNO-T, Harvard Apparatus). ET-1 was measured in the culture media by ELISA.

Results: NO production by HUVEC after 24 and 48 hr was stimulated above control levels by fetal serum collected during and up to 120 min after fetal CPB (P≤.01). Serum collected from fetuses that were surgically instrumented, but not yet subjected to CPB, decreased NO levels below controls (P≤.01). Stimulation of ET-1 after 24 and 48 hr of HUVEC culture peaked with serum collected at 30 min after fetal CPB (P≤.01 compared with control), but was elevated above control levels at each collection time point (P≤.05, Table 1).

Conclusions: Fetal CPB releases serum proteins that elevate endothelial cell NO and ET-1 production during and for at least 120 min after CPB. Although the specific regulatory proteins remain to be identified, the NO and ET-1 pathways share circulating mediators and participate in a feedback loop to modulate vascular tone.

Endothelial Cell Response to Fetal Serum after 48 hr of Culture

	Control	Pre-CPB	CPB Prime	30' CPB	30' Post-CPB	120' Post-CPB
Nitric Oxide (nM)	59.7±5.3	49.8±3.5[a]	61.2±5.8	83.4±4.1[a]	98±4.5[a]	89.2±3.8[a]
Endothelin-1 (pg/mL)	161±3	251±9[a]	214±31	253±18[a]	258±9[a]	218±42[a]

[a] P<.05 vs Control

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Chronic Hypoxia Increases Nitric Oxide Generation from Inducible Nitric Oxide Synthase (iNOS) in Fetal Guinea Pig Hearts. LaShauna Evans, Loren P Thompson. *Obstetrics, Gynecology and Reproductive Sciences, University of Maryland, Baltimore, MD, USA.*

Background: Intrauterine hypoxia is a leading cause of fetal morbidity and mortality. Nitric oxide (NO) is important in the regulation of cardiovascular function and derived from three NOS isoforms, neuronal (nNOS), endothelial (eNOS), and inducible (iNOS). Our lab has previously shown that intrauterine hypoxia increases iNOS expression and NO generation in fetal guinea pig hearts. To test the hypothesis that hypoxia-induced upregulation of NO is linked to cardiac iNOS expression, a selective iNOS inhibitor, L-NIL (L-N6-(1-Iminoethyl)-Lysine), was administered *in vivo* to fetal guinea pigs and NO levels measured in fetal hearts.

Methods: Pregnant guinea pigs were exposed to either normal room air (normoxia; 21%O_2) or 10.5%O_2(hypoxia) in a hypoxic chamber for 14 days prior to term (term=65d). L-NIL was administered to pregnant normoxic and hypoxic guinea pigs via their drinking water at a dose of 1-2mg/kg/d for 10 days. At ~60d gestation, pregnant sows were anesthetized and near-term fetuses removed via hysterotomy. The fetal hearts were excised, weighed, and normalized to their respective fetal body weights. Left cardiac ventricles were obtained and frozen in liquid N_2 and stored at -80°C until ready for analysis. The effect of total NO product (NO_2^- and NO_3^-, NOx) of left ventricles of fetuses exposed to normoxia (n=4), hypoxia (n=5) and hypoxia plus L-NIL (n=4) was quantified by a commercial Fluorometric NO Assay kit.

Results: Intrauterine hypoxia significantly reduced fetal body weight by 18% and increased placenta/fetal body wt by 33% as expected for hypoxic stress. Hypoxia induced a slight increase in heart/fetal body wt by 4%. Fetal cardiac NOx levels (pmoles/mg) were increased by hypoxia (68.4±11.2) by 1.8 fold compared to normoxic controls (37.3±12.1). L-NIL significantly decreased (P<0.05) NOx levels in hypoxic hearts by 58% (68.4±11.2 vs 29.0±9.3; hypoxic vs hypoxic+L-NIL, respectively).

Conclusion: L-NIL inhibits iNOS-derived NO generation in the hypoxic fetal guinea pig heart. Since previous study in our lab showed a significant increase in iNOS expression but a decrease in eNOS and no change in nNOS expression, we hypothesize that hypoxia upregulates cardiac NO generation via the iNOS pathway. Further study is needed to identify the important role of cardiac iNOS in the adaptive response of fetal hearts to chronic intrauterine hypoxia.

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Relation of Base Deficit in Blood and Extracellular Fluid to Worsening Lactic Acidemia with Repetitive Umbilical Cord Occlusions (UCOs) in the Ovine Fetus Near Term. Martin Frasch,[1] Roy Mansano,[2] Michael Ross,[2] Robert Gagnon,[1] Bryan Richardson.[1] *[1]ObGyn, CHRI, Univ Western Ontario, London, Canada; [2]ObGyn, Harbor-UCLA Med Ctr, Torrance, CA.*

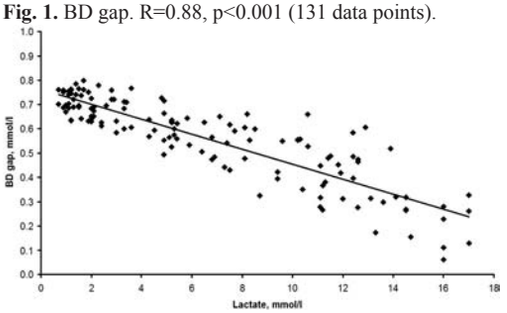
Objective: Fetal asphyxia-mediated metabolic acidosis results in a pH decrease and an increase of lactate and base deficit in blood (BD_{blood}) and extracellular fluid (BD_{ECF}). It is not clear whether BD_{blood} and BD_{ECF} are similarly altered with worsening fetal acidosis. In the present study we sought to study the dynamic relations of BD_{blood} and BD_{ECF} to lactate in the ovine fetus subjected to repetitive UCO with worsening acidemia.

Methods: Ten near-term fetal sheep (125±2dGA) underwent chronic preparation with brachial artery catheters and placement of an inflatable umbilical cord occluder. Following a baseline recording period, fetuses underwent a series of mild (1min every 5min), moderate (1min every 3min) and severe (1min every 2min) UCO with each series lasting 1h or until fetal arterial pH decreased to <7.0. Fetal arterial blood was sampled at baseline, immediately before and during the first mild, moderate and severe UCO and at 20min intervals during the moderate and severe UCO. BD gap (BD_{blood} - BD_{ECF}) was studied in relation to lactate. Presented as means±SEM.

Results: Lactate correlated to BD_{blood} (R=0.92, p<0.001). BD_{ECF} correlated strongly with BD_{blood} (R=1, p<0.001). BD_{ECF} increased by 0.55±0.03 mmol/l more than BD_{blood} from baseline to pH nadir, with BD gap therefore decreasing with increasing lactic acidemia (Fig. 1). Lactate, BD_{blood}, BD_{ECF} and BD gap correlated to pH (R=0.86, R=0.95, R=0.95 and R=1, respectively, all p<0.001) and pH could therefore be predicted with any of the four acid-base parameters.

Conclusion: The increases of BD_{blood} and BD_{ECF} and the decrease of BD gap with increasing lactic acidemia suggest a relatively more rapid accumulation of [H+] in ECF during UCO of increasing severity. This may be because the metabolic build-up of acidosis occurs primarily in the tissues and the endothelial permeability for [H+] is impeded during increasing acidosis with ATP depletion thus decreasing [H+] movement from ECF to plasma.

Fig. 1. BD gap. R=0.88, p<0.001 (131 data points).



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The Chemokine Stromal Derived Factor-1 (SDF-1) Constitutes a Putative 'Homing' Factor for Human Umbilical Cord Blood Cells under Hypoxic-Ischemic Conditions in Rat Brains. Katja Rosenkranz,[1] Sandra Kumbruch,[1] Katrin Lebermann,[1] Katrin Marschner,[2] Arne Jensen,[3] Rolf Dermietzel,[1] Carola Meier.[1] *[1]Department of Neuroanatomy and Molecular Brain Research, Ruhr-University Bochum, Bochum, Germany; [2]Department of Obstetrics and Gynecology, St. Elisabeth-Hospital, Bochum, Germany; [3]Department of Obstetrics and Gynecology, Knappschaftskrankenhaus, Ruhr-University Bochum, Bochum, Germany.*

Previous studies have shown that intraperitoneal transplantation of human umbilical cord blood (hUCB)-derived mononuclear cells led to the specific 'homing' of these cells to a hypoxic-ischemic brain lesion in perinatal rats. Motor deficits resulting from the lesion were alleviated upon transplantation. Thus, the presence of hUCB cells at the lesion site seems to be a major prerequisite for their potential beneficial effect. However, the mechanisms of cell 'homing' are still unclear. In this study, we focused on elucidating mechanisms underlying the specific migration of hUCB-derived mononuclear cells to the brain lesion.

One possibility to induce cell 'homing' are chemotactic signals present at the lesion site. The CXC chemokine stromal derived factor-1 (SDF-1), which was previously shown to be a potent chemoattractant for directed migration of other stem and progenitor cells, is a putative candidate in our lesion paradigm.

Therefore we investigated the spatial and temporal expression of SDF-1 in brain hemispheres with or without hypoxic-ischemic lesion. SDF-1 expression was substantially increased at the lesion site during the investigated period of fourteen days after the insult. Furthermore, HLA-positive hUCB cells were mainly detected in SDF-1 expressing brain regions and we were able to show that these cells express the SDF-1 receptor CXCR4 on their surface. The functional implication of SDF-1 in directing hUCB cell migration was determined by application of neutralizing SDF-1 antibodies in vivo, resulting in a reduced number of hUCB-derived mononuclear cells at the lesion site.

With these functional effects, together with the observed timing and location of its expression, the involvement of the chemokine SDF-1 in hUCB cell 'homing' seems conceivable.

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Novel Pathways in Inflammation-Induced Fetal Brain Injury. Michal A Elovitz, Jinghua Chai. *Obstetrics and Gynecology; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

Intro: While survival of extremely preterm infants continues to improve, the number of children with cognitive impairment and/or cerebral palsy is increasing. If preterm birth (PTB) cannot be prevented, then strategies to identify and treat fetal brain injury in the setting of a PTB must be investigated. These studies were performed to explore novel pathways involved in fetal brain injury in the setting of inflammation-induced PTB.

Methods: CD-1 mice on E15 receive intrauterine injection of lipopolysaccharide (LPS) or saline. 6 hours after injection, fetal brains from the left upper horn were harvested. 2 fetal brains were removed from each dam with 6 dams per treatment group. 12 separate RNA samples were prepared and used for microarray (MA) analysis. All protocols were conducted as described in the Affymetrix GeneChip Expression Analysis Technical Manual in the MA Core Facility using the MOE 430Av2 chip. Data analysis was performed using Significance Analysis for Microarray (SAM). The brain samples from the same dam were considered as dependent samples. Pathway analysis and functional annotation clustering tools were used with DAVID. Validations studies using QPCR with fetal brain samples (n=10-14 per group) were performed.

Results: While there was significant differences in gene expression between LPS and saline exposed fetal brains, variability existed even between pups from the same dam. 542 genes were significantly differentially expressed between LPS and saline brains ($P<0.01$). With a P value of <0.0001, 40 genes were differentially expressed. Pathway analysis revealed significant involvement of 1) the cadeherin, cadherin-like, cell fraction and calcium binding (enrichment score 4.03) and 2) ribosomal processing, RNA metabolism, pyrimidine metabolism (enrichment score 2.2). Specifically, genes involved in neurogenesis, synaptic function, and neuronal and glial metabolism were most differentially regulated. QPCR confirmed observed fold changes in 6/10 genes analyzed. Inflammatory pathways were not differentially regulated.

Conclusions: Current theories regarding fetal brain injury in PTB focus on activation of inflammatory processes as essential events. This data suggests that long-term neurological injury in a PTB may be secondary to altered neuronal function, metabolism and/or communication. Disruptions in these pathways should be explored as key mechanisms to adverse neurological outcomes in preterm neonates and should be targets for future investigations.

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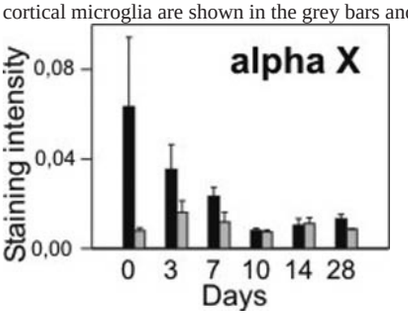
Regulation of Microglial Activation in the Developing Murine Brain. Mariya Hristova, Virginia Zbarsky, Daniel Cuthill, Adam Wallace, Donald Peebles, Gennadij Raivich. *Institute for Women's Health, University College London, London, United Kingdom.*

Introduction: The aim of this study was to assess the process of microglial differentiation in developing white matter, an area of the brain that is particularly vulnerable to damage pre-myelination (approx 32 weeks gestation). Microglia form a distinctive non-neuronal component. Although related to peripheral macrophages they undergo highly specific processes of regional maturation and differentiation inside the brain with a slimming of the cell body, development of very elaborate crenulated arborised branches and downregulation of most macrophage activation markers. This process is relatively rapid in most grey matter brain regions, but is retarded in and around the subcortical white matter (SWM) giving rise to the phagocytic fountains of microglia (FoM).

Methods: We examined the process of deactivation and morphological differentiation in the cortex and SWM of mice 0-28 days after birth (P0-P28) using confocal microscopy for monoclonal antibodies against alpha and beta integrin subunits and the costimulatory factor B7.2, colocalised with standard microglial marker IBA1.

Results: Strikingly, only the FoM macrophages, but not cortical microglia, strongly expressed typical activation markers alpha-5, alpha-6, alpha-M, alpha-X, beta-2 and B7.2. The data for alpha-X are shown as an example in the figure; cortical microglia are shown in the grey bars and SWM in black.



FoM activation was maximal at P0, decreased linearly over P3 and P7 and disappeared at P10. This process followed the presence of ingested phagocytic material but correlated only moderately with ramification, demonstrated by non-ramified but inactive P0 cortical microglia and formation of stubby processes in P3 FoM.

Conclusion: these data describe strong and selective biochemical activation of FoM phagocytes in P0-P7 SWM, roughly equivalent to early 3rd trimester human foetal development. This presence of highly active phagocytes in the neighbourhood of vulnerable WM could play an important role in the genesis of axonal damage in the foetus and premature neonate.

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Developmental Profile of Myelin Basic Protein (MBP) Expression in White Matter Tracts of Fetal Sheep Brain. Iwa Antonow-Schlorke,[1] Christine Gey,[1] Alexandra Helgert,[1] Thomas Muller,[2] Harald Schubert,[2] Otto W Witte,[1] Peter W Nathanielsz,[3] Matthias Schwab.[1] *[1]Dept. Neurol.; [2]Inst. Lab. Animal Science, Friedrich Schiller Univ. Jena, Germany; [3]Center Pregnancy Newborn Res., Dept. ObGyn., Texas Univ. Health Sciences Center, San Antonio, TX, USA.*

MBP plays a key role in central myelination (Baumann and Pham-Dinh, Physiol Rev 2001) as essential myelin protein (Moscarello, in: Cell biology and pathology of myelin. New York Plenum Press 1997, pp. 13-25). MBP is expressed in mature oligodendrocytes (Jakovcevski and Zecevic, Glia 2005). Although myelination in the fetal sheep brain, a model extensively used to evaluate fetal development, has been described using conventional staining techniques (Barlow, J Comp Neurol 1967) the use of specific MBP staining allows a more precise determination of the onset of myelination (Antonow-Schlorke et al., Reprod Sci 14.1, 2007, 248A).

Aim: To use MBP expression to determine the trajectory of development of different white matter tracts of fetal sheep brain.

Methods: The ontogenetic profile of MBP expression was estimated in 47 healthy fetuses at 0.27 (n=6), 0.40 (n=5), 0.53 (n=5), 0.63 (n=6), 0.75 (n=12), 0.87 (n=9) and 0.93 (n=4) of gestation (term 150 days). After brain fixation and embedding in wax, sections at the level of the optic chiasm were

stained with a monoclonal anti-MBP antibody using the ABC-technique. Immunohistochemical distribution of MBP was morphometrically assessed in the dorsal internal capsule and cortical white matter tracts, i.e. the lateral centrum semiovale, superficial white matter and median corpus callosum using an image analysis system (Scion Image 6.21, NIH, USA).

Results: MBP expression of various fetal white matter structures started at different time points; initially in the internal capsule at 0.53 of gestation followed by the cortical white matter structures at 0.63 of gestation. Cortical myelination advanced from the cortical deep white matter via superficial white matter to the corpus callosum reflecting different rates of progression.

Conclusions: The onset of MBP expression estimated here explicitly antedates previous observations in sheep (Barlow, 1969) demonstrating the higher sensitivity of MBP immunostaining compared to conventional myelin staining techniques (Urban et al., Anat Histol Embryol 1997). Data clearly reflect distinct myelination periods for different brain structures.

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Doppler Flow Velocity Waveforms Derived from the Chicken Embryonic Heart at Developmental Stages Comparable to First Trimester of Human Pregnancy Demonstrate Changes in Embryonic Cardiac Function. Annelien M Oosterbaan,[1] Nicolette TC Ursem,[1] Piet C Struijk,[1] Aton FW van der Steen,[2] Eric AP Steegers.[1] *[1]Obstetrics & Gynecology, Erasmus MC, Rotterdam, Netherlands; [2]Biomedical Engineering Thorax Centre, Erasmus MC, Rotterdam, Netherlands.*

The way the embryonic heart functions before cardiac morphogenesis is completed is still subject of many studies. To gain more insight into the early cardiac structure-function relationship, Doppler blood flow velocity waveforms at four different locations in the embryonic chicken heart during cardiovascular development were assessed. We collected waveforms using high frequency ultrasound biomicroscopy with a 55-MHz transducer at HH stages 18, 21 and 23 which are comparable to humans at 5 to 8 weeks of gestation. Waveforms were obtained at the inflow tract, the primitive left ventricle, the primitive right ventricle, and at the outflow tract in ten different embryos per stage. By exploring the time relation between the waveforms, cardiac cycle events were outlined. Our results demonstrate that stage and location dependent, intracardiac blood flow velocity waveforms can be obtained in the chicken embryo which reflect stage dependent pumping mechanisms. The blood flow profiles assessed at the four locations in the embryonic heart demonstrated a developmental related increase in velocity. In the primitive ventricle the passive filling wave decreased, whereas the active filling wave increased resulting in a decreased P to A ratio in the course of time. High frequency derived cardiac blood flow velocity characteristics support previous findings that the embryonic heart functions like a suction pump at early development and transforms towards another pumping mechanism at later developmental stages. These findings are of importance for the interpretation of human first trimester cardiac velocimetry studies.

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Normal Changes in the Diameter and Perimeter of the Fetal Thymus by Fetal Gender. Juan Antonio De Leon-Luis,[1] Francisco Gamez,[1] Pilar Pintado,[1] Joaquin Santolaya-Forgas,[2] Ricardo Perez-Fernandez,[1] Luis Ortiz-Quintana.[1] *[1]Department of Obstetrics and Gynecology, Hospital General Universitario Gregorio Maranon, Madrid, Spain; [2]Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA, USA.*

OBJECTIVE: In neonates (De Felice C et al. J Pediatr 1999; 135:384) and fetuses (Di Naro E et al. Am J Obstet Gynecol 2006; 194:153) thymus sizes bellow the 5th percentile have been linked to congenital infection. In this study we aimed at determining if the size of the thymus was different in male and female fetuses.

MATERIAL AND METHODS: The fetal thymus was measured between 24 and 37 weeks gestation as described by Zalel Y et al Prenat Diagn 2002; 22:114 and J.Y. CHO et al. Ultrasound Obstet Gynecol 2007; 29: 634. T-test was used for comparisons between groups.

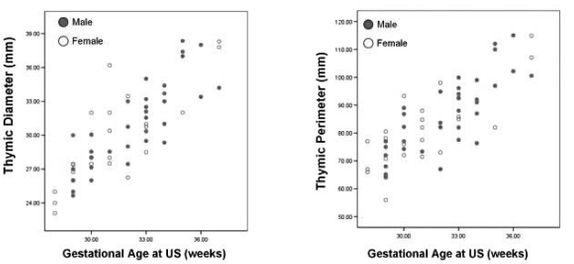
RESULTS: Summary statistics are shown in Table 1. Changes in the diameter of the thymus with gestational age by fetal gender are depicted in Figure 1a. Changes in the perimeter of the thymus with gestational age by fetal gender are depicted in Figure 1b.

CONCLUSIONS: This data suggests that thymus development as seen by ultrasound is similar in male and female fetuses.

Comaparison of thymus development by fetal gender

	Gender	Number of cases	Mean	Std. Deviation	p.value
GA at US (w)	Female	24	31.08	3.7	
GA at US (w)	Male	35	31.5	3.3	0.74
GA at delivery (w)	Female	24	39.1	3.8	
GA at delivery (w)	Male	35	38.8	2.9	0.60
Birth weight (gms)	Female	24	3073	652	
Birth weight (gms)	Male	35	3279	766	0.23
Diameter of the thymus	Female	24	29.6	4.7	
Diameter of the thymus	Male	35	31.4	4.6	0.16
Perimeter of the thymus	Female	24	85.3	14.7	
Perimeter of the thymus	Male	35	91.1	16	0.17

Development of the fetal thymus by fetal gender



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Impairment of Pre-Myelinating Oligodendrocytes Substantially Contribute to Glucocorticoid Sensitivity of Myelination. Iwa Antonow-Schlorke,[1] Alexandra Helgert,[1] Christine Gey,[1] Thomas Muller,[2] Harald Schubert,[2] Peter W Nathanielsz,[3] Otto W Witte,[1] Matthias Schwab.[1] *[1]Dept. Neurol.; [2]Inst. Lab. Animal Science, Friedrich Schiller Univ. Jena, Jena, Germany; [3]Center Pregnancy Newborn Res., Dept. ObGyn., Texas Univ. Health Sciences Center, San Antonio, TX, USA.*

Repetitive administration of betamethasone (BM) routinely used in perinatal medicine delays corpus callosum myelination in fetal sheep (Huang, Int J Dev Neurosci 2001). The timing of onset of myelination varies between brain structures.

Aim: To examine if susceptibility of myelination to BM depends on gestational age and brain region.

Methods: BM effects were evaluated in fetal sheep brains at 0.63 (n=6), 0.75 (n=3) and 0.87 (n=6) gestation (G) 48 h after the onset of one BM course to the ewe i.m. (2x110 µg/ kg maternal body weight 24 apart corresponding to 2x12 mg BM to a 70 kg pregnant woman). Persistency of BM effects was evaluated at 0.87 G after one BM course at 0.73 G (n=5) or two courses at 0.71 and 0.75 G (n=5). We used MBP as a marker for mature pre-myelinating and myelinating oligodendrocytes and myelin. MBP immunoreactivity (IR) was assessed in the dorsal internal capsule, lateral centrum semiovale, superficial white matter and median corpus callosum using an image analysis system.

Results: The impact of BM on MBP IR depended on gestational age. BM resulted in an acute loss of MBP IR in all white matter structures examined at 0.63 G ($p<0.05$), a stage when mainly pre-myelinating oligodendrocytes contribute to MBP IR. The duration of MBP susceptibility to BM differed between the cerebral white matter structures. MBP was sensitive to BM in the internal capsule from 0.63 to 0.87 G, in the centrum semiovale and superficial white matter at 0.63 and 0.75 G and, in the corpus callosum at 0.63 G. Loss of MBP IR was not detectable three weeks after fetal BM exposure. Repetition of BM treatment reduced MBP IR in the centrum semiovale by 40.6 % ($p<0.01$).

Conclusions: Single courses of BM have transient effects on early myelination mainly affecting pre-myelinating oligodendrocytes. The time course of the effect depends on brain region. The study provides evidence for disturbances of cerebral white matter after antenatal glucocorticoid treatment at the dose used clinically that might be crucial for the manifestation of cognitive and motor deficits in preterm children.

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Fetal Cerebral Blood Flow, Oxygenation, and Electrocorticographic Activity: The Role of Adenosine in Hypoxia-Mediated Responses. Stephen J Lee, Takuji Tomimatsu, Jorge Pereyra-Pena, Douglas P Hatran, Jonathon M Ross, Grant A McAuley, Lawrence D Longo. *Center for Perinatal Biology, Departments of Physiology and Obstetrics & Gynecology, Loma Linda Univ, Loma Linda, CA, USA.*

Background. For the fetus, the roles of arterial blood gases are recognized to be critical in the regulation of cerebral blood flow (CBF) and cerebral metabolic rate for oxygen ($CMRO_2$), cortical tissue PO_2 (tPO_2), and electrocorticographic (ECoG) activity. In addition, metabolites such as adenosine (ADO) are important in this regard. Nonetheless, the relation of adenosine and its metabolites to these indices of cerebral oxygenation are not well defined. In an effort to elucidate these interrelations, we tested the null hypothesis that acute hypoxic-associated cerebral oxygenation and related variables are not closely associated with adenosine.

Methods. By use of a laser Doppler flowmeter with fluorescent O_2 probe, and with ECoG electrodes, in near-term fetal sheep (n = 14) we measured laser Doppler CBF (LD-CBF), cortical tPO_2, ECoG root mean square (RMS) voltage, spectral edge frequency (SEF_{90}), *et cetera* in response to 20 min ADO receptor blockade alone, followed by 40 min moderate isocapnic hypoxia with continued ADO-R blockade.

Results. LD-CBF, cerebral O_2 delivery, tPO_2, and several other variables correlated highly with ECoG state. During the normoxic control period, blockade of adenosine-receptors (ADO-R) by 8-SPT, but not caffeine, decreased LD-CBF 10±1% ($p<0.05$), associated with a shift from high voltage low frequency (HVLF) to low voltage high frequency (LVHF). Continued ADO-R block and superimposed hypoxia (PaO_2 = 12±1 Torr) was associated with a LD-CBF increase of only 26±3% (as opposed to the usual increase of 44±4%), and the variation between HVLF and LVHF disappeared with a decrease of SEF_{90} ($p<0.05$).

Conclusions. For the normoxic near-term fetus with ADO-R inhibition by 8-SPT, LD-CBF decreased significantly. Acute fetal hypoxia with ADO blockade was associated with a significant shift and loss of variation in ECoG state and attenuated hypoxic-induced increase in LD-CBF. Thus, we reject the null hypothesis that in such fetuses, inhibition of adenosine-mediated cerebral vasodilation demonstrates no compromise in cerebral oxygenation. (Supported by USPHS HD-03807).

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Dephosphorylated Cofilin-1: A New Mechanism by Which Chronic Hypoxemia (HPX) Might Cause Fetal Brain. Y Dong, W Hou, Q Zhu, J Stites, CP Weiner. *Obstetrics and Gynecology, University of Kansas School of Medicine, Kansas City, KS, USA.*

OBJECTIVES: The most common cause of perinatal brain injury is chronic hypoxia/ischemia. The mechanisms are complex and poorly understood. Applying proteomics tools, we identified a set of hypoxia- related proteins in the fetal guinea pig (GP) brain (companion abstract). One protein, cofilin-1, which regulates the rapid cycling of actin assembly and disassembly, was dramatically altered by chronic brain HPX. Herein, we test the hypothesis that confilin-1 modifications during HPX contributes to brain damage via apoptosis and is an example of an adaptive response that becomes maladaptive.

METHODS: Time-mated GPs were housed in a chamber from 50d to 65d (term) with either room air (NMX) or 10.5% or 12% O_2. Fetal brains were removed and sections prepared for double staining specific to Bax and dephosporylated cofilin-1. Total protein was isolated and equal amounts loaded on a SDS gel, separated by electrophoresis, and transfered to PVDF membranes probed with primary antibodies to dephosphorylated and phosphorylated cofilin-1, Bax and Bcl-XL, and incubated with second antibody. Protein signals were detected, quantified by densitometry, and normalized to β-actin. Total RNA was isolated, reverse-transcribed, and quantified by real-time PCR using SYBR Green I labeling. Expression was calculated by the ΔΔCT method using 18s for control. Melt analysis was performed to confirm specificity of the amplification, and efficiency determined by the slope of the standard curve.

RESULTS: Periventricular Bax co-localized with dephosphorylated cofilin-1. HPX decreased phosphorylated cofilin-1 (NMX vs. 12% O_2 vs. 10.5% O_2: 108.2±1.7 vs. 79.8±1.8 vs. 64.7±2.2, respectively, n=4, $p<0.05$). Unanticipated was the finding HPX up-regulated dephosphorylated cofilin-1 (NMX vs. 12% O_2 vs. 10.5% O_2: 104.6±4.4 vs. 129.4±5.1 vs. 147.9±2.5, respectively, n=4, $p<0.05$). HPX increased cofilin-1 mRNA (NMX vs. 12% O_2 vs. 10.5% O_2: 2.3±0.3 vs. 7.3±0.2 vs. 7.9±0.2, respectively, n=4). HPX also increased Bax and Bcl-XL mRNA ($p<0.05$). All changes were proportional to the degree of HPX.

CONCLUSIONS: Cofilin-1 is modified by chronic HPXat transcriptional, translational, and posttranslational levels. Its co-localization with markers of neuronal apoptosis suggest cofilin-1 modification contributes to HPX brain damage via increased apoptosis.

ACKNOWLEGEMENT: Supported by grants from the PHS (R01 HL049041-12, cpw) and CDC grant (U7DP00187-03, cpw).

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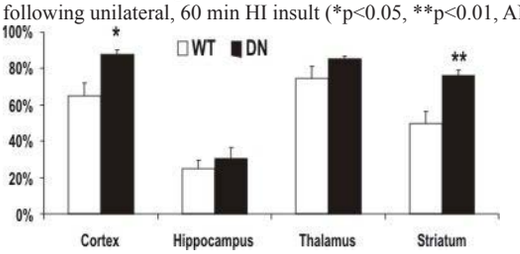
The Role of Mitogen Kinase Kinase (MEK) and Its Inhibition in Perinatal Brain Injury. Marya Hristova,[1] Virginia Zbarsky,[1] Giles Kendall,[1] Milan Makwana,[1] F Miller,[2] D Kaplan,[2] Gennadij Raivich,[1] Donald Peebles.[1] *[1]Institute for Women's Health, University College London, London, United Kingdom; [2]Sick Childrens Hospital, University of Toronto, ON, Canada.*

Introduction: The mitogen activated protein kinase (MAPK) signalling pathway is upregulated in perinatal hypoxic-ischaemic brain. The role of the extracellular signal-regulated kinase (ERK) cascade, a pivotal component of MAPK signalling, remains unclear with reported actions ranging from mitogenic and trophic effects to a neuronal death-promoting role. The aim of this study was to assess the role of neuronal ERK activity using selective neuronal inhibition in a perinatal model of hypoxic-ischemic brain injury.

Methods: we explored the effects of selective neuronal inhibition of ERK activation using transgenic mice expressing dominant-negative (dn) MEK1, the upstream activator of ERK1/2, which was under the control of the pan-neuronal Talpha1 alpha-tubulin promoter. P-ERK immunoreactivity was quantified following a hypoxic inschemic (HI) insult in P7 C576Bl/6 mice, caused by unilateral occlusion of the carotid artery, followed by hypoxia with 8% oxygen for 60 mins at 36°C. The volume of surviving brain on the affected hemisphere was expressed as a % relative to the control side.

Results: Expression of the MEK1 dn was associated with a reduction in ERK1/2 activation following hypoxia ischaemia, as assessed by p-ERK immunoreativity. Compared with the wild type, littermate controls, MEK1dn animals exhibited significantly decreased volume of forebrain damage brain following unilateral, 60 min HI insult (*$p<0.05$, **$p<0.01$, ANOVA).



Similar protective effects of MEK1dn were observed in cerebral cortex, hippocampus, thalamus and striatum when compared to wild type littermate controls and correlated with a significant reduction in microglial activation in all brain areas.

Conclusion: Overall, these results suggest that neuronal MEK1 and its downstream signals have an important death-inducing role in this model of perinatal brain injury and could serve as potential targets for therapeutic intervention.

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Melatonin Rescues the Detrimental Effects on Placental Efficiency of Maternal Undernutrition but Not of Maternal Hypoxia. JA Hansell,[1] CE Blanco,[2] DA Giussani.[1] *[1]Physiology, Cambridge University, United Kingdom; [2]Pediatrics, Maastricht, Netherlands.*

The presence of melatonin receptors in the placenta suggests a functional role in development (Iwasaki et al. *J Pin Res* **39:** 261, 2005). Melatonin protects against ischaemia/reperfusion-induced oxidative damage in rat placenta (Okatani et al. *J Pin Res* **31:** 173, 2001). Complicated pregnancy is often characterised by reductions in oxygen and nutrient delivery, and both conditions promote oxidative stress (Halliwell & Gutteridge. *Free Radicals in Biology & Medicine,* OUP, 2004). However, whether melatonin protects placento-fetal development during hypoxic or undernourished pregnancy is unknown. We investigated in rats the effects on placental and fetal growth of maternal treatment with melatonin in hypoxic and undernourished pregnancy.

Methods: On pregnancy day 15, Wistar rats were divided: control (21% O_2) ± melatonin (5μg.ml^{-1} drinking water), hypoxia (10% O_2) ± melatonin, and undernutrition (40% reduction in food intake) ± melatonin (n=7 per group). On day 20, dams were anaesthetised, the pups, placentae and fetal brain were weighed.

Results: Relative to controls (fetal weight: 3.73±0.05g; brain/body weight

ratio: 46.2±1.2mg/g; n=76), both hypoxic (2.94±0.03g; 58.4±1.1mg/g, n=80) and undernourished pregnancy (3.05±0.04g; 53.0±1.1mg/g, n=80) promoted asymmetric IUGR (P<0.05), without affecting placental weight. Melatonin treatment had no effect on IUGR, but it decreased placental weight in normoxic (0.43±0.01, n=95), hypoxic (0.44±0.01, n=98) and undernourished (0.41±0.01, n=81) pregnancies relative to untreated controls (0.48±0.01, n=76; P<0.05). When fetal body weight was expressed relative to placental weight, a measure of placental efficiency, melatonin prevented the fall in the ratio in undernourished, but not hypoxic pregnancies (Fig.1).

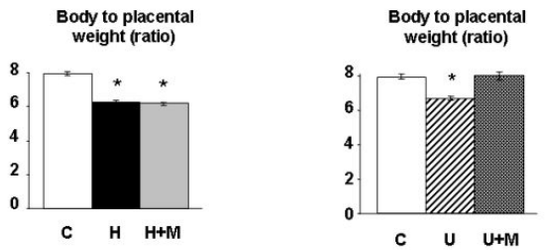

Fig.1. Melatonin treatment on fetal: placental weight in hypoxic and undernourished pregnancy. C, control; H, hypoxia; U, undernourished; M, melatonin. *P<0.05 *vs.* C; ANOVA+ Dunn's.

Conclusion: Melatonin rescues the detrimental effects of maternal undernutrition but not of maternal hypoxia on placental efficiency.

British Heart Foundation and BBSRC.

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Profile of Midkine, a Heparin-Binding Growth Factor, in Human Amniotic Fluid and Amnion. Hitoshi Ishimoto,[1] Takayuki Higuchi,[1] Kazuhiro Minegishi,[1] Satoshi Asai,[1] Seon Hye Kim,[1] Mamoru Tanaka,[1] Robert B Jaffe,[2] Yasunori Yoshimura.[1] *[1]Department of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo, Japan; [2]Center for Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA.*

Objective: Midkine (MK) is a 13-kDa heparin-binding growth factor with various functions including cell proliferation, migration, differentiation and angiogenesis. MK expression is strictly regulated in temporal sequence; it is highly expressed during midgestation. We recently studied MK expression in the midgestation human fetus, and showed that the highest MK expression were observed in the adrenal gland, brain, lung and kidney. In the present study, we investigated the profile of MK in human amniotic fluid (AF) and amnion (AM).

Methods: Amniotic fluid and amniotic membranes were collected at diagnostic amniocentesis, preterm no labor, and term no labor. MK protein levels were analysed by western blot. Expression of transcripts encoding MK and putative MK receptors were examined by RT-PCR and real-time quantitative RT-PCR (qPCR).

Results: 1) Western blot analysis demonstrated abundant MK protein in the human AM; MK levels were higher at midgestation than at term (3-fold: 16wks vs. 37wks). 2) Tissue transglutaminase known to polymerize MK was abundant in AF. 3) qPCR revealed that MK mRNA was not expressed in AM whereas it was highly expressed in the fetal adrenal, kidney and lung (positive controls). 4) Among the receptors implicated in MK signaling, low-density lipoprotein receptor-related protein and syndecan-4 were expressed in AM while protein-tyrosine phosphatase, anaplastic lymphoma kinase, and syndecan-3 were not.

Conclusions: Abundant MK protein in the midgestation AF is likely to be derived from the fetus. MK in AF may play a role in feto-amniotic communications and/or development of fetal organs exposed to AF.

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Long Term Expression of Human Factor IX after Ultrasound-Guided Fetal Gene Therapy in Sheep. Anna L David,[1] Jenny McIntosh,[2] Amit Nathwani,[2] Terry Cook,[3] Michael Boyd,[4] Boaz Weisz,[1] Victoria Wigley,[1] Khalil Abi-Nader,[1] Charles Rodeck,[1] Donald Peebles.[1] *[1]Institute for Women's Health, University College London, London, United Kingdom; [2]Department of Haematology, University College London, London, United Kingdom; [3]Department of Pathology, Imperial College, London, United Kingdom; [4]Biological Sciences Unit, Royal Veterinary College, London, United Kingdom.*

Introduction: Gene therapy for human factor IX (hFIX) deficiency is an attractive option as hFIX antibodies prevent adequate hFIX replacement therapy in up to 15% patients and only 1% hFIX levels are needed to provide cure; however, adult gene therapy trials with adeno-associated virus (AAV) show only short term hFIX expression. We hypothesized that long term hFIX expression could be achieved in fetal sheep using an AAV vector, without stimulating an immune response to the transgenic hFIX protein.

Methods: We injected AAV8 hFIX vector ($1 – 9 \times 10^{12}$ p/kg) into the peritoneal cavity of fetal sheep under ultrasound guidance in early (n = 3) or late (n = 4) gestation. Fetal blood was retrieved by ultrasound guided sampling from the intra-hepatic umbilical vein. Fetal and lamb blood was tested for hFIX expression using ELISA, antibody responses using functional assays, and for liver damage up to a year after birth. Vector spread was detected in maternal and fetal tissues by quantitative PCR analysis.

Results: The highest level hFIX was detected 18 days after late IP injection (44% and 28% normal human levels). Early gestation IP injection gave 8.7% and 1.8% at 3 and 21 days after injection. hFIX levels dropped rapidly correlating with the increase in size of the fetal liver and lamb. However, hFIX was detectable at a low levels (0.7%) 1 year after birth in early and 4 months after birth in late gestation injected lambs. Up to 1 year after birth, liver function tests and bile acid levels were normal, showing no evidence of liver pathology. No functional antibodies to the hFIX protein or AAV vector were detectable. High vector levels were detected in the fetal liver, and other peritoneal organs; no vector was present in fetal gonadal tissue.

Conclusion: hFIX expression is detectable up to 1 year after delivery of AAV vector to the fetal sheep using a clinically applicable method. This is the first study to show long term hFIX expression after fetal gene therapy in a large animal. Further work will include testing for immune tolerance to exogenous hFIX protein in these animals.

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Fetal Carriage of the 4G/4G Plasminogen Activator Inhibitor Type 1 (PAI-1) Genotype Is a Risk Factor for Fetal Thrombotic Vasculopathy and Has Functional Consequences. I Birgitta Sundell, Roberto Romero, Sun Kwon Kim, Jung-Sun Kim, Juan Pedro Kusanovic, Francesca Gotsch, Offer Erez, Sonia Hassan, Chong Jai Kim. *Perinatology Research Branch, NICHD, NIH, DHHS, Detroit, MI, USA.*

Objective: Fetal thrombotic vasculopathy (FTV) is characterized by changes in the chorionic villi, frequently termed "avascular chorionic villi". FTV is associated with adverse pregnancy outcome, yet, the pathophysiology is unknown. An association between the 4G/4G PAI-1 genotype and ischemic disease (stroke and myocardial infarction) has been reported. Therefore, we conducted a study to determine if there is an association between fetal PAI-1 4G/5G promoter polymorphism and fetal thrombotic vasculopathy.

Methods: A genetic association study was conducted. It included 94 cases of fetal thrombotic vasculopathy and 188 controls matched for gestational age. Genotyping of fetal DNA was performed by PCR. PAI-1 activity in fetal plasma was also determined in a subset of patients (56 cases and 128 controls).

Results:

Characteristics of Study Population and PAI-1 Genotype

Variables	Controls (n=188)	Cases (n=94)	P Value
§IUFD	0 (0)	4 (4.3)	0.012
Preeclampsia/Eclampsia	17 (9.0)	13 (13.8)	0.219
Chronic hypertension	7 (3.7)	5 (5.3)	0.531
*IUGR	24 (12.8)	22 (23.4)	0.023
Genotype			<0.001
5G/5G	43 (22.9)	6 (6.4)	
4G/5G	99 (52.7)	26 (27.7)	
4G/4G	46 (24.5)	62 (66.0)	

Values expressed as n (%)

§IUFD, Intrauterine fetal demise; *IUGR, Intrauterine growth restriction

Factors Associated with FTV in Multiple Regression Analysis

Selected variables	Groups	Odds ratio†	95% Confidence interval	P value
IUGR	No§	1		
	Yes	2.82	1.32-6.04	0.007
Genotype				
	5G/5G§	1		
	4G/5G	2.3	0.84-6.31	0.106
	4G/4G	12.41	4.56-33.75	<0.001

§Reference Group

†Adjusted for materinal age, smoking history, preeclampsia or eclampsia, chronic hypertension and gestational diabetes

Conclusion: 1) Fetal 4G/4G PAI-1 genotype is a powerful risk factor for fetal thrombotic vasculopathy (OR=12.41); 2) Fetal carriage of this polymorphism has functional consequences: an increase in fetal plasma PAI-1 activity; 3) A fetal thrombophilic state is likely to predispose to the development of ischemic lesions of the placenta, as well as fetal and adult vascular thrombotic disease; 4) We propose that some disorders attributable to fetal programming may have a genetic basis.

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Urocortin 2 Effects on Estradiol (E2) Secretion and P450 Aromatase mRNA and Protein Expresion in Cultured Human Placental Trophoblasts. Alberto Imperatore,[1,2] Felice Petraglia,[2] John RG Challis.[1] [1]*Departments of Physiology, Obstetrics and Gynaecology and Medicine, University of Toronto, Toronto, ON, Canada;* [2]*Department of Paediatrics, Obstetrics, and Reproductive Medicine, University of Siena, Siena, Italy.*

Objective: Placental estrogens play a pivotal role in the endocrine control of pregnancy and may be involved in the key changes occuring during parturition. It has been established that CRH interacting with CRH receptor 1 has a positive effect on estrogen production throughout pregnancy. Urocortin 2 (Ucn 2), a novel peptide of the CRH family binding exclusively CRH receptor 2, is expressed by human placenta and the aim of the present study was to evaluate the influence of Ucn 2 on estrogen biosynthesis in cultured trophoblast cells. **Methods:** Cultured term placental cells were treated with various concentrations of Ucn2 in the presence of the estrogens precursors dehydroepiandrosterone sulphate (DHEA-S), androstenedione or testosterone. Estradiol secretion was measured in the culture medium using a specific ELISA, P450arom mRNA and protein expression were evaluated by real time PCR and western blot analysis respectively. **Results**: The addition of Ucn2, in presence of DHEAS significantly increased E2 levels at 4, 8 and 12 hours treatment, while in presence of androstenedione and testosterone an increase in E2 secretion was detected only at 12, 24 and 36 hours (at different Ucn2 doses). Both P450arom mRNA and protein were up-regulated in presence of each estrogen precursor and the addition of Ucn2 caused a synergistic increase. Anti-sauvagine 30 (a CRH type 2 receptor antagonist) resulted in a significantly attenuated Ucn2 effects on E2 secretion and on P450arom mRNA and protein expression. **Conclusions**: The present study supports a possible role of Ucn2 on placental E2 biosynthesis: E2 secretion and P450arom transcript and protein expression were significantly increased after Ucn2 treatment. In conclusion, the CRF family may play a major role on placental steroidogenesis, stimulating DHEAS secretion in fetal adrenals by CRH and controlling placental estrogen biosynthesis through Ucn2. A possible influence on the mechanisms of parturition may be hypothesized.

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Increased Expression of KiSS-1 Gene in Preterm Placenta. Letizia Galleri, Michela Torricelli, Chiara Voltolini, Fernando M Reis, Felice Petraglia. *Department of Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Siena, Italy.*

Introduction Placenta expresses a large number of peptides involved in delivery. Neuropeptides, cytokines, are expressed and secreted by human placenta at the time of preterm delivery. Human placenta is a major source of KiSS-1, a 54-amino-acid peptide encoded by a putative metastasis suppressor gene. KiSS-1 acts on its placental G protein-coupled receptors (KiSS-1R); this peptide stimulates release of oxytocin in rats, the most potent known uterine stimulant, suggesting a possible role of KiSS-1 in the mechanisms of labor.

Aim of study The aim of this study was to evaluate placental expression of KiSS-1 at preterm labor.

Material and methods Placental tissue and plasma samples (both maternal and fetal) were collected at term in the absence of labor (TNL), at term spontaneous vaginal delivery (TL), and at preterm labor (PTL). Changes in placental mRNA expression were determined by real-time quantitative RT-PCR analysis. KiSS-1 protein levels were measured by specific immunoenzymatic assay (ELISA).

Results Placental KiSS-1 mRNA expression was significantly higher ($P<0.0001$) in PTL than in TNL and in TL. However, maternal and fetal plasma KiSS-1 levels did not differ among TNL, TL, and PTL samples.

Conclusion The present study showed that placental KiSS-1 mRNA expression is increased in preterm delivery. Further studies are needed to better understand the role of KiSS-1 on cascade leading term and preterm labor.

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Hypoxia Enhances FGF2- and VEGF-Stimulated Human Placental Artery Vascular Endothelial Cell Proliferation. Kai Wang,[1,2] Jing Zheng.[1] [1]*Department of Ob/Gyn, University of Wisconsin-Madison, Madison, WI, USA;* [2]*Department of Biochemistry, Dalian Medical University, Dalian, LiaoNing, China.*

Placental angiogenesis is essential for placental development, which occurs under a hypoxic environment (~ 2-8 % O_2) during normal pregnancy. It has been reported that in transformed human dermal microvascular endothelial cells, hypoxia activates ERK1/2, which stabilizes hypoxia-inducible transcription factor-1α (HIF-1α). However, signaling mechanisms governing placental angiogenesis under hypoxia is largely unknown. Herein, we tested whether hypoxia affected FGF2- and VEGF-stimulated cell proliferation partly via activating ERK1/2 and stabilizing HIF-1_α protein levels in human placental artery endothelial (HPAE) and transformed human placental microvascular endothelial (HPME) cells. **Methods:** Cells cultured under normoxia (~20% O_2) or hypoxia (3% O_2) for 2 days were treated with FGF2 and VEGF. After 3 days, the number of cells was determined. Activation of ERK1/2 and levels of HIF-1_α protein were determined by Western analysis. **Results:** Under normoxia, FGF2 and VEGF stimulated ($p < 0.05$) cell proliferation and induced (≤ 5 min, $p < 0.05$) ERK1/2 phosphorylation in HPAE and HPME cells. Hypoxia promoted ($p < 0.05$) FGF2- and VEGF-stimulated cell proliferation in HPAE cells, whereas hypoxia blocked ($p < 0.05$) such actions in HPME cells. Hypoxia enhanced FGF2-, but not VEGF-induced ERK1/2 phosphorylation in HPAE cells. In contrast, hypoxia promoted ($p < 0.05$) VEGF-, but not FGF2-induced ERK1/2 phosphorylation in HPME cells. Hypoxia increased ($p < 0.05$) HIF-1_α levels in the HPAE cells: first detected at 0.5 hr and maintained up to 24 hr. HPME cells had high basal levels of HIF-1_α (3 folds; $p < 0.05$) compared with HPAE cells. Hypoxia did not alter HIF-1_α levels up to 4 hr and decreased ($p < 0.05$) HIF-1_α levels at 24 hr in HPME cells, **Conclusions:** In HPAE cells, hypoxia enhances FGF2- and VEGF-stimulated cell proliferation possibly partially via promoting activation of different protein kinases. This stimulatory effect is associated with increased HIF-1_α protein levels. However, inhibition by hypoxia on FGF2- and VEGF-stimulated HPME cell proliferation is associated with relatively high HIF-1_α levels in the first 4 hr and decreased HIF-1_α levels at 24 hr. Thus, these data suggest that different signaling mechanisms are involved in hypoxia-modulated growth factor-induced placental angiogenesis in which an increase in HIF-1_α levels plays a critical role.

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A Time-of-Day Difference in Rat Placental Corticotrophin Releasing Factor Expression: Placental Giant Trophoblast Cells Behave like Central Nervous System Neurons. Jayaraman Lakshmanan, John D Richard, Bindu A Cherian, Sharon K Sugano, Avish Arora, Thomas Magee, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Corticotrophin releasing factor (CRF) is a well established neurohormone involved in the initiation of the stress response. We recently documented that rat placenta is analogous to human placenta in the expression of CRF-mRNA and protein, and that placental CRF release may contribute to in utero meconium passage. Time-of-day variation in CRF expression is a highly recognized phenomenon at the brain cellular sites of CRF synthesis. We sought to determine whether CRF expression in rat placenta is subject to time-of-day variation.

Method: Time-dated pregnant rats were obtained on day 12 of gestation (Term=22 days), housed in under 12h-12h light-dark conditions (lights on 0600). Lab chow and water were continuously available. On day 18, pregnant rats were quickly anesthetized by exposure to isoflurane, abdomen opened and fetuses and placenta exteriorized either at 0545-0600hr (zeitgeiber time, ZT0) or 1245-0100hr (ZT6). Individual placentas were processed either for RNA extraction or immunohistochemical investigation. The levels of CRF-mRNA were assessed by PCR using rat specific PCR primers. PCR bands were subsequently cloned and sequenced. Bouin's solution fixed paraffin sections of placenta were subjected to CRF immunohistochemistry with antibodies specific to rat species and intensity of immunostaining was analyzed using Image pro-Plus software and expressed in arbitrary units (AU).

Results: One specific PCR band of 339 bp size was consistently identifiable in placenta harvested at ZT6 but not at ZT0. Nucleotide sequence of the PCR band confirmed its identity as CRF-mRNA. Intensity of CRF-immunostaining was significantly greater at ZT6 in giant trophoblast (GT) cells (GT-CRF: ZT0= 0.176 ± 0.122 AU, ZT6= 0.272 ± 0.014 AU, P=0.007) but not in spongiotrophoblast cells (STC) (STC-CRF: ZT0 = 0.218 ± 0.025 AU, ZT6= 0.162 ± 0.011AU, P=NS) or labyrinth cells (LBC) (LBC-CRF: ZT0= 0.148 ± 0.003, ZT6 = 0.149 ± 0.015 AU, P=NS) .

Conclusion: Similar to adult brain, rat placenta expresses CRF mRNA and protein. Time-of-day variation of CRF expression originally seen in central nervous system neurons is also identifiable in giant trophoblasts cells at ZT6. These findings suggest that stress-mediated placental CRF release, and potentially fetal meconium passage, may be dependent upon time-of-day.

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Stimulation of AQP1 Gene Expression by Cyclic AMP Agonists in Human Trophoblast Cells: Potential Modulation of Maternal-Fetal Water Transfer. Louiza Belkacemi, Marie H Beall, Thomas R Magee, Margaret Pourtemour, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objectve: Transplacental water flow is essential for the provision and maintenance of fetal body water and amniotic fluid. Water flow across membranes is regulated by aquaporin (AQP) water channels in many tissues. Thus AQP1, expressed in the placenta, is a candidate to regulate maternal to fetal fluid exchange. Maternal beta-mimetics have been hypothesized to augment fetal growth by increasing the availability of nutrients and perhaps water. As cAMP acts as a second messenger to increase expression of selected AQPs in other tissues, we sought to determine if betamimetics acting through cAMP could modulate placental AQP1, and potentially influence placental water transfer.

Methods: Trophoblastic cell cultures were established in first trimester-derived extravillous HTR-8/Svneo cells and term placenta-like trophoblast carcinoma cell line JEG-3. Cultures were treated with SP-cAMP, a membrane-permeable and phosphodiesterase resistant cAMP, and forskolin, an adenylate cyclase stimulator, in doses of 0.5, 5 and 50 µM for 2 hrs (AQP1 mRNA expression) and 20 hrs (AQP1 protein expression). For time course experiments, cells were incubated with 5 µM of either SP-cAMP or forskolin for 2, 10 and 20 hrs at 37°C, 5% CO_2. After cell harvest, mRNA and protein expression were assayed using real time PCR and Western blotting.

Results: SP-cAMP and forskolin increased AQP1 mRNA expression in both cell lines after 2 hrs ($p<0.05$) in a dose-dependent manner. Protein expression paralleled the increase seen in the mRNA. 5 µM of SP-cAMP and forskolin stimulated AQP1 mRNA expression after 2 hrs in HTR-8/Svneo cells and after 10 hrs in JEG-3 cells ($p<0.05$). 5 µM of either SP-cAMP or forskolin stimulated AQP1 protein expression in both cell lines after 10 hrs ($p<0.05$) and the expression remained high at 20 hrs.

Conclusion: AQP1 gene expression in trophoblast cells is up-regulated by cAMP agonists. These results suggest that maternal beta-adrenergic agonists or antagonists may modulate maternal-fetal water flux via modulation of AQP water channels.

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Rat Placenta Expresses Corticotrophin Releasing Factor-Binding Protein and mRNA. Jayaraman Lakshmanan, Thomas R Magee, Bindu Cherian, Sharon K Sugano, Hanalise Huff, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Corticotrophin releasing factor- binding protein (CRF-BP), a 37 kDa secreted glycoprotein, was originally isolated from human plasma. It binds CRF and urocortin I with an equal or greater affinity than does the CRF receptor. CRF-BP expression has been reported in target tissues such as brain and placenta. Based on its ability to inhibit CRF actions in vitro, it is speculated to function as a "gate keeper" for CRF-initiated stress responses. We recently documented expression of CRF and Urocortin-1 in rat placenta. In the present study we sought to establish the expression of CRF-BP in rat placenta by immunohistochemical and PCR-analyses.

Methods: Placental tissues (n=20) collected from Sprague-Dawley pregnant rats on day 21 of gestation (term=22) were either fixed in 4% paraformaldehyde solution (pH 7.4) and paraffin embedded or processed for total RNA extraction. Paraffin sections of five micron thickness were subjected to immunohistochemical analysis with goat polyclonal antibodies to human CRF-BP precursor (sc-1824 Santa Cruz Biotechnology, CA) by avidin-biotin-peroxidase complex technique. The structure of cloned human CRF-BP precursor exhibit significant amino acid sequence homology among all species studied. Immunoreactivities on the sections were quantified using Image Pro 4.01 software and staining intensity (OD/area) expressed as arbitrary units (AU). All values are expressed as mean±SEM. For PCR, cDNA was synthesized and PCR amplified with a one step RT-PCR kit. Fragments were gel purified, clone into plasmid vector and DNA sequenced.

Results: The CRF-BP antibody elicited strong positive staining in decidua, giant trophoblasts, spongiotrophoblasts and labyrinth cells. The results of image analyses revealed (AU): decidua: 0.191±0.010, giant trophoblast cells: 0.139±0.029, spongiotrophoblasts: 0.120±0.003, fetal membranes: 0.128±0.009. PCR analyses identified a single 434 bp band, consistent with CRF-BP.

Conclusion: Our study establishes for the first time that rat placenta is analogous to humans in that both express CRF-BP mRNA and protein. Immunohistochemical findings reveal that CRF-BP protein expression occurs at multiple sites within the placenta.

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Expression of Per2 Clock Protein in Rat Placenta: An Internal Timer for Placental Functions. Jayaraman Lakshmanan, Reuben Lakshmanan, Sharon K Sugano, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Corticotrophin releasing factor (CRF) expression time-of-day variation occurs in giant trophoblast cells on the maternal side of the rat placenta. This temporal change in CRF expression pattern is very similar to that of central nervous system neurons, suggesting that placental cells may use a similar mechanism to read time of the day. The self-sustaining rhythm generating capacity of the suprachiasmatic nuclei is believed to be derived from cell-autonomous, transcriptional feed-back loops dependent on a number of canonical clock genes. In the present study we sought to determine whether rat placenta expresses period gene 2 (Per 2), one of the clock/cycle related genes.

Methods: Time-dated pregnant Sprague-Dawley rats were received on day 13 of gestation and housed in a controlled environment (0600-1800h lights on) with free assess to food and water. Placentas collected on days 20 and 22 of gestation (term=22days) between 0930 and 11.30 am were fixed in 4% paraformaldehyde and paraffin embedded. For each gestational ages a total of 12 placentas from 6 different pregnant rats were used. Paraffin sections (3 sections per placenta) were subjected to immunohistochemical analysis with antibody to Per2 (1:200, Santa Cruz biotechnology, CA) by ABC technique and 3, 3'diaminobenzidine as a chromagen. Sections were examined under microscope, imunostaining quantified by Image Pro 4.01 software and expressed in arbitrary units (AU). Data are presented as mean ± SEM. Statistical significance was analyzed by ANOVA with a P value < 0.05 as significant.

Results: Positive staining for Per2 was seen in giant trophoblasts (GT), spongiotrophoblasts (ST), and labyrinth (LB) cells. The relative intensities (AU) were as follows: Day 20: GT: 0.138±0.029, ST: 0.181±0.012 and LB: 0.160±0.005 (P=NS) and Day 22: GT: 0.125±0.015, ST: 0.180±0.026 and LB: 0.203±0.009 (LB > GT, ST, $P<0.05$).

Conclusion: The present results indicate that rat placental cells, devoid of any neural innervations, express Per2 clock protein, similar to central nervous system neurons. Based on the differences in the relative intensities between trophoblasts and labyrinth cells on day 22 of gestation, we conclude that fetal-maternal interactions in Per2 regulation disappear at term (or at the time of birth.). Our findings imply that Per2 may function as internal physiological modulator in placenta.

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Upregulation of Placental Nuclear Glucocorticoid Receptor Expression Is a Most Rapid Response to Maternally Administered Betamethasone at Early Morning Hours. Jayaraman Lakshmanan, John D Richard, Sharon K Sugano, Raina Khan, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: The rat placenta is a newly recognized site of corticotrophin releasing factor (CRF) expression, and studies in our laboratory have demonstrated a time-of-day variation of CRF expression in giant trophoblast cells. Glucocorticoids (GCs) are the only known positive regulators of placental CRF expression in humans. To determine the mechanism(s) underlying placental time-of-day CRF variation, we examined the effect of maternal administration of betamethasone (a synthetic glucocorticoid) on nuclear GC receptor expression in placental cells.

Method: Time-dated pregnant rats (n=6) on day 21 of gestation were given a single subcutaneous injection (at 5:45 AM) of betamethasone (350µg/kg body weight) while control pregnant rats (n=6) received saline. Dams were exposed to isoflurane anesthesia at 10:45 AM, and placentas harvested, fixed in Bouin's solution and paraffin embedded. Sections were subjected to immunohistochemical analysis with GC-receptor antibody (sc-8992, Santa Cruz Biotechnology), with immunoreactive material identified by ABC technique using 3, 3' diaminobenzidine as a chromagen. Percentages of GC-nuclear

receptor (GC-NR) positive cells and their intensities (OD/area) were quantified using Image pro 4.01 plus software. All values are expressed as mean ± SEM. Statistical analysis was by ANOVA with $p<0.05$ considered significant.

Results: In placentas of saline exposed pregnant rats, isolated labyrinth (LB) cells (<1%) were positive to GC-NR. No positive staining for GC-NRs was seen either in giant trophoblasts (GT) or in spongiotrophoblasts (ST). Betamethasone administration was associated with a significant and marked increase in GC-NR staining in all three placental cell types ($p<0.05$). Among the cells, GT (69±10%) demonstrated a greater percentage of cells expressing GC-NR than did ST (34±5%) or (LB=38±5%) ($p<0.05$), though there was similar immunoreactive intensities (GT: 0.216±0.017, ST: 0.238±0.031, LB: 1802±0.022 AU; p=NS)

Conclusion: Our findings indicate that all placental cells respond to GC with upregulation of GC-NR with an enhanced response among GT cells. These results suggest that endogenous or exogenous maternal stress-induced GC exposure may influence signaling responses within both maternal and fetal placental compartments.

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Ovine Placenta at Very-Preterm Gestation Expresses Corticotrophin Releasing Factor (CRF), CRF-Binding Protein (CRF-BP) and Glucocorticoid Receptors (GR). Jayaraman Lakshmanan, John D Richard, Guo L Liu, Sharon K Sugano, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: In humans, the placenta is the major source of maternal and fetal plasma CRF. Based on its critical functions, CRF is considered as a "placental clock" of parturition. We recently reported that ovine fetal as well as maternal plasma contain measurable amounts of CRF at near-term but not at very preterm gestation. We interpret the absence of CRF in plasma at very preterm gestation is either due to lack of placental CRF expression and/or placental CRF release. Here, we examined the expression status of CRF, CRF-BP (a known specific binding protein for CRF and a known regulator of CRF functions in human placenta) and GR (a known positive regulator of CRF expression in human placenta) in ovine placenta collected at very-preterm gestation.

Method: Placenta harvested from time-dated pregnant ewes on 118±2 days gestation were fixed in Bouins's solution and processed for paraffin embedding. Paraffin sections were subjected to immunohistochemical analysis with polyclonal antibodies specific to ovine CRF(1:300-1:500, Phoenix Pharmaceuticals, CA,) CRF binding protein (1:100-1:200, Sc 20630) and GR (1:100-1:200, Sc: 8992, SantaCruz biotechnology, CA). Immunoreactive material on the sections were identified as brown staining by ABC technique using diaminobenzidine as chormagen. Immunoreactive material was quantified by image analysis using Image Pro 4.01 Plus software and the immuoreactive intensity (OD/area) expressed as arbitrary units (AU).

Results: Strong positive staining for CRF (0.363±0.033 AU), CRF-BP (0.505±0.003 AU) and nuclear-GR (0.383±0.015AU) were noticed in syncytiotrophoblast cells in all placental sections obtained from pregnant ewes at118 days gestation. Control sections exhibited no positive staining.

Conclusion: Our findings indicated that ovine placenta at very-preterm gestation expresses CRF, CRF-BP and GR. These results suggest that absence of measurable CRF in maternal and fetal plasma at very-preterm gestation is not due to lack of placental CRF expression but rather due to the absence of regulated CRF secretory mechanisms.

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Rat Placenta Expresses Urocortin I Protein and mRNA. Thomas R Magee, Michael G Ross, John D Richard, Sharon K Sugano, Jayaraman Lakshmanan. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Urocortin I (UCN-1), a 40 amino acid neuropeptide belongs to corticotrophin releasing factor (CRF) stress hormone family. In humans, the placenta and several gestational tissues have been reported to express UCN-1 protein and UCN-1 mRNA. A number of published studies indicate that UCN-1 is similar to CRF in expression pattern and biological functions. We recently reported that rat placenta is a site of CRF protein and CRF mRNA expression. In the present study we sought to determine whether rat placenta expresses UCN-1 protein and UCN-1 mRNA.

Methods: Placenta (n=18) collected from pregnant rats at 21 day gestation were either fixed in Bouin's solution and processed for paraffin embedding or placed in RNA later preservative and frozen for RNA extraction. For immunohistochemical localization, five micron thickness paraffin sections were cut and immunostained with rabbit polyclonal antibodies to UCN-1 (1:500 to 1:750, Sigma) by standard ABC technique. Control sections were incubated with omission of UCN-1 antibody. Immunoreactive materials on the sections were identified using 3, 3' daminobenzidine as chromagen. Immunostaining intensity (OD/area) was quantified by Image-Pro Plus software and expressed in arbitrary units (AU). For PCR, cDNA was synthesized and PCR amplified with a one step RT-PCR kit. Fragments were gel purified, cloned into a plasmid vector and DNA sequenced. All values are given as mean ± SEM.

Results: UCN-1 polyclonal antibody elicited strong immunostaining in placental trophoblast cells with variable intensity. The pattern of immunostaining is as follows: Giant trophoblast cells: 0.334±0.319 AU, spongiotrophoblast cells: 0.318±0.013 AU and labyrinth cells: 0.233±0.139 AU. The relative intensity in labyrinth cells was significantly lower than the other two cell types ($P< 0.01$). PCR analyses revealed the presence of a single band of 379 bp, consistent with UCN-1 mRNA.

Conclusion: Similar to human placenta, rat placenta expresses UCN-1 protein and UCN-1 mRNA, with most expression localized to the maternal side trophoblast cells. These results support our hypothesis that rat placenta can be used as a model to understand the role of this peptide in feto-maternal stress.

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The Role of the Nuclear Hormone Receptors FXR, PXR and CAR in Placental Bile Acid Homeostasis in Normal and Pathological Pregnancy. Victoria Geenes, Peter Dixon, Selina Raguz, Jenny Chambers, Kishore Bhakoo, Catherine Williamson. *Imperial College, London, United Kingdom.*

Background: Obstetric cholestasis (OC) is a pregnancy specific liver disorder characterised by raised maternal serum bile acid levels and associated with adverse fetal outcome. The aetiology of OC is complex and not fully understood, but the fetal complications are likely to result from an accumulation of bile acids in the fetal circulation.

Bile acids are the toxic end products of hepatic cholesterol metabolism and are synthesised from 12 weeks gestation. In common with other waste products, accumulation in the fetal compartment is prevented by excretion across the placenta into the maternal compartment. However, studies of maternal and cord serum from normal and OC pregnancies have suggested that bidirectional transfer of bile acids is possible.

Hepatic bile acid transport and metabolism is regulated by members of the nuclear hormone receptor family, namely FXR, PXR and CAR, but the mechanisms for regulating placental transfer are unknown.

Objectives: This study used placenta from normal and cholestatic pregnancies to investigate the expression of genes involved in hepatic bile acid homeostasis.

Methods: Villous trophoblast samples from 6 OC and 7 normal pregnancies (NP), and 3 human livers were collected and preserved in RNAlater. Explant cultures were prepared from a further 4 NP placentas and cultured for 6 days at 8% oxygen. On day 5 they were treated with chenodeoxycholic acid, lithocholic acid or vehicle for 24 hours prior to fixing in RNAlater. Total RNA was extracted using Trizol, and reverse transcribed to cDNA. Quantitative real-time PCR was performed using SYBR Green. Target gene mRNA abundance was calculated from a standard curve and normalised to L19.

Results: The expression of FXR, PXR and CAR, the nuclear hormone receptors responsible for hepatic bile acid homeostasis and several FXR target genes (SHP, MDR3 and BSEP) was found to be very low in NP, and unaffected by the presence of maternal cholestasis. Furthermore, the expression of these genes could not be induced by bile acid treatment in an *in vitro* model.

Conclusions: Here we have shown that the nuclear hormone receptors (FXR, PXR and CAR) involved in hepatic bile acid homeostasis are expressed at very low levels in normal and pathological pregnancy and are thus likely to be of limited importance in placental bile acid transfer.

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A Placental Phenotype for Obstetric Cholestasis. Victoria Geenes,[1] Jenny Chambers,[1] Jo Wyatt-Ashmead,[2] Kishore Bhakoo,[1] Catherine Williamson.[1] *[1]Imperial College, London, United Kingdom; [2]Hammersmith Hospital, London, United Kingdom.*

Background: Obstetric cholestasis (OC) is a pregnancy specific liver disorder that affects 0.7% of pregnant women in the UK. Biochemically, OC is characterised by liver dysfunction with elevated maternal serum bile acids (SBA), and clinically by an increased risk of fetal complications including fetal distress, meconium staining of the amniotic fluid, preterm labour and sudden intrauterine death.

The aetiology of OC is complex and not fully understood, but the fetal complications are likely to result from the toxic effects of bile acids, which can cause vasoconstriction in the placenta and fetal cardiac dysrrhythmias.

Furthermore, in a rodent model of OC, bile acids cause oxidative stress in the placenta and a reduction in litter size. These changes are absent in animals treated with ursodeoxycholic acid (UDCA), a drug used to treat OC. However human data are lacking.

Objectives: This study used whole placenta and explant cultures to investigate the effects of bile acids on human placental architecture and to establish whether UDCA protects the placenta against bile acid induced damage.

Methods: Villous trophoblast samples were collected from 15 OC and 5 normal pregnancies (NP) and fixed in formalin. Explant cultures were prepared from a further 4 NP placentas, and cultured for 6 days at 8% oxygen. On day 5 a subset were treated with UDCA overnight, and on day 6 the explants were treated with taurocholic acid, taurochenodeoxycholic acid or vehicle for 6 hours prior to fixing. 5 μM sections were stained with haematoxyllin and eosin. Slides were reviewed by a Perinatal Pathologist and syncytial knots (SK) counted.

Results: OC tissues showed marked alterations in morphology, including congestion of the terminal villi, and loosening of the stroma and fibrotic change of the membranes of the stem villi. There were significantly more SK the OC samples (Mann-Whitney U Test p=0.024). Furthermore, SK were increased in explants treated with bile acids, but not those treated with UDCA prior to the addition of bile acids.

Conclusions: Here we describe several morphological abnormalities of the placenta associated with maternal cholestasis. These changes were confirmed using a placental explant model, which also showed that UDCA protects the placenta against bile acid induced damage. In summary, this study indicates that UDCA is likely to protect the fetus in OC.

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3D Ultrasound Assessment of Placenta Volume in the First Trimester in Pregnancies from Assisted Reproduction Technologies (ART). Simona Boito,[1] Emanuela Taricco,[1] Tatyana Radaelli,[1] Luca Mandia,[1] Dario Consonni,[2] Veronica Cozzi,[1] Inga Sandaite,[1] G Ragni,[3] Giorgio Pardi,[1] Irene Cetin.[1] *[1]Institute of Obstetrics and Gynecology, IRCCS Policlinico Mangiagalli & Regina Elena Foundation, University of Milano, Milan, MI, Italy; [2]Epidemiology Unit, IRCCS Policlinico Mangiagalli & Regina Elena Foundation, University of Milano, Milan, MI, Italy; [3]Assisted Reproduction Unit§, IRCCS Policlinico Mangiagalli & Regina Elena Foundation, University of Milano, Milan, MI, Italy.*

Objective. The placenta has complex metabolic and endocrine activities and is essential for the growth and survival of the fetus in utero. Ultrasound is the most sensitive and less invasive method to evaluate placental size, morphology and function. The three-dimensional approach allows to calculate placental volume in the I and II trimester of pregnancy. Pregnancies from assisted reproduction technologies (ART) show an increased rate of pathologies potentially related to placental insufficiency such as intrauterine growth restriction and preterm delivery.

Aim. To determine placental volume in ART pregnancies in a cross sectional study in the first trimester of gestation.

Design. Using three-dimensional ultrasound machine (GE 730) placental volume measurement from ART pregnancies (n=26; 10-13 wks) were compared with data from normal controls (n=26) matched for gestational age.

Data were analysed with software stata 9.

Results. Mean placental volume was 46.2 ml (SD±23.9) in ART pregnancies and 58.4 ml (SD±22.9) in normal controls. Mean difference resulted in 12.12192 ml (-1-25.19835) and was statistically different (P=0.047). Multiple linear regression analysis showed a statistically significant interaction (P=0.04) between gestational age and case status, i.e. differences in placental volume increase significantly with advancing gestational age between cases and normal controls. Mean gestational age at birth was not essentially different between the two subsets (39.1 weeks ± 1.6) however a statistically significantly lower mean fetal birthweight was found in ART pregnancies: 2945 g (SD±537) vs 3226.(SD±294.4) (P=0.044).

Conclusions. Placental volume is slightly decreased in ART pregnancies. Measurements of placenta volume by 3D ultrasound may play a role in identifying the degree of placental growth early in gestation in a risk population.

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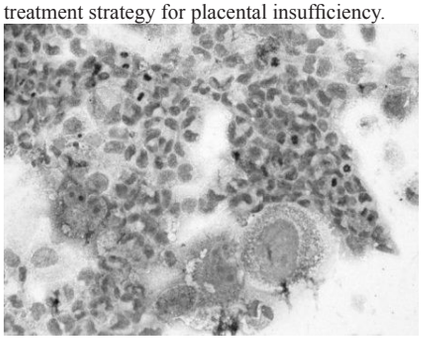
In Vitro Effects of Adenovirus-Mediated Gene Transfer of Insulin like Growth Factor-1(Ad-IGF-1) in Trophoblast Cells. Mounira Habli,[1] Datis Alae,[2] Foong Yen Lim,[2] Jignesh Parvadia,[2] Timothy Crombleholme.[2] *[1]Obstetrics and Gynecology; [2]Pediatric Surgery, University of Cincinnati/Children's Hospital, USA.*

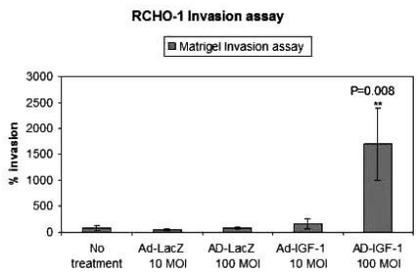
Objective:Recently, Ad-IGF-1 corrected both fetal and placental weights in rat model of fetal growth restriction (FGR) . To elucidate the mechanism of action of Ad-IGF-1;we examined the effect of Ad-IGF-1 on trophoblast proliferation, differentiation and invasion.

Methods: RCHO-1 trophoblast cell line derived from rat choricarcinoma was used. Ad-IGF-1 and β galactosidase transgene (Ad-LacZ) were given at MOI of 10:1 and 100:1 in all the experiments.Transduction efficiency was assessed 24 hr after infection with Ad-LacZ by β galactosidase enzyme activity. The invasiveness of RCHO-1 was measured by using BDMatrigel Invasion Chamber kit. RCHO-1 proliferation cell density was assessed by crystal violet assay. Morphologic analysis of RCHO-1 differentiation in response to treatment (differentiated cells are multinucleated giant cells) was assessed byimunocytochemistry staining using placental lactogen-1 antibodies(specific hormone produced by giant cells).

Results: 100% efficiency of gene transfer of Ad-LacZ to RCHO-1 cells was observed at both MOI's.There was no significant difference in proliferation between treated &control group regardless of the dose at 12 and 24 hrs. Ad-IGF-1 induced morphologic differentiation of trophoblast cells compared to control (Fig1) at 24 hrs.Ad-IGF-1 induced higher rate invasion in a dose dependent fashion as compared to control(Ad-LacZ) group(16% at 100MOI vs 0.8% in control p<0.008)(Fig2).

Conclusion: Ad-IGF-1 gene transfer induces differentiation and invasiveness of trophoblast cells. These may be the mechansim of correction of FGR in rat model of placental insufficiency. Placental gene transfer may be an effective treatment strategy for placental insufficiency.





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Alternative RNA Splicing of the Endothelin Receptor A in Different Cell Types of Human Placenta. Martina Dieber-Rotheneder, Ursula Hiden, Gernot Desoye, Mila Cervar-Zivkovic. *Department of Obstetrics and Gynaecology, Medical University Graz, Graz, Austria.*

Background and Hypothesis: Endothelin-1 is a polypeptide with a wide range of functions. In the placenta it acts as a potent regulator of vasotonus on endothelial and smooth muscle cells, whereas on the trophoblast it regulates cell proliferation, invasion and apoptosis. Endothelin-1 action is differently signaled through two endothelin receptor (ETR) subtypes, ETR-A and ETR-B. Several alternative splice variants of ETR were identified. Here we hypothesize that the ETR-A splicing varies with gestational age and is different in trophoblast vs endothelial cells of the human term placenta.

Methods: mRNA of placental tissue from first trimester (pregnancy terminations, missed abortions) and term of gestation, first trimester and term trophoblasts, arterial and venous placental endothelial cells as well as cell lines representing first trimester trophoblast (ACH3P) and term placental endothelial cells (HPEC) were analyzed for full length ETR-A and known as well as unknown splice variants by sqRT-PCR using primers spanning the exons 2-5. The predominant DNA bands in agarose gel were excised and sequenced.

Results: All tissues and cells expressed full length ETR-A. In all tissues an additional Δ3Δ4-deletion variant was identified which was not found in the isolated cells. Trophoblasts expressed another yet unidentified splice variant. The endothelial cells expressed a Δ3-deletion variant and a novel splice variant,

which was identified as partial Δ2 – partial Δ3 deletion. This novel splice variant was found regardless of the arterial or venous origin of the cells as well as in the cell line (SV40-transformed). In tissues und trophoblast no difference in splicing was found between first trimester and term.

Conclusion: Gestational age does not alter splicing of endothelin receptor-A. Splicing is different between trophoblasts and endothelial cells. The endothelial cells contain a novel splice variant. The differential splicing may allow maternal and fetal endothelin-1 to induce different effects on the two major cell types of the placenta.

(supported by grant 11258, Jubilee Fund, Austrian National Bank, Vienna).

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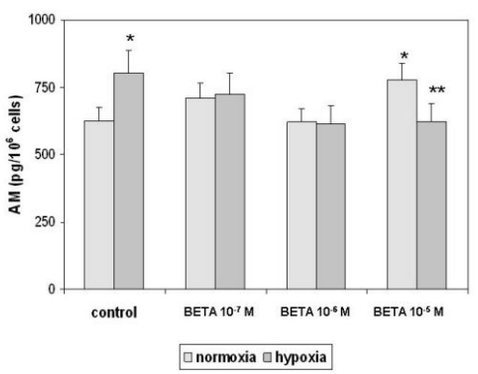
Adrenomedullin Production and Secretion by Human Trophoblast Cells Are Regulated by Glucocorticoids and Hypoxia. Francesca Ciardo,[1] Katia Pacioni,[1] Emanuela Marinoni,[1] Giovanna Corona,[1] Massimo Moscarini,[1] Alfredo Patella,[2] Romolo Di Iorio.[1] *[1]Department of Gynecology, Perinatology and Child Health, University "La Sapienza", Rome, Italy; [2]Department of Obstetetrics and Gynecology, University of Ferrara, Ferrara, Italy.*

Objectives: Adrenomedullin (AM) is produced by intrauterine tissues and is involved in the regulation of implantation, placental hemodynamics and endocrine function. Circulating AM is increased in pregnancy complications such as preeclampsia and intrauterine growth retardation. We investigated whether AM output by human trophoblast cells is regulated by hypoxia and/or glucocorticoids.

Study design: trophoblast cells obtained by human placentas at term (n=7) were cultured in presence or absence of hypoxia (3% O_2) and treated with or without betamethasone at the dose of 10^{-5}, 10^{-6}, 10^{-7}M. Media and cells were collected at 48h and at 2, 8 and 24h from syncytiotrophoblast cultures. AM was measured in cultured media by specific RIA kit. Protein expression in trophoblast cells was evaluated with immunohistochemistry and western blot.

Results: hypoxia stimulated AM output and protein expression by cytotrophoblast and syncytiotrophoblast cells. Betamethasone induced an increase in AM production and secretion in a time- and dose-dependent manner (figure). Effects of hypoxia were partially reversed by betamethasone in a dose and time-dependent fashion.

Conclusions: AM production in trophoblast cells is up-regulated by hypoxia and glucocorticoids, independently. Increased AM levels in pregnancy complications characterized by placental insufficiency might derived by an increase in AM placental secretion stimulated directly by hypoxia or indirectly by an increase in fetal cortisol levels.



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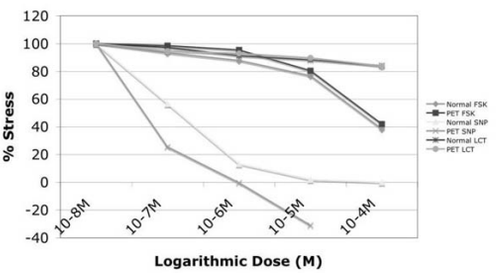
Vasomotor Responsiveness of Placental Arteries in Preeclamptic and Uncomplicated Pregnancies. Julie A Scott,[1] Kathryn Reed,[1] Padmini Komalavilas,[2] Colleen M Brophy.[2] *[1]Obstetrics & Gynecology Division of Maternal Fetal Medicine, University of Arizona, Tucson, AZ, USA; [2]Center for Metabolic Biology, Arizona State University/VAMC, Phoenix, AZ, USA.*

Preeclampsia complicates 5-8% of pregnancies, and while associated with significant maternal and fetal morbidity and mortality, the mechanisms responsible for preeclampsia are not completely understood. Recent advances suggest there are imbalances of pro- and antiangiogenic factors, present from the time of implantation affecting vascular responsiveness of the fetal placental unit and maternal vasculature. In this study, we investigate vasomotor responsiveness of placental arteries from normal and preeclamptic (PET) pregnancies using an in vitro muscle bath contractility assay. Transverse rings of placental arteries were cut and equilibrated in the muscle bath containing a bicarbonate buffer aerated with 95% O_2/5% CO_2 at 37°C. Viability was demonstrated by contraction to 110mM KCL prior to all experiments. Cumulative logarithmic dose dependent responses to four different contractile agents: PGF2α, serotonin, carbochol, and norepinephrine were compared. Placental arteries precontracted with PGF2α at half maximal concentration were relaxed with three vasorelaxants: sodium nitroprusside (SNP), forskolin (FSK) and lactate (LCT). Agonist studies revealed contraction to both PGF2α and serotonin but not to carbochol or norepinephrine. There were no statistically significant differences between the responses of normal and PET arteries. Both Normal and PET arteries demonstrated heightened responsiveness to sodium nitroprusside compared with forskolin and lactate. This study reveals that the placental vessels are more sensitive to sodium nitroprusside, that mediates relaxation through nitric oxide - cyclic GMP signal transduction pathway, than forskolin that induces relaxation through the cyclic AMP pathway.

Figure 1: Vasorelaxant Comparisons in PGF2α Contracted Placental Artery



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Utilization of an *Ex Vivo* Placental Perfusion Model To Predict Potential Fetal Exposure to Carboplatin Utilized in the Treatment of Cancer during Pregnancy. Stacy A Langmeyer,[1] Scott Mosley,[1] Susan Ramin,[2] Judith Smith.[1,2] *[1]Gynecologic Oncology, UT MD Anderson Cancer Center, Houston, TX, USA; [2]Obstetrics, Gynecology, and Reproductive Sciences, UT Health Sciences Center at Houston Medical School, Houston, TX, USA.*

Cancer complicates approximately one in 1,000 pregnancies. Depending on the type of cancer and trimester, patients can terminate the pregnancy or choose treatment such as chemotherapy. Since there is limited knowledge on the safety of chemotherapy on fetal tissues *in utero*, clinicians cannot efficiently analyze the risks of particular anticancer agents when deciding on a course of therapy. Since carboplatin is among the most common anticancer agents used for treatment of cancer during pregnancy the primary objective of this study was to evaluate if carboplatin will readily cross to placental barrier and determine total potential platinum fetal exposure.

This project utilizes an *ex vivo* placenta perfusion model to determine the concentration of carboplatin that crosses the human placental barrier. Placentas are obtained within 15 minutes of spontaneous delivery and re-perfused. Two carboplatin concentrations were selected of 1000 ng/mL and 5000 ng/mL to represent clinically relevant maternal plasma concentrations. Antipyrine was used as the internal control. Serial samples were collected every 10 minutes for total 60 minutes in open-open model then experiment repeated with closed circuit model. Antipyrine concentrations were determined by HPLC methods. Platinum concentrations in media samples were determined with a validated atomic absorption assay. A cell culture-based approach will be used to determine whether cell cycle or apoptotic proteins are altered (p53, BAX, bcl-2, AIF, and pro-caspase-3) using Western blotting as a detection method.

A total of two placentas have been completed at the low carboplatin concentration and one at the high concentration. The mean carboplatin clearance was 0.67 +/- 0.01 mL/min and 1.04 +/- 0.15 mL/min at the low and high carboplatin concentrations, respectively. An estimated 50% increase in the transport fraction was observed in the high concentration experiments compared to the low concentrations model. Fetal carboplatin exposure ranged from 60 to 300 ng/mL.

The placental perfusion experiments conducted at carboplatin 1000 and 5000 ng/mL indicate that carboplatin crosses the placenta through simple diffusion. The toxicology assays are ongoing to determine potential effect on fetal tissues.

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Amniotic Fluid Urocortin Concentrations at Mid-Gestation May Predict Preterm Delivery. Michela Torricelli, Letizia Galleri, Chiara Voltolini, Elisa Faldini, Francesca Filosomi, Fernando Reis, Giovanni Centini, Felice Petraglia. *Department of Pediatrics, Obstetrics, and Reproductive Medicine, University of Siena, Siena, Italy.*

Preterm delivery represents one of the predominant causes of perinatal mortality and morbidity, and is one of the most unpredictable gestational disturbances. Urocortin is a peptide expressed by trophoblast and gestational tissues (amnion and chorion), whose maternal levels correlate with gestational length, since they are increased at preterm delivery and decreased in post-term pregnancy, when compared to term pregnancy. The addiction of urocortin enhances contractility of human myometrial strips, suggesting a possible role on uterine contractility. High maternal urocortin levels in threatened preterm delivery correlates with the timing of delivery, suggesting a possible role in the predictivity pf preterm delivery.

In the present study urocortin concentrations in amniotic fluid collected at mid gestation for amniocentesis were correlated with gestational age at delivery.

A case-control study with amniotic fluid obtained from healthy 60 women undergoing amniocentesis for genetic indications was performed; urocortin concentrations were measured by a specific ELISA.

Amniotic fluid urocortin concentrations resulted significantly lower (P= 0.0025) in women delivering preterm (n=20; Ucn= 1.23 ± 0.07 pg/ml) (M ± SE) than in those delivering at term (n= 40; Ucn= 0.77 ± 0.11pg/ml).

In conclusion, the present preliminary data showed that amniotic fluid urocortin concentrations at mid gestation may represent predictive marker of preterm delivery.

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Modulation of Bax Expression between Physiological and Pathological Human Placentas throughout Pregnancy. Annunziata Mastrogiacomo,[1] Elisabetta Federico,[1] Francesca Caprio,[1] Maria Teresa Schettino,[1] Gabriele Coppola,[2] Antonio De Luca,[2] Luigi Cobellis.[1] *[1]Department of Obstetrics and Gynecology, Second University of Naples, Naples, Italy; [2]Department of Medicine and Public Health, Section of Anatomy, Second University of Naples, Nales, Italy.*

Hypothesis

Apoptosis is intimately involved in placental homeostasis, growth and remodeling and the apoptotic rates increase progressively during normal pregnancy as part of normal placental development. Moreover, apoptosis increases in pregnancies complicated by some pathologies such as preeclampsia, fetal growth restriction, diabetes. In the present study, we describe differences in the expression of pro-apoptotic protein Bax, in first trimester voluntary termination of pregnancy, first trimester abortion (reserved abortion), caesarean birth, spontaneous birth, preeclampsia and diabetes.

Material and methods

Human placental samples were obtained with informed consent from patients undergoing surgery such as first trimester voluntary termination of pregnancy (n=15), first trimester abortion (miscarriage) (n=15), delivery section (n=15), spontaneous birth (n=15), preeclampsia (n=15) and diabetes (n=15). The gestation period ranged from 5 to 40 weeks. The specimens were immediately fixed in formalin for immunohistochemistry.

Results

We first observed a strong increase of Bax expression in the cytotrophoblast, stroma, endothelial cells and decidua of placentas of the first trimester abortion compared to the low/moderate Bax immunopositivity in all the placental compartments during the first trimester voluntary termination of pregnancy. Secondly, we showed a more intense immunopositivity for Bax in the third trimester spontaneous birth respect to the third trimester caesarean birth. Thirdly, we observed an increase of Bax expression in preeclamptic placentas compared to the normal full-term placentas. On the contrary, we observed a moderate Bax expression in diabetic placentas only slightly decreased compared to the normal full-term placentas.

Conclusion

Our results seem to suggest that deregulation of apoptotic turnover may lead to placental dysfunction and pathologies.

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Unlocking the Potential of Proteomics: Achieving Relative Quantification of Proteins Recovered from Placental Explant-Conditioned Culture Media Using an Optimised Mass Spectrometry-Based Workflow. Richard T Blankley, Nicola J Robinson, Ian P Crocker, Philip N Baker, Jenny E Myers. *Maternal & Fetal Health Research Group, The University of Manchester, United Kingdom.*

Objectives: Maternal endothelial activation in preeclampsia (PE) is attributed to the release of unknown factors from a hypoperfused placenta. The application of proteomic technologies such as mass spectrometry promises the reward of identifying and characterising these factors. Using a placental explant-conditioned culture media model system we have developed a proteomics workflow to obtain relative quantification of proteins released into placental explant culture media.

Methods: Term villous explants were cultured in serum-free conditions and exposed to differing oxygen concentrations (6% & 1%) to mimic physiological and non-physiological intervillous O_2 tensions. The D4 media was concentrated, immunodepleted to remove fibrinogen (using Beckman IgY-12 columns) and proteins labelled using an ITRAQ (Applied Biosystems) kit following the manufacturer's protocol. Labelled peptides were fractionated by strong cation exchange and then analysed using LC-MALDI on a 4800 MALDI-TOF/TOF (Applied Biosystems) mass spectrometer. Data was analysed using Protein Pilot 2.0 (Applied Biosystems).

Results: When matched pooled (n=6) media samples were subjected to our proteomics workflow over 590 proteins were identified with a calculated false positive identification rate of <1%. Of these proteins a total of 29 display a statistically significant difference in protein levels between the 1% & 6% O_2 samples (p=<0.001). From a list of ~40 proteins of interest we selected 7 proteins for validation using ELISA/Western blotting to measure their relative abundance in both pooled and individual explant media samples. Interleukin 8 is an example of a low-abundance differentially expressed protein identified in our proteomic workflow and subsequently validated by ELISA; IL-8 (pg/ml) in 6% O_2 : 43.7, range 30.8 to 47.3; 1% O_2: 103.0, range 57.6 to 141.0; values are median with interquartile range. *** P<0.0001, Mann-Whitney test (n=40)

Conclusions: We demonstrate a successful reduction to practice of a relative quantitative proteomics strategy applied to placental explant-conditioned culture media. Having optimised and validated this proteomic workflow we will apply the same methods to compare proteins released by normal and preeclamptic placentas.

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Differential Expression of Dysferlin and Myoferlin during Trophoblast Syncytial Differentiation. JM Robinson,[1] WE Ackerman,[2] DD Vandre.[1] *[1]Physiology & Cell Biology, Ohio State University, Columbus, OH, USA; [2]Obstetrics & Gynecology, Ohio State University, Columbus, OH, USA.*

A proteomics screen of human placental microvillous syncytiotrophoblast (STB) revealed the expression of dysferlin (DYSF), a membrane repair protein associated with certain muscular dystrophies (Vandré et al., 2007). A second ferlin protein, myoferlin (MYOF), was also discovered which has not yet been characterized in the placenta. In human C2C12 myoblasts, DYSF and MYOF are reciprocally expressed as a function of differentiation into multinucleate syncytial myotubes, with DYSF predominating in differentiated cells. We hypothesized that DYSF and MYOF would show a similar reciprocal expression pattern in human trophoblasts as a function of syncytialization.

METHODS Term placentas from uncomplicated pregnancies were obtained with informed consent (N=8) and either fixed for immunofluorescence (IF) labeling or flash frozen for subsequent immunoblotting (IB). Human trophoblastic (BeWo, JAR, and JEG-3) and other cell lines (MRC-5, HL-60, HUVEC) were cultured in the absence or presence of 20 μM forskolin or solvent control for 0-3 days. DYSF and MYOF expression was examined by RT-PCR, IB, and IF.

RESULTS IB validated proteomics data showing MYOF expression in term placenta. By IF, MYOF labeling was predominant in apical and basal STB plasma membranes. MYOF was expressed in trophoblastic cell lines (BeWo, JAR, and JEG-3), cultured endothelium (HUVEC), and a fibroblast cell line (MRC-5), but was not detected in a leukemic cell line (HL-60). DYSF was constitutively expressed in JAR, but minimally expressed in unstimulated BeWo. Following forskolin-induced syncytialization, we observed a time-dependent increase in DYSF expression in BeWo concomitant with increasing syncytin-1 levels. By IF, DYSF expression was restricted to BeWo cells in syncytial structures. In contrast, MYOF expression was robust in mononuclear BeWo cells and not down-regulated over the course of 3 days of differentiation.

CONCLUSIONS Two ferlin-family genes are expressed in trophoblasts.

Fusion-competent BeWo cells behave similarly to cultured myoblasts and CTBs with regard to DYSF expression, which is restricted to syncytializing cells. MYOF, in contrast, is expressed constitutively in BeWo and other models. While both proteins are likely to function in STB plasma membrane repair (e.g., following syncytial sprouting), the relative contributions of each to this process awaits clarification.

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Features of Chronic Placental Insufficiency Are Significantly More Common in Small for Gestational Age Placentas from Infants with Intrauterine Growth Retardation. Emily King,[1] Violetta Kolesnikova,[1] Carri Tillotson,[2] Jean-Marie Guise,[3] Terry Morgan.[1] *[1]Pathology; [2]Center for Biostatistics; [3]Obstetrics and Gynecology, OHSU, Portland, OR, USA.*

Background: There is a strong association between intrauterine growth restriction (IUGR) and small for gestational age (SGA) placentas (Heinonen et al. Placenta (2001), 22:399-404). The cause is likely multifactorial, but we hypothesize that chronic uteroplacental insufficiency may play a role. Our objective was to test whether SGA placentas from human neonates with IUGR show significantly more pathologic features of placental insufficiency compared to controls.

Design: We performed a retrospective review of 242 consecutive singleton placentas submitted to OHSU pathology (2005-06), excluding elective terminations and spontaneous abortions before 22 weeks gestation. Clinical records were reviewed and pregnancy outcomes recorded, including: 1) neonatal sex; 2) weight (IUGR calculated by routine methods), 3) trimmed weight of the placenta (SGA calculated by routine methods), 4) gross placental infarctions, 5) gestational age at delivery, and 6) maternal features (e.g. race, gravida). Controls were defined as cases within the series without SGA or IUGR (e.g. submitted for meconium, infection, etc). Histologic sections were scored by two pathologists while blinded to clinical diagnoses as positive or negative for features of placental insufficiency, including accelerated villous maturation (AVM), chorangiosis, and microscopic infarctions. Significant associations were tested by χ^2 analysis and logistic regression for multiple variables.

Results: Similar to prior reports, we observed a strong association between IUGR and SGA placentas (χ^2 30.5; p <0.0001). This relationship was independent of maternal race, fetal sex, and parity, although it was more common in primigravidas. AVM and placental infarctions were significantly more frequent in SGA placentas with superimposed IUGR.

Diagnosis	Controls (n=173)	IUGR (n=31)	SGA Placenta (n=17)	IUGR + SGA (n=21)
AVM	43%	65%*	71%**	86%***
Infarction	23%	55%**	35%	43%*
Chorangiosis	20%	10%	12%	19%

Conclusions: Our data show that pathologic features of chronic placental insufficiency are common in SGA placentas from neonates with IUGR, suggesting that decreased nutritional blood flow to the fetoplacental unit may be a common cause of growth restriction.

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Evaluation of Gene Expression Profiles in Leukocytes from Non-Human Primates (Papio spp.) as Biomarkers of Placental Function. Natalia E Schlabritz-Loutsevitch,[1,2] Matthew P Johnson,[3] Gene B Hubbard,[2] Peter W Nathanielsz,[1,2] Eric K Moses.[3] *[1]Obststerics and Gynecology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; [2]Southwest National Primate Research Center, Southwest Foundation for Biomedical Research, San Antonio, TX, USA; [3]Department of Genetics, Southwest National Primate Research Center, Southwest Foundation for Biomedical Research, San Antonio, TX, USA.*

Introduction: Messenger RNA expression peripheral blood cells (PBC) has been recently used as biomarkers of environmental exposures (ionizing radiation or tobacco), physiological conditions (stress) and diseases (hypertension, neurological disorders). PBC evaluation is a useful diagnostic tool in an era of individualized medicine. Since there is an urgent need for non-invasive methods for determination of fetal (F) and placental (P) function, this study was designed to evaluate the genes differently and commonly expressed in P tissue and leukocytes in maternal (M) and F circulation.**Material and Methods**. P (n=5), F (n=3) and M blood (n=3) were obtained during cesarean section in pregnant baboons at term. Total RNA from a buffy coat pellet was isolated using a modified procedure of the QIAGEN RNeasy Mini Kit (QIAGEN). Anti-sense RNA (aRNA) was synthesized and purified using the Illumina RNA Amplification Kit (Ambion, USA). Hybridization of aRNA to Illumina Sentrix Human Whole Genome (WG-6)BeadChips and subsequent washing, blocking and detection was performed using Illumina's BeadChip 6´2 protocol. Samples were scanned on the Illumina BeadArrayer 500GX reader using Illumina BeadScan image data acquisition software (ver. 2.3.0.13). Differential gene expression data analysis was performed using Illumina BeadStudio software (ver. 1.5.0.34).

Results. The detection level of gene transcripts using illumina methodology with control human RNA was 6000-7000 at p<0.0001. 4329 gene transcripts were detected in F blood and 2957 in maternal blood. 1452 transcripts were uniquely expressed in fetal blood. 130 transcripts were found in M, but not in F leukocytes. The number of gene transcripts expressed in P tissue was 4825 and of these 2953 genes were not expressed in M leukocytes. **Conclusion.** Despite white blood cells trafficking through the P barrier there is a set of unique genes expressed only in P or in the M or F circulation. The application of these genes as the biomarkers of P barrier function still need to be evaluated.

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Can Placental Histology Establish the Timing of Meconium Passage? Maddalena Incerti,[1] Silvia Malberti,[1] Francesca Bono,[2] Massimiliano Greco,[1] Sara Consonni,[1] Alessandro Ghidini,[1] Anna Locatelli.[1] *[1]Obstetrics and Gynaecology, University of Milano-Bicocca, Monza, Italy; [2]Pathology, University of Milano-Bicocca, Monza, Italy.*

Objective: To evaluate if a relationship exists between duration of placental exposure to meconium in vivo and histologic evidence of severity and extent of meconium uptake by macrophages.

Study design: From a cohort of 353/7069 (5%) consecutive singleton liveborn infants delivered at term with thick meconium-stained fluid, 193(55%) had placental histologic examination performed, and in 50/193 the timing of meconium appearance after membrane rupture was documented. Placental histologic examination quantitatively evaluated the intensity of meconium uptake by resident macrophages based on the number of macrophages/field, and the extent of uptake based on histologic location, graded in a score 0 to 3.

Results: Mean interval between meconium appearance and delivery was 136.7± 126.7 min (range 10-510). After exclusion of 6 cases in which severe placental inflammation interfered with analysis, meconium uptake by macrophages was documented in 40/44 cases at the level of amniochorionic membranes, in 34/44 cases at the placenta, and in 18/44 cases at the umbilical cord. There was no correlation between the interval meconium appearance-to-delivery in relation to presence of meconium in the membranes (P=0.53), in the placenta (P=0.78), in the cord (P=0.71), or score of severity of meconium uptake (P=0.76). The results did not change after correcting for gestational age, oligohydramnios, presence of placental acute inflammatory or vascular lesions.

Conclusion: There is no relationship between duration of placental exposure to meconium and the extent and intensity of its uptake by macrophages in cases with exposure up to 8.5 hours.

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Transfer of Bisphenol A across the Human Placenta. Biju Balakrishnan,[1,2] Kimiora Henare,[1,2] Eric Thorstensen,[1,2] Murray D Mitchell.[1,2] *[1]The Liggins Institute, University of Auckland; [2]National Research Centre for Growth and Development, Auckland, New Zealand.*

Introduction: There are growing concerns over the effects of developmental exposure to the xenoestrogen Bisphenol A (BPA). Animal studies have shown that BPA is transferred through the placenta and can cause deleterious effects to the fetus. The presence of BPA in feto-placental tissues in humans has also been reported. However, a detailed study of the time-course of BPA transfer across the human placenta has not been performed. The aim of this research was to study the transfer of BPA in ex-vivo perfused human placental tissues.

Methods: A dual recirculating single cotyledon perfusion was used to monitor the placental transfer of BPA. BPA (10ng/ml), antipyrine (AP) (40µg/ml), and FITC dextran (FITC-DX) (12.5µg/ml) were added to the maternal perfusate, and perfusion was continued for 4 hours. Perfusate samples were collected from both reservoirs at timed intervals and analysed. BPA, AP, and FITC-DX were determined by HPLC with fluorescent detection, HPLC with UV detection, and spectrofluorometry respectively. The viability and metabolic activity of the placentae were assessed by measuring β-hCG (ELISA), glucose utilization, and lactate production (autoanalyzer). Fetal pressure, pH, flow rates and fluid shifts were monitored continuously.

Results: The biochemical validation parameters (glucose consumption, lactate production and β-hCG secretion) indicated that the placental tissue was metabolically active and viable throughout the 4-hour perfusion period. The physical parameters observed (fetal pressure <50 mmHg, pH range 7.2-7.6) were in concordance with other published works for placental perfusion. Membrane integrity was confirmed by fluid shifts from either circuit of

<5ml/hr, and by <2% materno-fetal transfer of FITC-DX. An observed AP transfer of 20-30% further validated our model. BPA first appeared in the fetal compartment within 30 minutes of perfusion and reached a peak of about 25-30% of maternal concentration within 3 hours of perfusion. This figure is likely to be an underestimate since it does not include conjugated BPAs.

Conclusion: This first study of BPA transfer in ex-vivo perfused human placental tissue shows that our model can serve as a useful tool to study the transfer kinetics and metabolism of BPA in human term placentae. We found that BPA rapidly crosses the human placenta at environmentally-relevant doses, with potentially harmful effects on the human fetus.

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Angiogenic Growth Factor Secretion by Uterine Natural Killer Cells in Co-Culture with Extravillous Trophoblast. Gendie E Lash,[1] Katsu Naruse,[1,2] Barbara A Innes,[1] Stephen C Robson,[1] Judith N Bulmer.[1] *[1]Institue of Cellular Medicine, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom; [2]Obstetrics and Gynaecology, Nara Medical University, Nara, Japan.*

OBJECTIVES. Uterine natural killer (uNK) and extravillous trophoblast (EVT) cells have been proposed to play roles in remodeling of uterine spiral arteries through secretion of various angiogenic growth factors. We have previously demonstrated that uNK cells are a significant source of angiogenic growth factors within the decidua, many of which alter with gestational age. However whether the secretion of these proteins is altered by interactions of uNK cells with EVT is unclear.

HYPOTHESIS. uNK cell angiogenic growth factor secretion is regulated by EVT in early pregnant decidua.

METHODS. Placental and decidual samples were collected from women undergoing termination of pregnancy with written informed consent (8-10 and 12-14 weeks gestation, n=12 each group). 0.2×10^6 $CD56^+$ uNK cells were positively selected from decidua and co-cultured with EVT (0.2×10^6) or cytotrophoblast (CTB; 0.2×10^6) purified from the same placenta for 24h in direct or indirect contact (n=6 each group). Angiogenin, Ang2, PDGF-BB, FGF-basic, TIMP1, ICAM1 and VEGF-A were measured by FAST Quant® Angiogenesis multiplex assay system, and VEGF-C, Ang1 and PlGF by ELISA. Results of uNK co-culture with EVT or CTB (negative control) at each gestational age were analyzed with Wilcoxon Rank test. In addition, the effect of direct and indirect co-culture of uNK cells with EVT at each gestational age was compared with Mann Whitney U test.

RESULTS. At 8-10 weeks gestation uNK secretion of Ang2 (P=0.03), ICAM1 (P=0.05) and PlGF (P=0.03) was increased in the presence of EVT compared with CTB. No differences were observed at 12-14 weeks gestation. In addition, at 8-10 weeks gestation uNK secretion of Ang2 (P=0.004), VEGF-C (P=0.007) and Ang1 (P=0.004) was increased in direct co-culture with EVT compared with indirect co-culture. At 12-14 weeks gestation uNK secretion of TIMP-1 (P=0.03) was reduced in direct co-culture with EVT compared with indirect co-culture.

CONCLUSIONS. uNK cell secretion of several key angiogenic growth factors was altered by direct culture with EVT. These data suggest that a membrane bound molecule (such as HLA-G) mediates this modulation of uNK cell activity.

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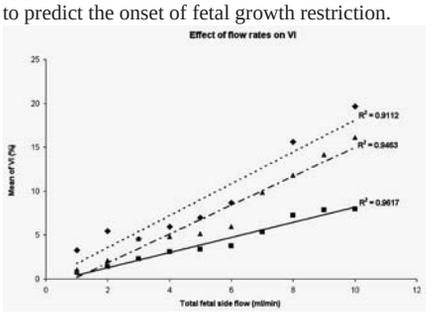
***In-Vitro* Dual Perfusion of the Human Placental Lobule as a Phantom To Investigate the Relationship between Fetoplacental Flow and 3-D Power Doppler Signal.** Nia W Jones,[1] Elizabeth S Hutchinson,[2] Paul Brownbill,[2] Ian P Crocker,[2] Diane Eccles,[3] George Bugg,[1] Nick Raine-Fenning.[1] *[1]Obstetrics and Gynaecology, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; [2]Maternal & Fetal Health Research Group, University of Manchester, United Kingdom; [3]GE Healthcare Ultrasound, Bedford, United Kingdom.*

Abnormalities in placentation and impaired placental circulation can lead to fetal growth restriction. 3-D ultrasound can be used to evaluate this through the quantification of the power Doppler signal that may be expressed as a percentage of colour voxels within a user-defined volume (VI: Vascularisation Index). We aimed to test the hypothesis that increased fetoplacental blood flow correlates with an increased VI, using the *in vitro* dual perfusion model of the human placental lobule. Three term lobules were dually perfused through both circulations with Earles bicarbonate buffer (EBB), and supplemented on the fetal-side only with adult erythrocytes, prepared to a 5% haematocrit. Following initial equilibration perfusion at normal flow values, fetal-side flow was varied between 1 and 10 ml/min, whilst maternal-side flow was held at 14 ml/min. Images were obtained with a 'Voluson i' ultrasound machine and a neonatal transducer (Pulse Repetition Frequency = 0.6Hz, Wall Motion Filter = low 1, and Gain = 0.0). Three 3-D datasets were acquired at each flow rate from each placental lobule and these were measured in triplicate using VOCAL. A sphere was centred on a visibly perfused cotyledon along the chorionic–decidual axis, with a diameter corresponding to placental thickness. Linear regression analysis was used to assess the relationship between the total fetal-side flow and mean VI. The mean VI showed a high degree of correlation with total fetal-side flow for each lobule (Figure 1) suggesting increased vascular perfusion and the inclusion of perfused vessels that cross the detection threshold with increased flow. This data provides qualifying information for translation to a clinical application, where early gestational fetoplacental blood flow will be assessed to predict the onset of fetal growth restriction.



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Expression of Heparin-Binding EGF-Like Growth Factor (HBEGF) in Term Chorionic Villous Explants and Its Role in Trophoblast Survival. Anthony N Imudia,[1] Brian A Kilburn,[1] Anelia Petkova,[1] Samuel S Edwin,[2] Roberto Romero,[2] D Randall Armant.[1,3] *[1]Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA; [2]Perinatology Research Branch, National Institute of Child Health and Human Development (NICHD), NIH, Bethesda, MD, USA; [3]Reproductive Biology & Medicine Branch, NICHD, NIH, Bethesda, MD, USA.*

Objective

Survival of first trimester cytotrophoblast cells depends on their upregulation of heparin-binding EGF-like growth factor (HBEGF), which is downregulated in placental tissues diagnosed with preeclampsia. We have examined the expression and cytoprotective activity of HBEGF in term villous explants subjected to hypoxic stress in vitro.

Methods

Non-pathological placentas were collected by cesarean section at term (n=6). Chorionic villous explants were prepared and cultured at either 20% or 2% O_2 and treated with the HBEGF antagonist CRM197 or recombinant HBEGF. Paraffin sections were assayed for trophoblast cell death by the TUNEL assay, proliferation by immunohistochemical labeling of nuclear Ki67 and HBEGF expression by semi-quantitative immunohistochemistry. Data were compared using ANOVA and the Student-Newman-Keuls posthoc test.

Results

CRM197 (10 mg/ml) increased trophoblast cell death after culturing villous explants 8 h at 20% O_2 ($P<0.05$), but only slightly affected proliferative capacity. Culture at 2% O_2 increased trophoblast cell death 100% above explants incubated at 20% O_2 ($P<0.05$). Trophoblast cell proliferation decreased after 24 h in explants cultured at either 20% or 2% O_2 ($P<0.05$). Exogenous HBEGF (1 nM) prevented the elevation of cell death during hypoxia ($P<0.05$) and maintained nuclear Ki67 expression at 20%, but not 2%, O_2. Contrary to first trimester trophoblast, HBEGF was not upregulated by hypoxia in term trophoblast.

Conclusion

The failure of term trophoblast to elevate HBEGF expression in response to hypoxia could contribute to their decreased survival at low O_2 compared to early gestation. Endogenous HBEGF signaling appears to facilitate survival of term trophoblast during villous explants culture. Exogenous HBEGF supplementation prevented cell death due to hypoxia and maintained trophoblast proliferation rates under in vitro conditions. Therefore, HBEGF, which is downregulated in preeclampsia, could have significant impact on trophoblast survival during late gestation.

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Conspicuous Meconium-Laden Macrophages in the Chorionic Plate Are an Independent Predictor of Clinically Significant Fetal Distress. Violetta Kolesnikova,[1] Emily King,[1] Carrie Tillotson,[2] Jean-Marie Guise,[3] Terry Morgan.[1] *[1]Pathology; [2]Center for Biostatistics; [3]Obstetrics and Gynecology, OHSU, Portland, OR, USA.*

Background: Meconium is a common indication for placental examination, present in approximately 30% of placentas routinely submitted to pathologists (Beebe et al. Obstet Gynecol 1996;87:771-8). Prolonged meconium exposure leads to accumulation of meconium-laden macrophages in the chorionic plate (estimated to require at least 3 hours). Our objective was to test whether prolonged meconium exposure is associated with clinically significant fetal distress.

Design: Retrospective review of 250 consecutive singleton placentas submitted to OHSU pathology (2005-06) was performed, excluding abortions before 22 weeks gestation, and placentas without representative sections of the chorionic plate. Clinical records were reviewed and pregnancy outcomes recorded, including: 1) evidence of fetal distress prompting C-section (non-reassuring fetal heart rate), 2) gestational age, 3) maternal diagnosis, 4) APGAR scores, and 5) neonatal length of hospital stay. Routine histologic sections were independently scored by two pathologists while blinded to clinical diagnoses and outcomes. Cases were scored as positive or negative for: 1) diffusely *conspicuous* meconium-laden macrophages in the chorionic plate (at least 1/hpf), 2) chorioamnionitis, and 3) features of placental insufficiency. Significant associations were tested by the Mann-Whitney U test for paired comparisons, X^2 analysis, and logistic regression for multiple variables.

Results: Conspicuous meconium staining of the chorionic plate was common (86/250, 34%) and was significantly more frequent in C-sections performed for fetal distress (19/35 cases, 54%) (X^2 7.1; p-value <0.01). Logistic regression modeling showed that this association was independent of chorioamnionitis and features of placental insufficiency. There was no association between meconium and neonatal outcome, including no difference in APGAR scores or length of hospital stay.

Conclusions: Our data support the hypothesis that meconium-laden macrophages in the chorionic plate are associated with fetal distress prompting C-section. Whether meconium is the cause or consequence of this distress is uncertain. However, given the acute time frame between clinical diagnosis and C-section, we suspect prolonged meconium exposure may be a significant cause of non-reassuring fetal heart rate changes.

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Apoptotic Index in Normal and Intrauterine Growth-Restricted Rat Placentas. Elissa Scotland, Tri Nguyen, S Chiang, Radmila Runic. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Intra-uterine stress caused by maternal food-restriction may have adverse fetal and placental effects. We sought to assess the effect of maternal food restriction during pregnancy on placental apoptosis.

Methods: Rat placentas were analyzed from control dams fed ad libitum and dams 50% food-restricted from day 10 of gestation. Placentas were harvested at embryonic days 16 and 20 (n=3). Placentas were fixed in 4% PFA and two methods of analysis were utilized to determine the proportion of apoptotic to non-apoptotic cells within maternal food restricted and control rat placentas: Immunohistochemistry (IH) using Fas-Ligand and in situ TUNEL (Terminal deoxynucleotidyl transferase biotin-dUTP Nick End Labeling). TUNEL: after rehydration, slides were subjected to Tdt enzyme. DAB was used to visualize brown apoptotic nuclei. DNA-se treated slide was used as a positive control. IH: primary rabbit polyclonal Fas-Ligand antibody was used at 1:100 dilution, after which secondary antibody linked to peroxidase and DAB staining was performed.

Results: Food-restricted placentas demonstrated 9.29% relative apoptotic index when compared to 2.95% in the control group. The IUGR IOD (integrated optical density) per unit area in the placental membrane was higher than in the control group. FasL demonstrates an IOD of 0.112 + 0.025 in food restricted placentas as compared to 0.049 + 0.003 per μm2 surface area in controls. Apoptosis was seen in the amnion as well as trophoblast (syncytialtrophoblasts and some cytotrophoblasts).

Conclusion: The increased apoptotic index in maternal food restricted placentas suggests that the accompanying IUGR may be a result of both maternal/fetal nutrient restriction and increased fetal stress. This study found that maternal food restriction during pregnancy affects placental apoptosis. There is more apoptosis in the IUGR placental bed at E16 than at E20, with the reverse being true for the placental membrane. More research is required to statistically validate the data. The suggested next step is to evaluate Fas and Fas-L and to address possible mechanisms of placental apoptosis.

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Immunohistochemical and Biochemical Characterization of Corticotrophin Releasing Factor-Binding Protein (CRF-BP) in Preterm Human Umbilical Cord. Jayaraman Lakshmanan,[1] Avish Arora,[1] Lilit Baldjyan,[2] Sharon K Sugano,[1] Olga Miadel,[2] Adegoke Adeniji,[2] Michael G Ross,[1] Calvin J Hobel.[2] *[1]Ob-Gyn, Harbor-UCLA Medical Center, Torrance, CA, USA; [2]Ob-Gyn, Cedars-Sinai Medical Center, Los Angeles, CA, USA.*

Background: CRF-BP is a 37kDa protein, that specifically binds corticotrophin releasing factor (CRF). The structure of cloned CRF cDNAs in all species examined predicts that the precursor is larger than the 37kDa in size and contains one N-glycosylation site. Marked reductions in plasma CRF-BP levels seen in pregnant women prior to both preterm and term delivery led to the notion that CRF-BP is a "gatekeeper" of CRF responses. Recently we identified CRF-BP expression in term human umbilical cord (UC) by immunohistochemical analyses.

Objective: To characterized the expression of CRF-BP by immunohistochemical and biochemical analyses in human UC.

Methods: Freshly obtained human preterm (28 to <37 weeks, n=6) umbilical cord (6 pieces of 3mm thickness taken at 1-2 cm intervals close to placenta) were fixed in Bouin's solution and paraffin embedded. Also, pieces of UCs were dissected, arteries and vein separated, weighed and frozen at -80C. For immunohistochemical localization, UC sections were subjected to immunostaining with polyclonal antibodies to human CRF-BP precursors. For western blot analyses, whole UC as well as isolated arteries and vein were homogenized in a buffer containing detergents and protease inhibitors. The homogenate supernatant proteins were subjected to western analyses by standard protocol. Immunoreactive protein bands were identified by chemiluminescent reagent.

Results: Both goat and rabbit CRF-BP polyclonal antibodies elicited weak to moderate positive staining in UC epithelial layers, vascular musculature and barely endothelial cells. They identified a strong 50kDa band in whole UC as well as in isolated artery and venous preparations. In addition minor immunoreactive protein bands of 37, 25, 20kDa in size were noticed in all three preparations.

Conclusion: We conclude that preterm UC expresses CRF-BP. We postulate that the 50kDa major band is either glycosylated CRF-BP precursor or glycosylated 37kDa mature protein. The low molecular weight immunoreactive proteins likely represent proteolytically processed, glycosylated CRF-BP precursor or a proetolytically processed mature 37Da CRF-BP. UC obtained at delivery could be useful as a tool to understand the critical functions of CRF-BP in feto-placental unit.

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Modulation of Matrix Metalloproteinase Secretion by Adenosine A3 Receptor in Preeclamptic Villous Explants. Young-Han Kim,[1] Yong-Sun Maeg,[2] Han-Sung Hwang,[1] Hyung-Min Choi,[3] Yong-Won Park.[1] *[1]Obstetrics and Gynecology, Yonsei University Medical College, Seoul, Korea; [2]Biochemistry, Yonsei University, Seoul, Korea; [3]Obstetrics and Gynecology, Medical College, Inje University Ilsan Paik Hospital, Korea.*

Objective: Adenosine, known to be released from inflammatory sites and tisse ischemia, has many important biologic roles. Four specific adenosine receptors have been cloned to date, termed A1, A2a, A2b, and A3. Recently our study has shown that increased A3 receptor in the trophoblast of preeclamptic pregnancy was noted and non-vascular and trophoblast-mediated A3 receptor may play an important role in the pathogenesis of preeclampsia. There are evidences of impaired trophoblast invasion related to matrix metalloproteinase (MMP) in preeclampsia and the relationship between adenosine receptor and MMP in other fields. The objective of this study is to evaluate the effect of MMP expression by adenosine A3 receptor in preeclamptic villous explants at different oxygen conditions.

Methods: Placental villous explants from normal (n=10) and preeclamptic (n=10) pregnancies were cultured at high (20%) and low (3%) oxygen levels for 5 days. Explants were analyzed for MMP-2/-9 and TIMP-1/-2 by RT-PCR and western blot. Preeclamptic villous explants in hypoxic culture condition were treated with A3 receptor agonist, Cl-IB-MECA and A3 receptor antagonist, MRE. MMP-2/ -9 expression was determined in a time- and dose-dependent manner by RT-PCR, western blot. Also MMP-2/-9 activity was evaluated by zymogram assay.

Results: There were significantly increased A3 receptor intensity and reduced MMP-2/-9 and TIMP-1/-2 expression at low oxygen level in normal and preeclamptic villous explants. Interestingly, in preeclamptic villous explants, after high oxygen culture MMP-2/-9 and TIMP-1/-2 expression were recovered to almost same level compared to those in normal villous explants. Treatment of preeclamptic villous explants with Cl-IB-MECA in low oxygen level resulted in a time- and dose-dependent enhanced expression of MMP-2/-9. This Cl-IB-MECA-induced expression of MMP-2/-9 was inhibited by pretreatment with MRE.
Conclusion: To our knowledge, this study is the first to evaluate modulation of MMP secretion by adenosine A3 receptor in preeclamptic villous explant. Our results provide evidence for the existence of functional adenosine A3 receptors in the trophoblast and suggest that adenosine A3 receptor will be investigated as a therapeutic target in preeclampsia.

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Cannabinoids Stimulate Prostaglandin Production in Human Gestational Tissues through a Tissue and CB1 Receptor-Specific Mechanism. Murray D Mitchell,[1] Timothy A Sato,[1] Anderson Wang,[1] Jeffrey A Keelan,[1] Anna P Ponnampalam,[1] Michelle Glass.[2] *[1]Liggins Institute; [2]Department of Pharmacology and Clinical Pharmacology, University of Auckland, Auckland, New Zealand.*
Introduction: It is well established that prostaglandins play critical roles in multiple aspects of pregnancy and that the fetal membranes are an important site of intrauterine prostaglandin production. Endocannabinoids have been implicated in the maintenance of pregnancy and parturition in women and are a source of arachidonic acid which is a substrate for the production of prostaglandins. The aim of the present study was to determine the effects of endocannabinoids on the production of prostaglandins in extraplacental membranes – amnion, chorion and decidua.
Methods: Explants of term amnion and choriodecidua were established and treated with endogenous endocannabinoids 2-arachidonoyl glycerol (2AG) and anandamide (AEA) and with the synthetic cannabinoid CP55,940, to determine the ability of these substances to modulate prostaglandin E_2 (PGE_2) production. PGE_2 was measured by radioimmunoassay. The explants were also treated with CP55,940 in the presence of either SR141716A (a selective antagonist of the cannabinoid receptor CB1) or NS398 (a COX-2 inhibitor), to determine whether any observed stimulation of PGE_2 production was mediated through COX-2 activity and/or the CB1 receptor. COX-1, COX-2, cPLA2 and PGDH protein levels were measured by western blotting.
Results: All three cannabinoids caused a significant increase in PGE_2 production in amnion but not in choriodecidua. However, separated fetal (chorion) explants responded to cannabinoid treatment in a similar manner to amnion, whereas maternal (decidual) explants did not. The enhanced PGE_2 production caused by CP55,940 was abrogated by co-treatment with either SR141716A or NS398, illustrating that the cannabinoid action on prostaglandin production in fetal membranes is mediated by CB1 agonism and COX-2. Preliminary data from western blotting show that cannabinoid treatment results in the up-regulation of COX-2 expression. However, there was no change in COX-1 expression and no evidence either for up-regulation of cPLA2 or for down-regulation of PGDH expression.
Conclusion: This study demonstrates a potential role for endocannabinoids in the modulation of prostaglandin production in late human pregnancy, with potentially important implications for the timing and progression of term and preterm labour and membrane rupture.

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Inactivation of VEGF Receptor-2, but Not VEGFR-3 or VEGFR-1, during the Peri-Implantation Period Prevents Normal Pregnancy Development in the Rodent through Disruption of Uterine Angiogenesis. Nataki C Douglas,[1] Hongyan Tang,[1] Raul Gomez,[1] Bronislaw Pytowski,[2] Daniel J Hicklin,[2] Jan Kitajewski,[1] Mark V Sauer,[1] Ralf C Zimmermann.[1] *[1]Division of Reproductive Endocrinology and Infertility, Columbia University, New York, NY, USA; [2]ImClone Systems, Inc., New York.*
Objective: VEGF is involved in the regulation of uterine angiogenesis and implantation in both rodents and non-human primates. VEGFR-1, R-2, and R-3 are expressed in the uterine decidua and are involved in the regulation of vessel formation in many systems. To determine if these receptors have a functional role in the regulation of post-implantation angiogenesis and pregnancy development, we examined the effects of blocking VEGFR-1, R-2, and R-3 function.
Design: Prospective animal laboratory
Material and Methods: To avoid effects of VEGF receptor neutralization on ovarian function, we utilized a progesterone replaced, ovariectomized mouse model. VEGF receptor blocking antibodies were administered on ED 3.75, prior to embryonic expression of these receptors. Embryonic development was evaluated on ED 10.5, blood vessel density and apoptosis on ED 7.5, and cellular proliferation on ED 5.5 (n=5 per time point in each group). ANOVA with Bonferroni correction was used to compare sample means.
Results: See Tables.
Conclusions: Neutralization of VEGFR-2 and VEGFR-3, but not VEGFR-1 resulted in a significant reduction in cellular proliferation and decidual angiogenesis. VEGFR-3 mediates decidual angiogenesis, but is not required for normal pregnancy development. In contrast, an intact VEGF/VEGFR-2 pathway is required for the decidual angiogenesis that mediates early pregnancy development.

Effect of VEGFR neutralization on ED 10.5

	Control	anti R-1	anti R-2	anti R-3
No. of implantation sites	10.7 +/- 1.2	11 +/- 0.9	11.0 +/- 1.1	14.0 +/- 0.6
No. of embryos	10.7 +/- 1.2	11 +/- 0.9	0	14.0 +/- 0.6
Mean embryo weight	0.138 +/- 0.01	0.124 +/- 0.02	N/A	0.165 +/- 0.02

(mean +/- SE)

Effect of VEGFR neutralization on EDs 5.5 and 7.5

	No. of embryos*	% blood vessels*	% cellular proliferation**
Control	10.2 +/- 1.0	19.1 +/- 0.9	43.6 +/- 0.6
anti R-1	9.5 +/- 1.1	15.5 +/- 1.0	40.0 +/- 0.5
anti R-2	7.8 +/- 1.2	9.1 +/- 1.0♦	25.7 +/- 0.9♦
anti R-3	8.4 +/- 1.6	13.1 +/- 0.7♦	37.5 +/- 1.2♦

♦p< 0.05, * ED 5.5, ** ED 7.5, (mean +/ - SE)

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3-Phosphoglyerate Dehydrogenase (PGDH) Is Regulated by HOXA10 during Early Implantation. Danielle Vitiello, Hongling Du, Robert Pinard, Hugh Taylor. *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT.*
HOXA10 encodes a transcription factor required for endometrial receptivity and embryo implantation. Our objective was to identify and to characterize those molecular markers regulated by HOXA10 in multiple cellular model-systems. Using microarray technologies, we identified putative HOXA10 target genes involved in early implantation. Liposome-mediated transfection delivering either empty vector or the same plasmid constitutively expressing HOXA10 was introduced into newly impregnated mice during laparotomy or layered onto cultured human endometrial stromal-cells (HESCs). RNA products from these *in vivo* and *in vitro* transfections were used to identify targets and to validate the microarray screen employing semi-quantitative real-time PCR (qRT-PCR). We identified 82 statistically-significant genes regulated by HOXA10 overexpression of which 57 genes were down-regulated greater than 2-fold when compared to controls. Cellular ontogenies of differentially-expressed genes include: cell adhesion molecules, signal transduction factors as well as metabolic regulators. Furthermore, we identified the 3-phosphoglycerate dehydrogenase gene, (PGDH) whose products are regulated by HOXA10 during implantation in both murine model systems in and cell culture. This genes codes for an enzyme critical to *de novo* L-serine biosynthesis via a phosphorylation-dependent pathway. Microarray analysis demonstrated a 2-fold expression decrease when HOXA10 is overexpressed. This diminution in PGDH expression was noted in the validation experiments using qRT-PCR and corroborated in HESC cells where the mRNA levels decreased to 40% when compared to controls. The repression of PGDH during the implantation window may represent a conservation of activity as secretory-phase protein synthesis may be suppressed in order to promote cellular differentiation and resultant implantation. These regulatory relationships identified in mouse implantation likely function to enhance uterine receptivity and may have a role in human implantation.

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Leukocyte HOXA10 Expression Correlates with Endometrial Receptivity in Mice and Humans. Danielle Vitiello, Elena Ratner, Hongling Du, Robert Pinard, Hugh Taylor. *Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Objective: HOXA10 is expressed in endometrium, where it is regulated by sex steroids and is necessary for endometrial receptivity and implantation. HOXA10 is also expressed in leukocytes. Here we hypothesized that HOXA10

would be regulated by sex steroids in both cell types. We further hypothesized a correlation between expression of HOXA10 in peripheral blood cell (PBCs) and endometrium in both mice and in humans.
Methods: Real-time PCR was used to determine differential expression of HOXA10 mRNA in U937 cells, a monomyelocytic cell line, in response to increasing concentrations of estradiol. To determine if HOXA10 is expressed in leukocytes *in vivo*, peripheral leukocyte HOXA10 mRNA expression was measured over sequential estrus cycles in mature CD1 nulliparous mice and correlated to vaginal smear-cytology. Additionally, peripheral leukocytes were isolated either from pregnant or normally-cycling women to assess HOXA10 mRNA expression.
Results: There was a direct, dose-responsive correlation between exposure to increasing estradiol and HOXA10 mRNA expression levels in U937 cells. In a murine model, we demonstrated that HOXA10 mRNA expression-levels varies throughout the estrus cycle with a marked increase in expression following vaginal plug detection. The nadir of HOXA10 expression is prior to proestrus and increased up to 2-fold during the receptive phase. This increased expression continues throughout gestation. The heightened expression in murine leukocyte-derived HOXA10 mRNA also is demonstrated across species. Our preliminary data suggests that the greatest fold-increase of expression occurs during the window of implantation of the secretory phase in normal, cycling women. This level was sustained in pregnancy. There appears to be a trend with the highest levels of expression associated with viable gestations. Women with attenuated expression profiles had non-viable gestations.
Conclusions: HOXA10 expression is regulated by sex steroids in both leukocytes and endometrium. The temporal pattern of peripheral HOXA10 transcript expression demonstrated in mice and humans mimics the differential RNA expression documented within the uterus. Leukocyte HOXA10 expression during the reproductive cycle in mice and humans is a marker of endometrial receptivity. Peripheral leukocyte expression of HOXA10 mRNA may correlate with implantation success.

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Analysis of Endometrial Secretions To Predict Pregnancy Following ART. Carolien M Boomsma, Annemieke Kavelaars, Marinus JC Eijkemans, Gijs Teklenburg, Bart CJM Fauser, Cobi Heijnen, Nick S Macklon. *Reproductive Medicine and Gynaecology and Laboratory of Psychoneuroimmunology, University Medical Center, Utrecht, Netherlands.*
Introduction
The purpose of this study is to assess the association between the intra-uterine cytokine expression profile at the time of embryo transfer and successful embryo implantation following IVF/ ICSI treatment.
Materials and methods
210 women undergoing IVF/ ICSI underwent endometrial secretion aspiration prior to embryo transfer. 16 known soluble mediators of implantation were measured using a multiplex immunoassay, namely IL1ß, IL5, IL6, IL10, IL12, IL15, IL17, IL18, TNFα, VEGF, IFNγ, Eotaxin, MCP-1, IP-10, Dkk-1 and HbEGF. MIF was determined using an ELISA. The total protein concentration was measured for normalization purposes. Data were log transformed to obtain normal distribution. Multivariable logistic regression analysis with a backward elimination procedure ($p<0.1$) was used, potential confounders (age, blood contamination, embryo quality) were included in a forward stepwise model.
Results
Ten mediators of the 17 analysed were detectable in 90-100% of the samples. IL15 was detectable in 76% of samples, DKK-1 68%, IL-10 56%, IL-17 54%, IL-5 35% and HbEGF was detectable in 23% of samples. IFN-γ was not detectable in any of the samples. Multivariable logistic regression showed only LogMIF concentrations to have a significant correlation with achieving clinical pregnancy ($p=0.036$). Higher MIF concentrations were correlated with a higher chance of conceiving.
Conclusion
Endometrial secretion analysis represents a novel means of assessing the intra-uterine milieu encountered by the embryo and offers new perspectives in the study of endometrial receptivity. In this large prospective study assessing an array of cytokines, MIF was found to be significantly correlated with pregnancy. MIF, macrophage migration inhibitory factor, is a cytokine with numerous proinflammatory, immunomodulatory, angiogenic and tissue remodelling properties. MIF induces the synthesis and secretion of matrix metalloproteinases by endometrial cells, which may contribute to embryo invasion. Its expression is particularly increased during the secretory phase, suggesting a role in reproductive processes. Analysis of aspirated endometrial secretions offers a direct clinical test of endometrial receptivity which can be applied during treatment cycles without disrupting implantation.

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Low Luteal Progesterone Levels Do Not Alter Midsecretory Endometrial Gene Expression or Histology. Steven L Young,[1] Rebecca S Usadi,[3] Bruce A Lessey,[2] Jeremy M Groll,[4] Marc A Fritz.[1] *[1]U. N. Carolina, Chapel Hill, NC; [2]Greenville Hospital, Greenville, SC, USA; [3]Carolinas Medical Ctr, Charlotte, NC; [4]Wright-Patterson Medical Ctr, WPAFB, OH.*
Endometrial receptivity and secretory differentiation require progesterone (P). It has been hypothesized that low P levels result in delayed endometrial differentiation and infertility due to reduced receptivity.
Objective: Test the effects of low luteal P on histologic and molecular markers of differentiation and function.
Methods: Normal cycling women (n=12) were treated with daily leuprolide (1.0 mg/d) beginning in the midluteal phase and continuing through the protocol. After menses, subjects received transdermal estradiol (E, 0.2 mg/d) for 20 days. After day 10 of E, subjects also received daily i.m. injections of P, randomized to 10 mg/d (sub-physiological) or 40 mg/d (physiological). Endometrial biopsy was performed after 10 days of combined E and P treatment. Additional untreated women had biopsies performed 10 days after spontaneous LH surge. Endometrial histologic dating was performed by two individuals according to the criteria of Noyes et al. mRNA levels were assessed using real-time RT-PCR.
Results: Mean(s.d.) of peak and trough P serum concentrations in the 40 mg/d P group were 18.2(5.1) and 9.4(4.8) ng/dL, respectively, while those in the 10 mg/d group were 7.0(3.0) and 3.4(1.0) ng/dL. There were no differences between treatment groups for histologic dating; the mean(s.d.) histologic date was 25.7(0.8), 24.4(0.9), and 24.8(1.3) for the 10 mg, 40 mg, and spontaneous cycle groups, respectively. There also were no differences among the three groups in mRNA levels of ten functional markers (ERα, PR, β3 integrin subunit, osteopontin, cyr61, EGR-1, FKB52, c-fos, and CD55), although variability of gene expression was greater in those who received 10 mg/d P than in those who received 40 mg/d P. There was also no correlation between serum progesterone level and gene expression or histologic date.
Conclusions: Sub-physiological levels of progesterone, in the range seen in ovulatory women, do not induce detectable changes in expression of marker genes or histological dating, although low P levels were associated with greater variability of gene expression. These data suggest that abnormalities in endometrial histologic development and function likely result from intrinsic abnormalities rather than from low levels of P secretion. (Supported by UNC Nova Carta Fund and NIH U54 HD-35041).

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Endometrial IP10 Attracts Trophectoderm through CXCR3 Interaction. Simcha Yagel,[1] Caryn Greenfield,[1] Hen Sela,[1] Jacob Hanna,[2] Irit Manaster,[2] Orna Singer,[3] Ronit Haimov-Kochman,[1] Shira Natanson-Yaron,[1] Diana Prus,[1] Benjamin Reubinoff,[2] Debra S Goldman-Wohl,[1] Ofer Mandelboim.[2] *[1]Obstetrics and Gynecology, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel; [2]Lautenberg Center for Immunology, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel; [3]Human Embryonic Stem Cell Research Center, Hadassah-Hebrew University Medical Centers, Jerusalem, Israel.*
Introduction: Implantation is initiated in part by attraction of the blastocyst to the endometrium lining the uterus. We hypothesized that this process is partly accomplished by chemokines expressed by the endometrium interacting with chemokine receptors on the blastocyst, suggesting that they play an important role in implantation.
Materials and Methods: Chemokine receptors were characterized in JEG cells, placental villi, primary trophoblast cell culture, trophectoderm cells derived from human ES cells, blastocyst trophectoderm, and 1st trimester placental tissue sections. Expression of chemokines was tested in decidua, endometrium, and Ishikawa and HEC-1 cell lines. Immunohistochemistry, intracellular staining, ELISA, FACS analysis, and RT-PCR were employed to characterize chemokine receptor and ligand expression. Functional testing was performed using transwell migration assays and in a nude mouse model using a matrigel gel plug cell attraction assay followed by FACS analysis.
Results: Trophoblasts demonstrated expression of various chemokine receptors, most prominently CXCR1 and CXCR3. Immunohistochemistry of trophoblast from placental villi plated on Matrigel expressed CXCR3 and CXCR1 as well as HLA-G. Noteworthy is that trophectoderm cells derived from hES cells treated with BMP-4 and JEG cells, and blastocyst trophectoderm expressed principally CXCR3. ELISA and immunohistochemistry showed that decidua and endometrium expressed chemokines IP10 and IL8. Migration assays demonstrated that IP10 significantly attracted various trophoblast and trophectoderm cells in vitro, and in the mouse model in vivo.

Conclusion: Taken together these results demonstrate the interaction between trophoblasts and endometrial cells is mediated by CXCR1 and CXCR3, and IL8 and IP10. These interactions are important in the attraction of trophoblasts at the feto-maternal interface. However, IP10-CXCR3 is the most relevant to early implantation as only CXCR3 is expressed consistently by trophectoderm.

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The Endometrial Proteome: Changes from Proliferative to Secretory Phase. Lois A Salamonsen, Jenny I-C Chen, Xian Mak, Peter J Stanton, David M Robertson, Andrew N Stephens. *Prince Henry's Institute of Medical Research, Melbourne, Vic, Australia.*

Global gene analyses have demonstrated major changes across the menstrual cycle, but which of these are reflected in the proteome is not known. This study aimed to globally assess proteins differentially expressed in the endometrium between the proliferative and. secretory phases.

2D PAGE analysis with DIGE minimal dye labelling was conducted across the pI range 4–7 on endometrial tissue from either the mid-proliferative or mid-secretory phases (n=4/group). Profiles were assessed using SameSpots software. Differentially expressed proteins were identified using MALDI-TOF MS and the interrelationship of proteins examined using Ingenuity software.

A total of 1010 spots were detected: 196 were differentially expressed ($p < 0.05$) with 17 spots having an overall false discovery rate <5% ($q < 0.05$). Hierarchical clustering analysis revealed that these 17 proteins lay within three main branches in the protein dendrogram. One cluster had proteins up-regulated in the proliferative phase and two contained proteins up-regulated in the secretory phase. The unique protein profiles were also revealed using principle component analysis (PCA): proteins clustered into two main groups, according to cycle phase. PCA thus indicated similar unique protein signatures as suggested by hierarchical clustering.

Thirty one of the differentially expressed proteins were identified using MALDI-TOF MS. These proteins could be grouped into seven categories, which included structural (7), transport (4), regulatory (2), membrane (2), enzyme (2), motor (1) and others (2). Proteins involved in matrix assembly and those needed for subsequent establishment of secretory endometrium were up-regulated in proliferative endometrium. Proteins important for cellular organisation and communication as well as products responding to environmental stress and the immune system were highly up-regulated during the secretory phase. Biological pathways were constructed based on the proteins identified. The top network for secretory endometrium clustered around TGF-b. Others related to inhibition of cell death / cell viability and leukocyte extravasation.

These studies provide a global approach to the cyclic changes of the endometrium and highlight the complex dynamics of protein expression in human endometrium.

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Hormonal Regulation of Prokineticins in the Human Fallopian Tube: Potential Regulators of Embryo Transport. AW Horne,[1] HN Jabbour,[2] P Lourenco,[1] S Wright,[2] S Battersby,[2] ARW Williams,[1] HOD Critchley.[1] *[1]Reproductive and Developmental Sciences, University of Edinburgh, Edinburgh, United Kingdom; [2]MRC Human Reproductive Sciences Unit, Queen's Medical Research Institute, Edinburgh, United Kingdom.*

Background: Understanding the factors regulating embryo transport in the fallopian tube (FT) has important clinical implications. Embryo retention in the tube due to FT dysfunction is thought to lead to tubal pregnancy, a considerable cause of morbidity and occasional mortality. Transport of the embryo through the FT is, for the greater part, accomplished by smooth muscle contraction. A group of multi-functional proteins and their receptors, called prokineticins, have been shown to affect smooth muscle function in other tissues, such as the intestine. The expression pattern of prokineticins, and their receptors, was examined in normal human FT obtained throughout the menstrual cycle, and the effect of the sex steroids on prokineticin expression was examined in an in-vitro model of the FT.

Methods: FT biopsies (n=18) and sera (for measurement of oestradiol and progesterone for endocrine staging) were collected from women undergoing gynaecological procedures for benign conditions. Using a combination of quantitative Taqman RT-PCR and immunohistochemistry, the mRNA and protein expression pattern of prokineticins, and their receptors, were examined in the FT throughout the menstrual cycle. Tubal explant culture was established using surgical tissue from the biopsies and exposed to varying concentrations, and time courses, of oestrogen and progesterone.

Results: Prokineticin 1 (PK1) and prokineticin 1 receptor (PKR1) mRNA are up-regulated in the progesterone-dominant mid-secretory phase. PK1 and PKR1 protein are expressed in the epithelium, smooth muscle and around the blood vessels of the FT. Stimulation of tubal explant cultures with a physiological concentration of progesterone showed an up-regulation of PK1 and PKR1.

Conclusions: Prokineticins show temporal variation in expression in human FT and appear to be regulated by progesterone. Their role in embryo transport needs to be investigated to further understanding of pregnancy complications, such as tubal pregnancy.

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Translating Mouse to Human: A Dynamic Model of Xenografted Human Endometrium. Alex J Polotsky, Liyin Zhu, Nanette Santoro, Jeffrey W Pollard. *Albert Einstein College of Medicine, Bronx, NY, USA.*

In the mouse endometrium, the hormonal environment controls cellular proliferation and cell cycle activity. Estradiol (E2) inhibits glycogen synthase kinase 3 beta (GSK-3β), resulting in nuclear accumulation of cyclin D1 and progression of the cell cycle, as well as DNA replication licensing. In utero administration of the GSK-3β inhibitor, LiCl, results in epithelial cell proliferation in the absence of E2 (Zhu, Pollard. PNAS. 2007;104:15847). In this study, we derived a functional model of xenografted human endometrium to perform mechanistic studies of human endometrial proliferation.

Methods: Human endometrial samples were obtained from volunteers aged 18-45. Immuno-compromised mice were transplanted with disaggregated/ recombined human epithelial glands and stroma under the kidney capsule. After 6 weeks of out-growth, mice were ovariectomized, and replaced with E2 or LiCl. Xenografts were harvested and processed for immunohistochemistry (IHC) and glandular labeling index (LI). T test was used to compare group means.

Results: 40-50% of engraftments were successful, resulting in a vascularized endometrium with characteristic architecture (A, B). Hoechst 33258 staining confirmed that xenografts were made up of human cells ©. E2 (E) induced significantly greater proliferation compared to control (D) as assessed by IHC for Ki67, with LI of 25.9±1.3 and 8.8±3.3, respectively, p =0.02). Mini-chromosome maintenance-2, a protein involved in DNA replication licensing, was more sensitive for E2-treated cells synthesizing DNA than was Ki67 staining (I). Estrogen (G) and progesterone receptors (H) were expressed in xenotransplant tissue, the latter being up-regulated by E2. LiCl (F) induced proliferation similar to E2 and greater than control (LI =18.4±6.4, p= 0.2 for E2 vs. LiCl).

Conclusion: Xenografted human endometrium provides a dynamic model of endometrial proliferation that is well suited for translational studies. Administration of LiCl in the absence of E2 induced glandular proliferation, supporting the notion that similar mechanisms are operative in human proliferation as in the mouse.

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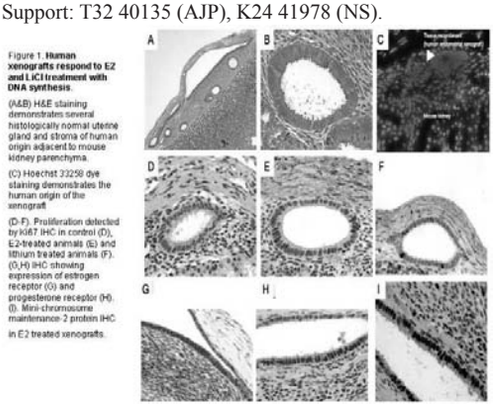

Figure 1. **Human xenografts respond to E2 and LiCl treatment with DNA synthesis.** (A&B) H&E staining demonstrates several histologically normal uterine gland and stroma of human origin adjacent to mouse kidney parenchyma. (C) Hoechst 33258 dye staining demonstrates the human origin of the xenograft. (D-F). Proliferation detected by Ki67 IHC in control (D), E2-treated animals (E) and lithium treated animals (F). (G,H) IHC showing expression of estrogen receptor (G) and progesterone receptor (H). (I). Mini-chromosome maintenance-2 protein IHC in E2 treated xenografts.

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Gonadotropin Releasing Hormone Agonist (GnRHa) Leuprolide Acetate (LA) Suppresses Proinflammatory Cytokine Expression Induced by the Ovarian Steroids in Human Endometrium. Xiaoping Luo, Orhan Bukulmez, R Stan Williams, Nasser Chegini. *Obstetrics and Gynecology, University of Florida College of Medicine, Gainesville, FL, USA.*

GnRH analogs have been extensively used in assisted reproduction. Although the main effects of GnRH analogs are via GnRH receptors on the pituitary gonadotrope, GnRH and GnRH receptors have been identified in many reproductive tissues including human endometrium, suggesting their potential action at endometrial level. In the present study, we examined the potential regulatory action of GnRHa, LA on sex steroid mediated gene expression

in human endometrium. Human endometrial surface epithelial (HES) cells and isolated endometrial stromal (ESC) cells were used as in vitro models. All experiments lasted for 24h. The cells were treated with estradiol (E_2, 10 nM, 24h), progesterone (P4, 10 nM, 24h), GnRHa (LA 1 μM, 24 h), E_2 (12h) followed by P4 (last 12h) and GnRHa plus E_2 plus P4 where, GnRHa added either first, second or last in order at 8h intervals. Total RNA was extracted, reversed-transcribed and subjected to real-time PCR simultaneously, measuring the expressions of IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p35, IL-12p40, IL-15, IFN-γ and TNFα. Both HES and ESC expressed majority of these cytokines with the exception of low to undetectable levels of IL-2, IL-4, IL-5 and IFN-γ. Treatment with E_2 and P4, either alone, or in combination significantly upregulated the expression of many of these cytokines at varying extend as compared to controls. However, GnRHa either alone or in combination with E_2 and P4 significantly diminished E_2, P4 or E_2 plus P4 induced mRNA expression of cytokines. The suppressive effects of GnRHa on some of the cytokines varied significantly by the order which GnRHa was introduced into the culture medium. We conclude that GnRHa by acting directly on the endometrial cells effectively suppresses the mRNA expression of several key proinflammatory cytokines upregulated by ovarian steroids. Our results imply that GnRHa therapy during assisted reproduction may modify endometrial receptivity via downregulation of proinflammatory cytokines induced by estradiol and progesterone.
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Expression of Myoferlin in Human Endometrium during the Menstrual Cycle. Pinar H Kodaman,[1] Tugba Altun,[1] William Murk,[1] Umit Kayisli,[1] Pascal N Bernatchez,[2] William C Sessa.[2] [1]*Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT, USA;* [2]*Vascular Biology and Therapeutics, Yale University School of Medicine, New Haven, CT, USA.*
Introduction: Endometrial angiogenesis is characterized by a rapid increase during the early proliferative phase that peaks midcycle, followed by a gradual decrease in the secretory phase, while menses involves generalized endometrial inflammation, necrosis, and vascular thrombosis. Vascular endothelial growth factor (VEGF), whose expression is regulated by sex steroids, is a mediator of endometrial angiogenesis. Recently, myoferlin, a 230 kD transmembrane protein, was identified in endothelial cells where it mediates VEGF-dependent endothelial cell proliferation, migration, and nitric oxide synthesis by affecting VEGF receptor-2 function and stability. Myoferlin also appears to play a role in vesicle trafficking and membrane repair. **Objective:** To characterize myoferlin protein expression in endometrium. **Methods:** Western analysis of cultured human endometrial stromal cells (HESC) and human endometrial endothelial cells (HEEC) treated with physiologic concentrations of estradiol and progesterone was performed using a polyclonal rabbit antibody against myoferlin. Subsequently, immunohistochemistry (IHC) was performed on human endometrium samples obtained from various stages of the menstrual cycle. **Results:** Myoferlin protein was expressed in HESCs and HEECs, but expression was not affected by sex steroids. IHC staining for myoferlin was specific, intense, and localized to the apical membrane of glandular epithelial cells and endothelial cells, with less intense staining in the stroma. H-score quantification showed that in endometrial endothelial cells, myoferlin protein expression was highest during the early proliferative and early secretory phases, while glandular and stromal myoferlin expression peaked during the late proliferative/early secretory phase. **Conclusion:** Myoferlin expression in human endometrium correlates with periods of greatest endometrial angiogenesis, and expression is not limited to endothelial cells, but also includes glandular and stromal cells. Given the involvement of myoferlin in VEGF signaling as well as membrane repair, understanding its role in human endometrium may further elucidate an understanding of endometrial development both under physiologic and pathologic conditions.

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The Human Embryo Is the Primary Regulator of Embryo-Endometrial Molecular Cross Talk during Early Implantation. Gijs Teklenburg,[1,2] Cobi Heijnen,[1] Esther Baart,[1] Karima Amarouchi,[1] Carolien Boomsma,[1] Janet Carver,[2] Helen Mardon,[2] Annemieke Kavelaars,[1] Nick Macklon.[1] [1]*Reproductive Medicine and Gynaecology, and Laboratory of Psychoneuroimmunology, University Medical Center, Utrecht, Netherlands;* [2]*Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Women's Centre, John Radcliffe Hospital, Oxford, United Kingdom.*
Introduction: Uterine receptivity and implantation are controlled by locally acting trophic factors and cytokines. In humans, the regulation of the embryo-endometrial dialogue beyond the early blastocyst stage is poorly understood. We hypothesized that the interaction between a healthy conceptus and the receptive endometrium is associated with a distinct local regulation of cytokine production favouring implantation.
Methods: 97 human embryos, cryopreserved at day 4 after fertilization and donated for research, were thawed and cultured under standard conditions until day five. Forty-two embryos from 18 donors developed to the blastocyst stage. Following removal of the zona pellucida, they were placed in individual co-culture on a confluent monolayer of endometrial stromal cells. On day 8, the developmental potential of each embryo was assessed as early arrested, late arrested or developing. Culture supernatants were analysed for concentrations of IL-1β, IL-5, IL-6, IL-10, IL-12, IL-15, IL-17, IL-18, TNF-α, MCP-1, IP-10, Eotaxin and HB-EGF using a Multiplex immunoassay. Day 8 supernatants from 29 culture systems in which no embryo had been placed were also analysed as controls.
Results: 11 out of 42 (26%) embryos continued to develop and were able to attach and invade into the stromal cell compartment of the co-culture environment. Twelve late arrested embryos showed signs of degradation on day 8 and the totally disintegrated embryos were assigned to the early arrested group. Supernatants from both early and late arrested embryo cultures contained significantly lower levels of a number of cytokines and growth factors in comparison to developing embryo cultures. Moreover, the levels of these mediators in co-cultures were significantly lower than those in non-embryo control stromal cell culture supernatants.
Conclusion: These data suggest a pivotal role of the embryo in embryo-endometrial cross talk. Whether reduced mediator expression in co-cultures reflects a selective down regulation of stromal cell cytokine and growth factor production is now being investigated.

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N-Cadherin Regulates Epithelio-Mesenchymal Transition and Endometrial Epithelial Cell Dynamics during Human Implantation. Hiroshi Uchida, Tetsuo Maruyama, Toru Arase, Masanori Ono, Takashi Kajitani, Maki Kagami, Hideyuku Oda, Sayaka Nishikawa, Yasunori Yoshimura. *Department of Obstetrics and Gynecology, Keio University School of Medicine, Shinjuku, Tokyo, Japan.*
Epithelio-mesenchymal transition (EMT) is thought to play a role in functional differentiation of endometrial epithelial cells during human implantation. Molecular mechanism of epithelial sheet remodeling caused by embryo invasion remains elusive. To address this, we investigated cellular dynamics of N-cadherin and vimentin, the two representative major markers of EMT, during implantation. In in vitro implantation assay using a human endometrial epithelial cell line, Ishikawa, and a human choriocarcinoma cell line, JAR (Uchida et al., Hum Reprod 2007), we pre-treated human Ishikawa cells with or without ovarian steroid hormones (17β-estradiol + progesterone; EP), FA-5 (N-cadherin blocking Ab), or suberoylanilide hydroxamic acid (SAHA), one of histone deacetylase inhibitors, which has a potential to improve in vitro implantation (ibid). Implantation or treatment with or without EP or SAHA enhanced the expression of N-cadherin and vimentin but down-regulated E-cadherin. Furthermore, treatment with EP or SAHA accelerated Ishikawa cell motility and increased the number and spreading area of JAR spheroids. In vitro implantation assay, the most prominent staining intensity of N-cadherin was observed just around the adhered spheroid from which its intensity decreased away. Functional blockade of N-cadherin by FA-5 resulted in the complete suppression of Ishikawa cell motility, the unique distribution of N-cadherin around JAR spheroids, and the spreading area of JAR spheroids, while it did not affect the number of the adhered spheroids. Human implantation consists of the multiple steps, including apposition, adhesion and penetration. Thus, these results collectively indicate that EMT may take place after the apposition and that N-cadherin may be required for the remodeling and EMT of the epithelial sheet during embryo invasion. N-cadherin may enhance the recruitment of spheroid-neighboring cells, suggesting its role in the covering-up of the invading embryo through acceleration of epithelial cell motility.

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Endocannabinoid Regulation in Human Endometrium. Jessica G Scotchie, Marc A Fritz, Steven L Young. *Obstetrics and Gynecology, University of North Carolina, Chapel Hill, NC, USA.*
Background: Research in mouse models demonstrates two cyclically regulated endocannabinoids produced in murine endometrium, anandamide and 2-

arachidonoyl glycerol (2-AG); both play critical roles in murine embryonic implantation. No studies of endocannabinoids in human endometrium have been performed.

Objectives: Determine menstrual cycle expression and localization of synthetic and degradative enzymes for anandamide and 2AG in human endometrium.

Methods: Human endometrium was collected from volunteers across the menstrual cycle (n=43). Quantitative RT-PCR was performed analyzing the expression of: N-acylphosphatidylethanolamine (NAPE) and fatty acid amide hydrolase (FAAH), the synthetic and degradative enzymes for anandamide, respectively; *sn*-1-diacylglycerol lipase-a (DAGLa), and b (DAGLb), the synthesis enzymes for 2AG; and monoacylglycerol lipase (MAGL) and cyclooxygenase-2 (COX2), the degradative enzymes for 2AG. The constitutive gene PPIA was used for comparison. Immunohistochemical localization of NAPE protein was performed using NAPE-PLD polyclonal antibody (Cayman Chemical, #10005430). ANOVA and student's t-test analysis performed on samples grouped by proliferative (PRO), early, mid-, and late secretory (ES, MS, LS) phases.

Results: mRNA expression of all enzymes responsible for synthesis and degradation of anandamide and 2AG was detected throughout the cycle. No significant cyclic change in NAPE, FAAH, or DAGLb gene expression was seen. A decrease in DAGLa gene expression in the MS and LS phases compared to the PRO and ES phases ($p=0.004$) was seen. MAGL gene expression was higher in the secretory phase than the PRO phase ($p=0.004$). COX2 gene expression was detected at low levels in the PRO, ES and MS phases, with marked increase in the LS phase ($p<0.05$ for all comparisons). Protein localization of NAPE showed a cytoplasmic epithelial location, with increased staining on PRO and ES samples compared to MS and LS samples. Immunolocalization of remaining proteins is ongoing.

Conclusion: This is the first report documenting the presence of endocannabinoid synthetic and degradative enzymes in human endometrium. Genes controlling anandamide expression do not fluctuate significantly across the cycle. However, 2AG's degradative enzymes increase in the secretory phase, suggesting that lower 2AG levels may be advantageous for embryo implantation. Our findings suggest that human endometrial endocannabinoid regulation differs from murine regulation.

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Interleukin-1 beta (IL-1β) Regulates IL-6 Signaling in Decidua-Implication in the Pathophysiology of Preeclampsia (PE). SJ Huang,[1] CF Yen,[2] CP Chen,[3] F Schatz,[1] CJ Lockwood.[1] *[1]Obstetrics, Gynecology and Reproductive Sciences, Yale University, New Haven, CT, USA; [2]Ob/Gyn, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan; [3]Ob/Gyn, MacKay Memorial Hospital, Taipei, Taiwan.*

Objective: Previously, we found much higher cytoplasmic immunoreactive IL-6 levels in the preeclamptic decidual cells than in adjacent interstitial trophoblasts. Such decidual cell-derived IL-6 contributes to the systemic endothelial cell dysfunction that elicits the proteinuria and hypertension of the maternal syndrome. IL-6 promotes the transition from innate to adaptive immunity. Moreover, by skewing monocyte differentiation from a dendritic to a macrophage phenotype, decidual IL-6 may promote the macrophage excess observed in the preeclamptic decidua. Macrophages impair trophoblast decidual invasion to foster incomplete spiral artery remodeling that elicits placental ischemia and hypoxia. The current study: 1) localized IL-6 mRNA levels in preeclamptic versus normal decidual sections; 2) evaluated mechanisms regulating IL-6 synthesis by targeting intracellular signaling pathways with specific inhibitors; 3) identified potential IL-6 targets by immunolocalizing the IL-6 receptor (IL-6R) to specific cell types in placental bed biopsies.

Methods: In situ hybridization localized IL-6 mRNA in normal versus preeclamptic decidua. IL-6R was immunolocalized in placental bed biopsies. Leukocyte-free first trimester decidual cells were incubated with E2 and MPA ± IL-1β (1 ng/ml) ± an inhibitor of p38 MAPK (SB203580) or protein kinase C (calphostin C) or NFκB (activation inhibitor III) for 24 hrs. An ELISA measured secreted IL-6 levels.

Results: IL-6 mRNA was present primarily in decidual cells with increased IL-6 mRNA levels observed in PE. Preferential expression of the IL-6R was observed on decidual cells in placental bed biopsies. Compared with basal IL-6 levels (0.22 ± 0.14 pg/ml/μg cell protein) by decidual cells, IL-1β enhanced IL-6 output by decidual cells (364.55 ± 160.29 pg/ml/μg cell protein). Only the p38 MAPK inhibitor significantly reduced this output to 39.50 ± 8.09 pg/ml/μg cell protein ($n=4$, $p<0.05$).

Conclusions: Our results indicate that inflammatory cytokine enhances IL-6 synthesis in decidual cells of the preeclamptic decidua by a mechanism involving p38 MAPK. Such IL-6 is likely to act as an autocrine/paracrine effector via decidual cell-expressed IL-6R to contribute to the macrophage excess observed in the preeclamptic decidua.

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Heparanase Is Up-Regulated by Estrogen and during the Secretory Phase of the Human Endometrium. Ronit Haimov-Kochman,[1] Shira Natanson-Yaron,[1] Caryn Greenfield,[1] Achinoam Lev-Sagie,[1] Lichtenstein Michal,[2] Haya Lorberboum-Galsky,[2] Israel Vlodavsky,[3] Simcha Yagel,[1] Arye Hurwitz.[1] *[1]OB/Gyn, Jerusalem, Israel; [2]Cellular Biochemistry Human Genetics, Hadassah Hebrew University Medical Centers, Jerusalem, Israel; [3]Cancer and Vascular Biology Research Center, Technion School of Medicine, Haifa, Israel.*

Introduction: Heparanase is an endoglycosidase that cleaves heparan sulfate (HS) proteoglycan of the extracellular matrix. The full-length proheparanase is activated by cleavage into an active isoenzyme, resulting in the release of HS-bound cell-differentiation factors, such as HB-EGF. The cycling endometrium involves remarkable steroid hormone-induced tissue remodeling. *In vivo*, increasing exposure to unopposed estrogen may lead to endometrial malignant transformation.

Aim: To investigate heparanase expression and regulation in the cycling endometrium.

Materials and methods: Heparanase mRNA levels were measured by quatitative RT-PCR in naturally menstruating women and in Hec1A, estrogen receptor (ER)-negative and Ishikawa, ER-positive endometrial carcinoma cell lines exposed to increasing doses of estradiol. Heparanase isoenzymes were localized by immunohistochemistry using specific anitbodies in murine endometrium and human normal, hyperplastic and malignant endometrium.

Results: Heparanase mRNA level increased 100fold in secretory phase (d21) compared to proliferative phase (d10) endometrium. Heparanase transcript levels increased 14fold during 8 hr culture in ER positive adenocarcinoma cell line exposed to increasing doses of estradiol, but not in Hec1A, ER negative cell line. Both heparanase isoforms were localized to murine glandular endometrium. Human glandular endometrium at both proliferative and secretory phases was immunoreactive with the active isoform of heparanase. Proheparanase was detected in basal membrane of endometrial glands and endometrial stroma during secretory phase. Along with malignant transformation of the endometrium the presence of proheparanase increased dramatically from none in stroma of normal and hyperplastic endometrium to abundance in malignant tumors.

Conclusions: Heparanase gene expression is higher during the window of implantation and up-regulated with estrogen in endometrial cells via ER *in vitro* and *vivo*. Heparanase is differentially localized in the secretory phase of the endometrium compared to the proliferative phase, suggesting a role for this molecule during the window of implantation in man.

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Interleukin-18 (IL-18) System mRNA and Protein Expression in the Human Fallopian Tube with Ectopic Implantation. Hong-Yuan Huang,[1,2] Tien-Hung Huang,[2] Chin-Jung Li,[1] Chyi-Long Lee,[1,2] Hsin-Shih Wang,[1,2] Yung-Kuei Soong.[1,2] *[1]Obstetrics and Gynecology, Chang Gung Memorial Hospital, Kwei-Shan, Tao-Yuan, Taiwan; [2]Obstetrics and Gynecology, Chang Gung University and School of Medicine, Kwei-Shan, Tao-Yuan, Taiwan.*

Objective: Ectopic pregnancy, an abnormal implantation of a fertilized ovum outside the uterine cavity, has been increasing in number at a staggering pace of all pregnancies. IL-18 system is one of the major cytokines involved in human endometrium during embryo implantation and might perform a defensive role against maternal immune response. Very little information is available regarding the expression and synthesis of cytokines in the pathogenesis of fallopian tube with ectopic gestation. The purpose of this study is to investigate IL-18 system expression in human fallopian tubes with ectopic pregnancy.

Methods: Paired segments of human fallopian tubes with ectopic implantation site and side portion close to ectopic gestation (n=7) were collected from women undergoing laparoscopic salpingectomy after informed consent and IRB approval. Segments of fallopian tubes from women undergoing tubal ligation (n=4) were used as control groups. Total extracted RNA was reverse transcribed and amplified by PCR using specific primers for GAPDH (94 bp), IL-18 (144 bp), IL-18BP (188 bp) and IL-18R (307 bp). Quantitative IL-18 and IL-18BP mRNA expression in human fallopian tube was determined by real-time PCR. To determine the presence of IL-18 system proteins, tissues were fixed and processed for immunohistochemical study. Data analysis was done with ANOVA and Pearson's correlation.

Results: IL-18 and IL-18BP as well as IL-18R mRNA were all expressed in tubal ectopic implantation and normal tubes. According to real-time PCR with C_T value quantification and $2^{-\Delta\Delta Ct}$ method, a significantly higher IL-18 expression in tubal ectopic implantation and lower ratio of IL-18 antagonist to agonist in portion close to ectopic implantation is demonstrated in comparison to normal tubes ($p<0.05$). Immunoreactive IL-18 system at the protein levels was also present in human fallopian tubes with ectopic implantation and normal tubes.
Conclusions: These results suggest that fallopian tube IL-18 system expression may play a crucial role during the process of early embryonic implantation. The expression and ratio of antagonist to agonist in fallopian tubes may indicate an earlier "dialogue " in human fallopian tubal gestation prior to uterine implantation.

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Activin beta-A Subunit, Follistatin and Follistatin-Like 3 Are Expressed in the Endometrium of Ovariectomized Rats and Regulated by Estrogen Replacement. Marcia C Ferreira, Ines KD Cavallo, Fernando M Reis. *Departments of Physiology and Obstetrics & Gynecology, UFMG, Belo Horizonte, MG, Brazil.*
Activin A is a growth factor expressed in the endometrium, where it modulates tissue remodelling and enhances decidualization. The effects of activin A are counteracted by two binding proteins, namely follistatin and follistatin-like 3 (FSTL3). While the endometrial expression of activin A increases during the secretory phase of menstrual cycle, the effects of ovarian steroids on these proteins and their mRNAs has not been assessed yet in postmenopausal women or in ovariectomized animals.
We have evaluated the effects of estrogen alone or estrogen plus progestin on the endometrial expression of activin beta-A subunit, follistatin and FSTL3 in ovariectomized rats.
Adult female Wistar rats (n=21) were ovariectomized and received one week later a single dose of estradiol benzoate (1.5 mg/kg body weight, i.m. injection), either alone (n=7) or associated with depot medroxyprogesterone acetate (2.4 mg/kg body weight, i.m. injection, n=7), or oil vehicle (control group, n=7). One week after the hormone or placebo treatment, the animals were sacrificed and their uteri were removed and processed by immunohistochemistry and real-time PCR. Data were normalized to the expression of ribosomal phosphoprotein P0 (RPP0) and analyzed with the delta-delta Ct method, ANOVA and Newman-Keuls test.
Activin beta-A subunit mRNA levels increased significantly in the uteri of rats treated with estradiol alone (7.4 fold increase over controls, $p<0.05$) and to the same extent in rats receiving estradiol plus medroxyprogesterone (6.1 fold increase over controls, $p<0.05$). This was accompanied by increase of beta-A subunit immunostaining in estradiol and estroprogestin-treated rats, which was noted only in the surface endometrial epithelium. Follistatin mRNA expression, conversely, showed a significant decrease in the groups treated with estrogen alone (0.5 fold compared to controls, $p<0.05$) and estrogen plus progestin (0.4 fold compared to controls, $p<0.05$), while follistatin immunostaining in the glandular epithelium was weaker in estradiol and estroprotestin-treated rats compared to controls. FSTL3 expression was similar in the 3 groups.
In conclusion, the expression of activin beta-A subunit increases and that of follistatin decreases following estrogen replacement in the endometrium of ovariectomized rats, and these effects are not further altered by the addition of progestin.

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Endometrial NK Cells Are a Unique Inert NK Subset until Pregnancy. Simcha Yagel,[1] Irit Manaster,[2] Jacob Hanna,[2] Ronit Haimov-Kochman,[1] Miri Godin,[2] Yuval Bdolach,[1] Caryn Greenfield,[1] Shira Natanson-Yaron,[1] Arye Hurwitz,[1] Debra S Goldman-Wohl,[1] Ofer Mandelboim.[2] *[1]Obstetrics and Gynecology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [2]Lautenberg Center for Tumor Immunology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.*
Introduction: We recently demonstrated that NK (natural killer) cells play a critical role in trophoblast migration and angiogenesis at the fetal maternal interface. NK cells populate the endometrium at the secretory phase of the menstrual cycle, the time of anticipated blastocyst implantation. Peripheral blood (pb) NK cells and decidual NK (dNK) cells express a variety of activating receptors, including NKp44, NKp30 and NKp46, collectively known as natural cytotoxicity receptors (NCRs), and NKG2D which regulate NK cell killing and growth factor production.
Aim: To compare endometrial NK cell (eNK) activating receptor expression and function to pbNK and dNK cells and endometrial ligand expression, with a focus on their roles in blastocyst implantation.
Patients and Methods: Subjects were IVF patients undergoing natural menstrual cycles. Endometrium samples were collected on treatment days 10 and 21. A lymphocyte profile of the endometrial cells and PB was performed. FACS analysis was performed on isolated endometrial NK cells, pbNK cells and dNK cells for CD56, CD16, NKp44, NKp30, NKp46 and NKG2D. NCR ligand expression was characterized on adherent endometrial cells using NCR-Ig fusion proteins and NKGD2-Ig and NKG2D specific ligands as well as control CCMI-Ig. Redirected killing assays and cytokine secretion assays of IFNγ, VEGF, PLGF, and IL-8 with and without IL-15 were performed.
Results: Endometrial lymphocytes of day 10 and 21 in these women are mostly CD56 bright CD16- NK cells, with a significant amount of T cells, similar to pbNK cells and in marked contrast to dNK cells. Unlike pbNK and dNK cells, eNK receptors do not express NKp30, NKp44. NKp46 and NKG2D are the only activating eNK receptor expressed. Like decidual cells, adherent stromal endometrial cells expressed the ligands for NKp30, NKp44 and NKG2D, suggesting that these NK cells have potential for activation. Finally eNK cells could not kill or secrete cytokines.
Conclusions: These findings of a unique activating receptor profile on endometrial NK cells, unlike that of dNK and pbNK, suggest that eNK cells are a special local population of NK cells that change dramatically and are activated at the onset of pregnancy.

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Variation in Platelet Activation throughout the Menstrual Cycle. Fiona C Denison,[1] Amy O Robb,[1] Imogen B Smith,[1] Nicholas L Mills,[2] Hilary OD Critchley,[1] David E Newby.[2] *[1]Centre for Reproductive Biology; [2]Centre for Cardiovascular Sciences, The University of Edinburgh, United Kingdom.*
BACKGROUND: Platelet-monocyte aggregation (PMA) is a sensitive and novel measure of platelet activation with important proinflammatory consequences including release of cytokines and chemokines. Previous studies using less sensitive techniques suggest that platelet activation alters during the menstrual cycle in response to circulating concentrations of sex steroids. The effect of sex steroids on circulating (c) PMAs during a single menstrual cycle is not known. OBJECTIVE: To determine whether cPMAs, platelet surface (ps) P-selectin and plasma (p) P-selectin vary through the menstrual cycle in response to changes in circulating sex steroid concentrations. METHODS: 10 healthy, nulliparous, pre-menopausal, non-smoking women (mean age 31 years), with regular menses (27-29 days) were studied. Subjects gave written informed consent and the study had ethical approval. Serial venous blood samples were taken at menstrual, follicular, periovulatory and luteal phases of a single cycle (days 1-3, 6-9, 13-15 and 20-22). cPMAs (monocytes positive for the platelet marker CD42a) were measured by flow-cytometry. psP-selectin expression was calculated on CD42a positive cells. Isotype-matched controls were used. Serum oestradiol (E) and progesterone (P), plasma and pP-selectin were measured by ELISAs. Data were analysed by one-way ANOVA with repeated measures and Bonferroni's post-tests for multiple comparisons.
RESULTS: Luteal phase P was >30 nmol/L in all women. Numbers of cPMAs and expression of psP-selectin were both significantly higher during menstrual compared with periovulatory phase of the menstrual cycle (25.59±2.15 vs. 16.65±2.88%, $p=0.01$ and 3.95±0.77 vs 2.10±0.37%, $p<0.05$, respectively). There was no significant difference in pP-selectin concentration during the menstrual cycle ($p=0.8$). There was no correlation between levels of serum E or P and numbers of cPMAs, expression of psP-selectin or pP-selectin concentration. CONCLUSIONS: Numbers of cPMAs and expression of psP-selectin are maximal at menstruation with neither numbers of cPMAs nor expression of psP-selectin correlating with serum E or P levels. This study suggests that activated platelets may potentially contribute to the inflammatory response at menstruation by releasing inflammatory mediators.

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Decidual Vascularization and the Occurrence of Miscarriages. Margreet Plaisier,[1] Pieter Koolwijk,[2] Victor WM van Hinsbergh,[2] Frans M Helmerhorst.[1] *[1]Department of Gynecology and Reproductive Medicine, Leiden University Medical Center, Leiden, Netherlands; [2]Department of Physiology, Institute for Cardiovascular Disease, VU University Medical Center, Amsterdam, Netherlands.*
Objective: Decidual vascular adaptation to pregnancy is important for the success of implantation and may be involved in the pathogenesis of miscarriages. Present study analyzed decidual vascularization and its regulation

by angiogenic factors and peri-cellular proteases in decidual secretory endometrium (DSE), decidua parietalis (DP), and basalis (DB) of miscarriage patients and matched controls. Comparison of these parameters between the two groups enabled hypothesizing about their correlation with the occurrence of miscarriages.
Methods: Decidua was obtained during 1st trimester termination of pregnancy (control group) and vacuum aspiration of missed abortions (case group). Vascularization was studied by CD34-immunohistochemistry. The expression of vascular endothelial growth factor-A, placental growth factor, flt-1, KDR, Angiopoietin-1, Angiopoietin-2, TIE-2 and the membrane-type matrix metalloproteinases MT1-, MT2-, MT3- and MT5-MMP were determined at mRNA and antigen level and CD56-positive uNK cells, CD68-positive macrophages, proliferation (Ki67) and apoptosis (activated caspase-3) were evaluated by immunohistochemistry in consecutive serial sections.
Results: The decidual vascularization pattern showed differences between cases and controls: i.e. fewer vessels with larger circumference in cases, and this correlated with the differential expression of various angiogenic factors and proteases at mRNA and antigen level. Moreover, the endothelial protein expression of flt1, KDR, MT2- and MT5-MMP was increased at the implantation site of cases. Ki67 and active caspase-3 showed similar levels in the two groups and also the immune cells, both uNK cells and macrophages, showed no differences at the implantation site between both groups.
Conclusion: Differences between cases and controls appeared not to be based on altered proliferation, apoptosis, and/or inflammation. The differences in vascularization pattern and in the expression of angiogenic factors and proteases between both study groups suggest a correlation between decidual vascularization and the occurrence of miscarriages.

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Human Decidual Natural Killer Cells Express the Receptors for and Respond to Adrenomedullin. Yaun-Lin Dong,[1] Hong Y Wen, Janice Endsley,[2] Alison Hogg,[2] Hui-Qun Wang,[3] Manubai Nagamani,[1] Chandra Yallampalli.[1] *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA; [2]Microbiology & Immunology, University of Texas Medical Branch, Galveston, TX, USA; [3]Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX, USA.*
Background: Natural Killer (NK) cells are the predominant lymphocytes present in human implantation site. Decidual NK cells express perforin, an essential molecule required for lysis. Formation of the placenta involves cooperation between maternal NK cells and fetal trophoblast cells that remodels the blood supply; however, the interaction between trophoblasts and decidual NK cells is largely unknown. Adrenomedullin (ADM) has been implicated in regulating early placental function and fetal growth.
Objective: To determine the role of multifunctional peptide ADM in the decidual NK cells and fetal trophoblast cells interactions.
Methods: Decidual and placental tissues were obtained from normal first-trimester pregnancies terminated for social reasons. Ethical approval to use these tissues was obtained from the IRB of University of Texas Medical Branch. Cell preparations containing all decidual mononuclear cells were isolated by collagenase enzymatic disaggregation. CD56 decidual NK cells were purified by magnetic bead isolation.
Results: 1) Immunohistochemical analysis showed that ADM is expressed primarily in decidual cells and trophoblast cells at the human implantation site; 2) Confocal imaging analysis demonstrated that decidual NK cells, which were identified by anti-CD56 staining, express ADM receptor components CRLR/$RAMP_2$/$RAMP_3$ and their mRNA expressions were futher confirmed by RT-PCR; 3) K562 target cell killing assay indicates that ADM inhibits cytokine IL-12/IL-15-induced decidual NK cell cytotoxicity; and 4) Immunofluorescent labeling and flow cytometric analysis revealed that ADM suppresses perforin expression by decidual NK cells.
Conclusion: Trophoblast-derived ADM inhibits decidual NK cell cytotoxicity via suppressing perforin expression, thus, our results provide evidence for a new paradigm of embryonic-maternal communication involving a ADM mediated interaction between decidual NK cells and fetal trophoblasts.

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HLA-G Does Not Alter Uterine Natural Killer Cell Secretion of Cytokines. Leandro G Oliveira, Gendie E Lash, Judith N Bulmer, Barbara A Innes, Roger F Searle, Stephen C Robson. *Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom.*
Background: We have previously demonstrated that co-culture with extravillous trophoblast cells (EVT) (expressing HLA-G) alters cytokine secretion by uterine natural killer (uNK) cells, particularly at 12-14 weeks gestation. We have also reported that uNK cells can stimulate EVT invasion, but only at 12-14 weeks gestation (not at 8-10 weeks gestation). In addition, uNK cell cytokine profiles alter with increasing gestational age. Other reports have suggested that EVT or HLA-G expressing cells may alter the expression of cytokines and angiogenic growth factors by uNK cells.
Hypothesis: HLA-G expressing cells alters uNK secretion of cytokines.
Methods: $CD56^+$ uNK cells were isolated from early pregnancy decidua (8-10 and 12-14 weeks gestation, n=10 each group) using enzyme digestion and positive immunomagnetic bead separation. The human B lymphoblastoid 721.221 transfected with either HLA-G1 (221G) or a mock cDNA (221cDNA) were obtained as a kind gift from Mr R Apps (University of Cambridge, UK). Isolated uNK cells were cultured in the presence or absence of the two cell lines in either direct or indirect contact (n=5 each group and each gestational age) for 24 hours. Cell supernatants were analysed for cytokines using a FASTQuant® TH1/TH2 multiplex protein assay (IL-6, IL-1β, IL-10, IL-2, IL-5, TNF-α, IL-13, Il-4, IFN-γ) or by standard ELISA (TGF-β1). The effect of direct co-culture of uNK cells with 221G compared with co-culture with 221cDNA at each gestational age was tested using Mann Whitney U test. The effect of co-culture of uNK cells with 221G in both direct and indirect contact was also tested using Mann Whitney U test.
Results: There was no difference in the level of cytokines secreted by the 221G or 221cDNA cells. Cytokine secretion by uNK cells was not altered after direct co-culture with either 221G or 221cDNA cells at either gestational age. In addition, direct or indirect co-culture of 221G or 221cDNA with uNK did not alter cytokine secretion at either gestional age.
Conclusions: HLA-G does not alter the secretion of cytokines by uNK cells from either 8-10 or 12-14 weeks gestation. Other EVT or decidua derived factors (including HLA-E) may be responsible for the alteration in secretion of cytokines by uNKs with increasing gestational age.

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Role of Natural Killer Lymphocyte NKG2D Receptor Pathway in Adhesion Development. Zeynep Alpay,[1] Sureyya Savasan,[2] Steven Buck,[2] Jiang Zhongliang,[1] Yaddanapudi Ravindranath,[2] Michael P Diamond,[1] Ghassan M Saed.[1] *[1]Obstetrics and Gynecology; Reproductive Endocrinology and Infertility, Wayne State University, Detroit, MI, USA; [2]Pediatric Hematology/Oncology, Children's Hospital of Michigan; Wayne State University, Detroit, MI, USA.*
Introduction: Natural killer (NK) lymphocytes are central to innate immunity and contribute to tissue homeostasis by eliminating altered cells. Their NKG2D receptor pathway plays a fundamental role in target elimination through binding NKG2D ligands on the cell surface. Reduction in the NKG2D ligand, ULBP2, expression is associated with immune resistance in neoplastic processes. We have previously shown that fibroblasts from adhesion tissue (AT) are characterized by increased extracellular matrix molecules and inflammatory cytokines compared with normal peritoneal (NP) fibroblasts. **Objective:** To determine if there is a difference in NK lymphocyte-mediated elimination between NP and AT fibroblasts and to investigate potential role of NKG2D pathway in this process. **Material and Methods:** Expression of NKG2D ligands; ULBP2, ULBP3, MICA, and MICB was evaluated by flow cytometry and western blot in primary cell cultures of fibroblasts from NP and AT, established from two patients. Peripheral blood NK lymphocytes (CD56+CD3-) from three healthy volunteers were isolated using MACS system with purity greater than 90% and kept in interleukin 15 overnight. Fibroblast elimination with and without ULBP2 blocking was investigated following 3-hour co-incubation with allogeneic NK lymphocytes using our established flow cytometric cell mediated cytotoxicity assay. Paired *t* test was used in statistical analysis. **Results:** The flow cytometry studies showed that NKG2D ligands (ULBP2, ULBP3, MICA and MICB) were lower in AT compared to NP fibroblasts, reaching a statistical significance in ULBP2 expression (P = 0.039). Western blot analysis also revealed a lower ULBP2 protein level in AT than NP fibroblasts. Furthermore, NK lymphocyte-mediated elimination was 20% lower in AT in comparison with NP fibroblasts. Blocking ULBP2 expression resulted in decreased NK lymphocyte-mediated NP fibroblast elimination by 27%, supporting the role of NKG2D receptor pathway in the process. **Conclusions:** Our results demonstrate that NKG2D pathway is operational in AT fibroblast resistance to immune elimination, and extends our prior observations of the potential role of immunological mechanisms in the pathogenesis of adhesion development.

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Evidence that Galectin-1 Has Undergone Intense Purifying Selection in Placental Mammals: A Potential Link between Placentation and Immune Response. Nandor G Than,[1] Roberto Romero,[1] Offer Erez,[1] Amy Weckle,[1] John Hotra,[1] Asad Abbas,[1] Chong Jai Kim,[2] Juan Pedro Kusanovic,[1] Francesca Gotsch,[1] Joaquin Santolaya-Forgas,[3] Kurt Benirschke,[4] Zoltan Papp,[5] Lawrence I Grossman,[6] Morris Goodman,[6] Derek E Wildman.[6] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Department of Pathology, Wayne State University, Detroit, MI, USA; [3]Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [4]Department of Pathology, University of California, San Diego, CA, USA; [5]First Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary; [6]Department of Molecular Medicine & Genetics, Wayne State University, Detroit, MI, USA.*

Objective: Galectin-1 is an anti-inflammatory lectin that has pleiotropic regulatory functions at the crossroad of innate and adaptive immunity. Human galectin-1 is expressed in the placenta and immune privileged sites and it has been implicated in establishing immune tolerance. The aim of this study was to examine the evolution and placental expression of the *LGALS1* gene in primates.

Methods: Seven primate nucleotide sequences were generated, aligned to 27 vertebrate orthologs from all classes and subjected to phylogenetic analysis. Deduced amino acid sequences were analyzed for functionally important substitutions. Placental galectin-1 expression was studied by immunohistochemistry and Western blot.

Results: 1) The *LGALS1* gene had high sequence identity among all investigated species. 2) Phylogenetic analysis revealed that intense purifying selection had been acting on the *LGALS1* gene in placental mammals (dN/dS=0.11); 3) Residues responsible for sugar binding or molecule stabilizing were highly conserved in primates. 4) Immunostaining showed a uniformly abundant and ubiquitous galectin-1 expression pattern in human, Old and New World Monkey and prosimian placentas, regardless the type of placentation.

Conclusion: The *LGALS1* gene has conserved sequence and placental expression pattern in primates that may suggest its important function in maternal-fetal immune interactions. These results support the view that immune interactions at the maternal-fetal interface evolved in concert with invasive placentation and that these interactions have been maintained regardless of the degree of placental invasion in primates and other mammals.

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Expression of Interleukin-23 in Human Endometrium throughout the Menstrual Cycle and Early Pregnancy. Yesim H Uz,[1,2] William Murk,[1] Umit A Kayisli,[1] Aydin Arici.[1] *[1]Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA; [2]Department of Histology and Embryology, Trakya University School of Medicine, Edirne, Turkey.*

Background: Interleukin-23 (IL-23) is a recently discovered heterodimeric cytokine, comprised by a novel p19 subunit and a p40 subunit shared by IL-12. It has biological activities that are similar to but distinct from IL-12, and is known to be involved in Th1/Th2 cell class switching and the regulation of cytokines such as IFN-gamma, IL-12, TNF-alpha, and IL-17. Early pregnancy is associated with alterations in the maternal immune response, such as changes in cytokine expression, and leukocyte recruitment and subtype switching. We hypothesized that expression of IL-23 in the human endometrium is menstrual cycle- and pregnancy-dependent.

Materials and Methods: Endometrial samples from women (n=44) undergoing surgery for benign gynecologic conditions, and decidual tissues from women (n=8) with clinically normal pregnancies terminated voluntarily in the first trimester, were obtained after receiving informed consent. Endometrial samples were grouped according to menstrual phase. Paraffin sections were stained with IL-23p19 antibodies and evaluated semi-quantitatively with HSCORE. Statistical analysis of the data was done using ANOVA, with $p<0.05$ considered significant.

Results: IL-23 immunoreactivity was predominantly located in the cytoplasm of both endometrial stromal (ESC) and glandular (EGC) cells. ESCs showed mild IL-23 immunoreactivity without significant changes in intensity throughout the menstrual cycle. On the other hand, first trimester decidual cells showed significantly stronger IL-23 staining compared to ESCs from non-pregnant endometrium ($p<0.001$). IL-23 immunoreactivity in EGCs was high in the late proliferative phase, as compared to other cycle phases and first trimester tissues ($p<0.001$). Moreover, EGCs from the early secretory phase ($p<0.05$) and first trimester tissues ($p<0.01$) showed higher IL-23 immunoreactivity compared to the early proliferative and late secretory phases.

Conclusions: This is the first study describing IL-23 expression in the human endometrium and decidua. These results suggest that IL-23 has a cycle-dependent expression in endometrial cells and may be involved in regulating cytokine expression and immune cell modulation during the menstrual cycle and early pregnancy.

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Decreased IL-2 Production, Signaling, and Gene Expression from T Cells in a Low Estrogen Environment. Lowell T Ku, Steven T Nakajima, Cicek Gercel-Taylor, Douglas D Taylor. *Obstetrics, Gynecology, and Women's Health, University of Louisville, Louisville, KY, USA.*

Objective: Estrogen appears appear to be a critical regulator of the immune system. Since hypoestrogenism is present in the postmenopausal woman, our objective was to determine whether T cell activation and function, defined as IL-2 production and signaling molecule expressions at the transcriptional and translational levels, were affected by a low estrogen environment.

Design: Prospective study in a University Research Laboratory.

Materials and Methods: Jurkat 6.1 T cells, initially grown in estrogen free media, were incubated in 4pM (representing postmenopausal levels) or 40pM (premenopausal levels) of estradiol (E_2) for 48 hours. Cells were either resting or activated with a phorbol ester, 4 β-phorbol 12 β-myristate 13 α-acetate (PMA), and ionomycin. Enzyme-linked immunosorbent spot assay (ELISPOT) was performed to analyze production of IL-2. Expression of signaling protein components, CD3ζ and JAK, were determined by Western immunoblotting. Real time-polymerase chain reaction was performed to quantify CD3ζ, JAK2, and JAK3 gene expression. A p value of <0.05 was considered significant.

Results: Jurkat cells exposed to 4pM E_2 and activated exhibited significantly diminished numbers of IL-2 producing colonies compared to T cells exposed to 40pM (55.7±1.2 vs. 75.3±0.7 colonies, $p<0.0001$). Analysis of cellular CD3ζ and JAK2 protein demonstrated that Jurkat cells incubated in 4pM E_2 expressed a 1.48-fold decrease in CD3ζ and 1.64-fold decrease in JAK compared with cells incubated in 40pM E_2 ($p<0.001$). These diminished protein levels appeared to be the consequence of suppressed transcription, as the mRNA levels of CD3ζ, JAK2 and JAK3 were significantly decreased in Jurkat cells incubated in low levels of estrogen (11.3, 213.3, and 13.3 fold, respectively, compared to 40pM).

Conclusions: Jurkat cells exposed to low postmenopausal estrogen levels produce significantly less IL-2 following activation, which was associated with a significant decrease in signaling proteins. The diminished levels of signaling proteins appear to result from decreased CD3ζ, JAK2 and JAK3 gene expression in the presence of low estrogen. These findings support the observation of decreased cellular immune response in postmenopausal women and may provide a basis for the increased risk of infections and cancer proliferation associated with aging.

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The Expression Pattern of 2 Novel Cytokines (IL-24 and 29) in Human Fetal Membranes. Judith Eckardt,[1,2] Stephen J Fortunato,[1] Holger Maul,[2] Ramkumar Menon.[1] *[1]The Perinatal Research Center, Nashville, TN, USA; [2]Womens' Hospital, University of Heidelberg, Heidelberg, Baden-Wuerttemberg, Germany.*

OBJECTIVE: Interleukin (IL) 24 and 29 are novel cytokines produced by various immunological cells in response to microbial antigens. The functions of these cytokines in reproductive system is unknown. This study examines the expression pattern of IL-24 and IL-29 in human fetal membranes from preterm and term births and in in vitro in normal term membranes in response to bacterial endotoxin (lipopolysaccharide-LPS).

METHODS: Fetal membranes collected (n=12) from cesareans at term (normal, not in labor) were placed in an organ explant system for 48 hours and were stimulated with LPS for an additional 24 hrs. Fetal membranes were also collected (n=10) either at preterm or term after vaginal deliveries. In a case -control study (preterm birth vs. normal term deliveries) amniotic fluids (AF) (n=200) were collected to document the role of IL-24 and IL-29 in PTB. Tissue expressions of IL-24 and IL-29 were studied by RT-PCR using specific primers. ELISA documented culture media and AF cytokine concentrations. Statistical analysis was performed using non-parametric Mann-Whitney U test.

RESULTS: Both IL-24 and IL -29 expressions were seen in fetal membranes in culture (in vitro) regardless of stimulation. In vivo in membranes from preterm and term deliveries and membranes at term not in labor also documented the expression of these cytokines. Culture media analysis documented higher concentration of IL-24 after LPS stimulation (LPS–65.5 vs. 7.7 pg/ml; p=0.006)

whereas no difference was noticed in IL-29 concentration (LPS-22.8 control-19.5 pg/ml; p=0.5) between the two groups. AF analysis, regardless of the status, did not document detectable concentrations of either of the cytokines (lower limit 7.65 pg/ml for both).
CONCLUSION: This is the first study to document the expression of two novel cytokines in laboring and non laboring human fetal membranes and also in membranes from preterm deliveries. IL-24 production was stimulated by LPS whereas IL-29 was not affected. These cytokines are not physiological components of AF and their role in fetal membranes is unclear. Higher IL-24 concentration in response to LPS but lack of its presence in term or preterm AF is suggestive of an autocrine immune response during pregnancy in response to a microbial antigen.

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Evidence for a Selective Migration of Fetus Specific $CD4^+CD25^{bright}$ Regulatory T Cells from the Peripheral Blood to the Decidua in Human Pregnancy. Tamara Tilburg,[1,2] Dave L Roelen,[2] Barbara J van der Mast,[1] Godelieve M de Groot,[1] Carin Kleijburg,[1] Sicco A Scherjon,[1] Frans HJ Claas.[2] *[1]Department of Obstetrics, Leiden University Medical Centre, Leiden, Netherlands; [2]Department of Immunohematologie and Bloodbank, Leiden University Medical Centre, Leiden, Netherlands.*
During pregnancy the maternal immune system has to tolerate the persistence of fetal alloantigens. Many mechanisms contribute to the prevention of a destructive immune response mediated by maternal alloreactive lymphocytes directed against the allogeneic fetus. Murine studies suggest that $CD4^+CD25^+$ T cells provide mechanisms of specific immune tolerance to fetal alloantigens during pregnancy.
Previous studies by our group demonstrate that a significantly higher percentage of activated T cells and $CD4^+CD25^{bright}$ T cells are present in decidual tissue in comparison with maternal peripheral blood in human pregnancy [(1)]. In this study we examined the phenotypic and functional properties of $CD4^+CD25^{bright}$ T cells derived from maternal peripheral blood and decidual tissue. Depletion of $CD4^+CD25^{bright}$ T cells from maternal peripheral blood demonstrates regulation to a 3rd party umbilical cord blood cells comparable to non-pregnant controls, whereas the suppression capacity to umbilical cord blood cells of her own child is absent. Furthermore, maternal peripheral blood shows a reduced percentage of $CD4^+CD25^{bright}FOXP3^+$ and $CD4^+CD25^{bright}HLA\text{-}DR^+$ cells compared to peripheral blood of non-pregnant controls. In contrast, decidual lymphocyte isolates contain high percentages of $CD4^+CD25^{bright}$ T cells with a regulatory phenotype that are able to down regulate fetus-specific and non-specific immune responses.
These data suggest a preferential recruitment of fetus-specific regulatory T cells from maternal peripheral blood to the fetal-maternal interface where they may contribute to the local regulation of fetus specific responses.
[(1)] Tilburgs T, Roelen DL, van der Mast BJ, van Schip JJ, Kleijburg C, de Groot-Swings GM, Kanhai HH, Claas FH, Scherjon SA. Differential distribution of $CD4^{(+)}CD25^{(bright)}$ and $CD8^{(+)}CD28^{(-)}$ T-cells in decidua and maternal blood during human pregnancy. Placenta. 2006 Apr;27 Suppl A:S47-53.

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Correlation between Progesterone, Prolactin and Cortisol with CD94/NKG2 and NKD2D Receptors in Peripheral Blood during the Menstrual Cycle. Erika A Martinez-Garcia,[1] Monica Vazquez-del Mercado,[1] Pedro E Sanchez-Hernandez,[2] Jose F Munoz-Valle,[1] Beatriz T Martin-Marquez,[1] Victor E Arana-Argaez,[1] Trinidad Garcia-Iglesias,[2] Luz MA Balderas-Pena,[3] Alicia Del Toro-Arreola,[2] Lourdes Nunez-Atahualpa,[4] Juan Velazquez-Rodriguez,[5] Laura Gonzalez-Lopez,[6] Jorge I Gamez-Nava,[3] Adrian Daneri-Navarro.[2] *[1]Instituto de Investigacion en Reumatologia y del Sistema Musculo Esqueletico, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; [2]Laboratorio de Inmunologia, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; [3]Unidad de Investigacion Medica en Epidemiologia Clinica, Centro Medico de Occidente, IMSS, Guadalajara, Jalisco, Mexico; [4]Facultad de Medicina, Universidad Autonoma de Guadalajara, Guadalajara, Jalisco, Mexico; [5]Unidad de Investigacion Medica de Zacatecas, IMSS, Zacatecas, Mexico; [6]Hospital Regional General 110, IMSS, Guadalajara, Jalisco, Mexico.*
There is evidence that the maternal immune system is influence by changes in the hormonal levels during the menstrual cycle (MC). So far, the information related to the levels of T, TReg, NK cells and receptors of activation and inhibitors is scarce.
Aim: To analyze the populations of T, TReg, NK cells and their receptors of peripheral blood of healthy women and their correlation with hormones during MC.
Material and Methods: We studied to 10 women not using hormonal contraceptives in the day 5th of the follicular phase and 21st of the luteal phase of the MC. PBMC subsets and their receptors were determined by flow cytometry and hormone levels by chemiluminescence method.
Results
We found that the progesterone and prolactin were positive correlated (rho= 0.802, $p<0.05$ and rho= 0.757, $p<0.05$, respectively) with CD94/NKG2 in T cells and negative correlated (rho= -1.00, $p<0.01$ and rho= - 0.857, $p<0.05$, respectively) with NK cells. Meanwhile cortisol was positive correlated (rho= 0.857, $p<0.05$) with the receptor NKG2D expressed in NK cells.
Conclusion
The results observed in this study in the luteal phase of MC on the expression of CD94/NKG2 inhibitor receptor and NKG2D activator receptor were related to a particular hormone (progesterone, prolactin and cortisol) might contribute to understanding the physiological role of the neuroendocrine axis on the expression of some receptors of the immune system in order to keep the homeostasis milieu of the MC.

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Novel Observations on Surfactant Protein-D (SP-D) in Human Pregnancy: Human Neutrophils Are a Source of SP-D. Sun Kwon Kim, Roberto Romero, Sam Edwin, Jung-Sun Kim, Yeon Mee Kim, Juan Pedro Kusanovic, Francesca Gotsch, Offer Erez, Sonia Hassan, Chong Jai Kim. *Perinatology Research Branch, NICHD, NIH, DHHS, Detroit, MI, USA.*
Objective: SP-D, a key component of the innate immune system, is detected in amniotic fluid (AF) and believed to originate in the fetal lung. However, SP-D is produced by other cells and therefore extra-pulmonary sources must be considered. The objective of this study was to determine the maternal and fetal plasma and AF concentrations of SP-D to gain insight into the behavior of this natural antimicrobial peptide in pregnancy. Moreover, we studied SP-D expression in maternal and fetal peripheral leukocytes.
Methods: Maternal and fetal plasma and AF samples were obtained from patients in the following groups: 1) term not in labor (TNL; n=20); 2) term in labor (TIL; n=31); 3) preterm labor without histologic chorioamnionitis (PTL; n=30) and 4) preterm labor with histologic chorioamnionitis (PTL-HC; n=27). SP-D concentration was measured by ELISA. SP-D mRNA expression in maternal and fetal leukocytes was evaluated by real-time qRT-PCR. Flow cytometry and confocal microscopy were used to study the localization of SP-D in leukocytes.
Results: 1) The AF SP-D concentration increased as a function of gestational age (mean, TIL: 52,055.7 ng/mL vs. PTL: 31,371.0 ng/mL; $p<0.05$); 2) In contrast, the maternal and fetal plasma SP-D concentrations decreased with advancing gestational age (mean, TIL: 375.8 ng/mL vs. PTL: 672.9 ng/mL; $p<0.05$, and TIL: 366.9 ng/mL vs. PTL: 1606.8 ng/mL; $p<0.001$, respectively); 3) The maternal plasma SP-D concentration was lower than that of fetal plasma (mean: 506.1 ng/mL vs. 1054.4 ng/mL; $p<0.001$); 4) However, SP-D mRNA expression in maternal leukocytes was 4.8 fold higher than that of fetal leukocytes ($p<0.001$); 5) Neutrophils (both maternal and fetal) expressed SP-D as demonstrated by flow cytometry and confocal microscopy.
Conclusion: 1) The concentrations of SP-D in the maternal and fetal circulation decreased with gestational age while the AF concentration increased; 2) The expression of SP-D mRNA is higher in maternal leukocytes than in fetal leukocytes; 3) We report for the first time that maternal and fetal neutrophils are a source of SP-D and propose that this molecule plays a role in host defense against infection and in the modulation of the maternal and fetal immune response.

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Impact of Intercourse on Pregnancy Rate after Intrauterine Insemination. Ingrid Cardenas, Alejandro Manzur, Rodrigo Macaya, Guillermo Durruty, Claudia Almendra, Gloria Gajardo. *Obstetrics and Gynecology, Pontificia Universidad Catolica de Chile, Santiago de Chile, Metropolitan Region, Chile.*
Introduction
Intrauterine insemination (IUI) is a fertility technique that allows for couples to have intercourse after the procedure is performed. It has been postulated that intercourse after IUI may increase the pregnancy rate by either endometrial

stimulation or because it may represent a second spermatic flow in the peri-ovulatory period. In the present study we evaluate the effect of intercourse on the pregnancy rate of patients undergoing IUI.

Material and methods:

From 2004 to 2006 675 patients were enrolled in the study. Every couple undergoing IUI was instructed at the moment of insemination to decide whether to have or not intercourse on the same day of the procedure. All couples were abstinent three to seven days before IUI. The information regarding intercourse was recorded the day after treatment. Ovulatory, insulin resistant, cervical, male, tubal and endometrial factors as well as parity and time of infertility were compared between the two groups. All these factors were analyzed based on number of follicles that ovulated in each group. Our principal outcome was to determine the pregnancy rate.

Results.

Intercourse was practiced by 65.8% of the couples. The global pregnancy rate was 15.4%. The pregnancy rate for the couples who had intercourse was 13.3% and 19.5% for those who did not have intercourse ($p<0.05$). Even though age, parity, time of infertility and stimulation protocols were similarly distributed in both groups, the proportion of tubal and endometrial factors were higher among those who had intercourse ($p<0.01$). When subjects with tubal and endometrial factors were excluded, the pregnancy rate between both groups (n=463) was similar (16.1% vs 19.6% for positive and negative intercourse, respectively). The average number of ovulatory follicles was 2.12 ± 1.1 for the group that had intercourse and 2.17 ± 1.3 for those who did not.

Conclusions

According to our results, intercourse after IUI does not improve pregnancy rate after this procedure is performed. Furthermore our study indicates that IUI does not interfere with sexual intimacy since almost 66% of the couples decided to have intercourse on the same day of the procedure.

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Maternal, but Not Fetal, Microchimerism Decreases with Increasing Gravidity in Parous Women. Hilary S Gammill,[1,2] Katherine A Guthrie,[1] Tessa M Robinson,[1] J Lee Nelson.[1,3] *[1]Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [2]Obstetrics and Gynecology, University of Washington, Seattle, WA, USA; [3]Rheumatology, University of Washington, Seattle, WA, USA.*

Objective: Microchimerism (Mc) is the presence of small numbers of foreign cells in an individual. Acquisition of Mc primarily occurs through transplacental cell trafficking during pregnancy and, in adult women, leads to persistent Mc, both from the mother (MMc) and from the fetus (FMc). We sought to investigate the effects of gravidity on MMc and FMc in healthy, parous women.

Methods: Mc was assayed in DNA extracted from peripheral blood mononuclear cells (PBMC). HLA-genotyping was first conducted and Mc quantified employing a Q-PCR assay targeting a non-shared maternal- or fetal-specific HLA polymorphism. Gravidity was dichotomized as a history of one pregnancy compared with two or more, and the prevalence of Mc was analyzed using logistic regression. Possible confounders were included as appropriate, including subject age and time since last pregnancy. Adjustment for possible correlation between values was also made when there were repeated measures for the same subject.

Results: For the MMc analysis, there were 35 subjects with 73 observations. For the FMc analysis, there were 68 subjects with 145 observations. Table 1 provides a summary of MMc and FMc by gravidity. MMc was significantly decreased with higher gravidity. FMc was not affected by gravidity.

Prevalence of Mc according to gravidity

	Gravida 1	Gravida 2 or more	Adjusted* OR (95%CI)
MMc	7/14 (50%)	5/48 (10%)	0.19 (0.04-0.79)
FMc	13/30 (43%)	36/115 (31%)	0.49 (0.21-1.17)

*Adjusted for possible correlation between values within a subject, subject age, and time from last pregnancy, as appropriate.

Conclusion: Increasing gravidity is significantly associated with a decreased prevalence of MMc. Despite additional sources of FMc, there does not appear to be an increase in FMc prevalence with increasing gravidity. The biology of Mc is incompletely understood, and the nature of MMc and FMc are likely to be different given that acquisition of the former, but not the latter, occurs within a nascent immune system. These data raise interesting questions when considered as interactions of acquired grafts within a host, including whether emergence and persistence of one dominant source of Mc may be most advantageous for an individual.

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Anti-IgD Antibody Treatment as a Novel Immunosuppressive Agent for Autoimmune Diseases and Its Effects on Th1/Th17 Gene Expression. Tommie G Nguyen,[1] Eileen D Gallery,[1,2] Jonathan M Morris.[1,2] *[1]Perinatal Research, Kolling Institute of Medical Research, Sydney, NSW, Australia; [2]Northern Clinical School, University of Sydney, Sydney, NSW, Australia.*

Objectives: Elevated T-helper cell type-1 (Th1) and type-17 (Th17) cytokine expression have a role in autoimmune diseases, allograft rejection and pregnancy-related complications. Thus, molecules that can shift the immunity away from Th1/Th17 responses toward a Th2 response represent a novel therapeutic treatment for these conditions. We have previously demonstrated that pregnancy is associated with a suppression of T-bet in peripheral T cells. In this study, we examined a novel effect of anti-IgD antibody on T-bet expression, Th1/Th17 gene expression in human peripheral blood mononuclear cells (PBMC) and its therapeutic effects in an animal model of collagen-induced arthritis. **Methods:** Human PBMC were isolated and then cultured in the presence of anti-IgD antibody at various time points followed by stimulation with PMA/Ionomycin (P/I) for 5 hrs. Gene expression was examined by RT-PCR, Western blotting and ELISA. For *in vivo* study, arthritis-prone DBA/J1 mice were induced to undergo joint inflammation by intradermal injections with bovine type-II collagen. These mice were then given 3 daily doses of 10 mg/kg of intravenous injection with anti-IgD antibodies as preventive or therapeutic treatments (n = 10 per group).

Results: Treatment with anti-IgD antibodies significantly suppressed P/I-induced expression of T-Bet (a master regulator of Th1 immunity), TNF-α (a classical pro-inflammatory Th1 cytokine), and IL-17 (a critical pro-inflammatory Th17 cytokine) in human PBMC. This suppression is highly specific to these genes because anti-IgD antibodies have no effects on the expression of IFN-g and IL-2 (two classical Th1 cytokines). *In vivo* experiment showed that anti-IgD antibody treatment markedly reduced clinical severity of joint inflammation when comparing the clinical score of control mice group (7.8 ± 1.1, mean ± s.e.m), preventive group (5.4 ± 1.2) and therapeutic group (3.5 ± 1.3) . **Conclusions:** Our study has demonstrated that suppression of T-bet by anti-IgD antibodies, similar to the changes seen in human pregnancy is a novel *in vivo* anti-inflammatory effect. Given the essential role of T-Bet, TNF-α and IL-17 in the pathogenesis of human autoimmune diseases, anti-IgD antibodies may represent a novel immunosuppressive treatment that needs further studies and evaluation.

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Differential Effects of Anti-Phospholipid Antibodies on First Trimester Trophoblast Cells. Vikki M Abrahams,[1] Melissa J Costello,[1] Shawna Joyce,[1] Michael J Paidas,[1] Larry W Chamley,[2] Jan J Brosens.[3] *[1]Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA; [2]Department of Obstetrics and Gynecology, University of Auckland, Auckland, New Zealand; [3]Institute of Reproductive and Developmental Biology, Imperial College London, London, United Kingdom.*

Objective: Women with circulating anti-phospholipid antibodies (aPL) are at risk for recurrent miscarriage, preeclampsia and preterm labor. aPL antibodies directly target the placenta by binding to phospholipids or phospholipid-binding proteins expressed on the surface of viable trophoblasts. The objective of this study was to determine the effects of aPL antibodies on first trimester trophoblast cells.

Methods: Two mouse IgG1 anti-human $Beta_2$-glycoprotein I monoclonal antibodies (mAbs), designated ID2 and IIC5, were used in these studies. The first trimester trophoblast cell line, HTR8, was incubated with either medium, a mouse IgG1 control, ID2 or IIC5 (5-40μg/ml), in the presence or absence of unfractionated heparin (100ng/ml). Trophoblast cell death and apoptosis was determined using a viability assay, Hoechst staining and a caspase activity assay. Cytokine production was evaluated by Multiplex analysis.

Results: Following a 48 hour incubation, significant trophoblast cell death was induced by IIC5 ($34.5 \pm 2.8\%$) and ID2 ($46.4 \pm 3.2\%$) at the high dose of 40μg/ml, when compared to the medium and mouse IgG controls ($p<0.001$). Hoechst staining showed that ID2- and IIC5-induced trophoblast cell death was a result of apoptosis. Moreover, ID2 and IIC5 induced a significant increase in caspase-3, -8 and -9 activity ($p<0.001$). Treatment of trophoblasts with heparin significantly inhibited the effects of IIC5 and ID2 on cell death by $46.5 \pm 12.8\%$ and $52.6 \pm 8.6\%$ %, respectively ($p<0.005$). Following a 72 hour incubation at lower concentrations (20μg/ml), treatment of trophoblast cells with ID2 or IIC5 resulted in a significant upregulation of IL-8, MCP-1, GROα production ($p<0.05$), and a significant reduction in IL-6 secretion ($p<0.05$).

Conclusion: This study demonstrates that at low levels aPL antibodies can modulate trophoblast cytokine production, while at higher levels, the same antibodies induce trophoblast apoptosis in a caspase-dependent manner. These findings shed new light on the mechanisms by which aPL antibodies may impact placental survival and function.

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Antigenic Targets for the Diagnosis of Premature Ovarian Failure. HC Bohler, C Gercel-Taylor, LT Ku, ST Nakajima, DD Taylor. *Obstetrics, Gynecology, & Women's Health, University of Louisville, Louisville, KY, USA.*

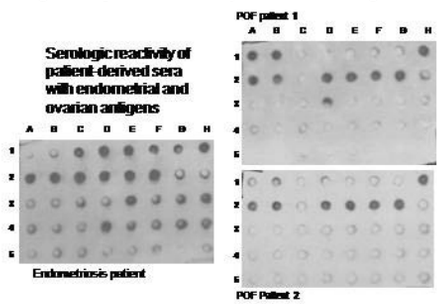
Objective: Premature ovarian failure (POF) is a premature depletion of ovarian follicles before the age of 40, affecting approximately 1% of women <40 years. The involvement of autoimmune mechanisms in POF has been suggested and similar mechanisms have been postulated for other ovarian pathologies, including idiopathic infertility, polycystic ovary syndrome (PCOS), or endometriosis. While the association of autoantibodies has been demonstrated for these ovarian pathologies, variation in specificity and frequency of false positivity have limited the diagnostic use of autoantibodies. The objective of this study was to develop an antigen array to differentiate antibody recognition patterns of POF from other infertility pathologies.

Design: Prospective study in a University Research Laboratory.

Materials and Methods: Patients diagnosed with infertility were included in this pilot study: endometriosis (n=6), PCOS (n=4) and POF (n=5). Autoantibodies were assayed by dot immunoblotting using an antigen array derived from endometrial and ovarian cells. For the cellular antigen preparations, solubilized total proteins were separated by reverse phase-HPLCquid chromatography and the individual proteins were blotted onto nitrocellulose membranes and reactivity visualized by peroxidase-labeled antihuman IgG.

Results: Patients with POF, endometriosis, and PCOS all exhibited autoantibodies reactive with these cellular proteins. While some antigenic reactivities were shared by all infertility patients, the pattern of antigen recognition was distinct for patients with POF. Patients with POF all recognized a common 6 antigenic proteins (row 2, antigens a,b,d-g).

Conclusions: Alterations in autoreactivity are observed in patients with the diagnosis of infertility; however, distinct patterns of autoantibody recognition can be demonstrated for patients with different pathologies. While this study needs to be expanded to reliably establish the specificity, sensitivity and positive and negative predictive values, patients with POF clearly exhibit a shared recognition pattern that may be useful a diagnostic marker.



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Genistein Reverses Immunologic Signal Transduction Suppression from Hypoestrogenism. J Preston Parry, Douglas D Taylor, Steven T Nakajima, Cicek Gercel-Taylor. *OB/GYN, University of Louisville, Louisville, KY, USA.*

Objective: Americans' consumption of nutraceuticals is one of the most rapidly expanding health markets, growing at a rate of 25% annually. Multiple nutraceuticals containing phytoestrogens have been marketed as "immune boosters" despite suboptimal evidence-based medicine to support such statements. As immunomodulatory therapies should affect downstream cytokine expression, the relative effects of estradiol and genistein in regulating expression of CD3-ζ and JAK3 were tested. These markers were chosen since they are central to T cell signaling. CD3-ζ is a critical transducer of TCR activation and regulates T cell proliferation and cytokine production. JAK3 upregulation is a specific marker for hematopoietic cell stimulation.

Methods: To test the immunomodulatory effects of phytoestrogens and estrogen, Jurkat 6.1 (T cell leukemia) cells were grown in estrogen-free, phenol red-free media for 72 hours. These cells were then exposed for 48 hours to 0 pM, 4 pM (postmenopausal), or 40 pM (premenopausual) of estradiol in the presence of increasing concentrations of genistein (0, 0.4, 1.5, 6.25, 25, and 100 μM). Cells were then solubilized and cellular protein quantitated. Protein concentrations were standardized and western blots for each set of culturing conditions were run in triplicate. CD3-ζ and JAK3 expression were quantitated following visualization with chemiluminescence by digital pixel quantification.

Results: Our findings show that in the absence of estradiol and at postmenopausal levels of estradiol, genistein induced a dose dependent increase in CD3-ζ reaching a maximum of 20 fold. Although cultivation of T cells in 40 pM of estradiol significantly increased the levels of CD3-ζ and JAK3 relative to hypoestrogenic conditions, the genistein mediated dose response was not observed.

Conclusions: These in vitro results indicate that genistein can at least partially reverse suppression of signaling molecules observed in postmenopausal estrogen environments. Clinically, this suggests that phytoestrogens may have greater immunomodulatory properties for postmenopausal females than those that are premenopausal.

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Maternal Serum IL-6 as a Biomarker of Acute Immunologic Rejection of Pregnancy. Joaquin Santolaya-Forgas,[1] Juan Deleon-Luis,[2] Isabel Galan.[2] *[1]Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA, USA; [2]Amarillo Women's Health Research Institute, Texas Tech University Health Science Center, Amarillo, TX, USA.*

OBJECTIVE: Markers of acute rejection of pregnancy are very scarce. In this study we aimed at determining if rapid changes in maternal serum concetration of a variety of biomarkers could be used for this purpose.

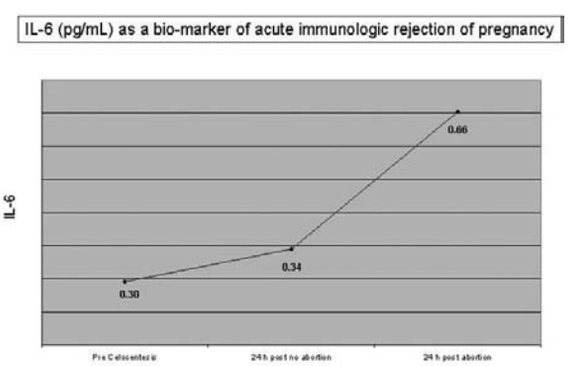
MATERIAL AND METHODS: We used an established baboon model for *in utero* stem cell therapy to introduce at 36-44 days from conception and via ultrasound-guided celocentesis, human Hematopoietic Stem Cells with different proportions of Natural Killer T-cells (NK). Maternal blood was collected before and 24 hours after celocentesis for quantification of hormones and IL-6 using solid phase, enzyme labeled, chemiluminescent sequential immunometric assays. Pearson correlation analysis was used for determination of significant changes from baseline ($p<0.05$).

RESULTS: All 9 animals survived their pregnancies. Seven animals receiving <5% concentration of NK delivered at term (~180 days gestation) while 2 animal receiving more than 7% concentration of NK had dead fetuses on ultrasound evaluation 24 hours after celocentesis. Table 1 depicts mean maternal serum concentration of the biomarkers investigated (all n.s.). Figure 1 shows mean IL-6 changes from baseline in continuing (n.s.) and rejected pregnancies ($p<0.05$).

CONCLUSIONS: We have described a model in which *in utero* graft vs host disease can be studied. These preliminary results suggest that of all the biomarkers investigated, IL-6 might be the most sensitive for detection of an acute rejection of pregnancy.

Biomarkers of acute immunologic rejection of pregnancy

Biomarker	Unit	Pre-Celocentesis (9)	24 hours-post	24 hours-post
			No abortion	Abortion
Free bhCG	ng,mL	0.01	0.02	0.02
Progesterone	ng/mL	6.7	10.8	8.6
Estradiol	pg/mL	77.9	126.4	101.5
Cortisol	ug/dL	16.9	19.1	21.2
IGF-II	ug/mL	1368.4	1556.3	1040.5
IGFBP-3	ug/mL	5.3	5.3	5.5



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A Transitory Increase in CD8+ T Cell Proliferation Augments Their Number during Pregnancy. Michelle Norton,[1] Peyman Bizargity,[1] Karen A Fortner,[2] Elizabeth A Bonney.[1] *[1]OB/GYN, University of Vermont, Burlington, VT, USA; [2]Medicine, University of Vermont, Burlington, VT, USA.*

Background: The activity of cytotoxic CD8+ T cells during pregnancy protects the mother and fetus from infection. However, pregnancy's effect on the proliferation and apoptosis of CD8+ T cells has not been clearly defined.

Objective: To determine if normal pregnancy changes the number of proliferating or apoptotic splenic CD8+T cells.

Methods: Female C57BL/6 mice were used unmated (UM) or underwent timed mating. One day prior to harvest, mice were i.p. injected with bromodeoxyuridine (BrdU), which is incorporated into replicating DNA. Harvested spleens were homogenized, enumerated, and stained for cell surface expression of CD8 and T cell receptor beta chain (TCRβ). Apoptotic cells were detected by treatment with terminal transferase and FITC-dUTP (TUNEL). The numbers of CD8+TCRβ+ cells that were BrdU+ or TUNEL+ were calculated from the percentage of positive cells obtained by flow cytometry and the absolute number of cells counted. For each experiment, the ratio of the number of positive cells in pregnant to UM mice was compared by ANOVA with Dunnett's post-test.

Results: At day 5 of pregnancy (n=5), the number of BrdU positive CD8+ T cells was two fold higher than that found in UM (n=12, $p<0.01$). The number of proliferating CD8+ T cells continued to be non-significantly elevated at day 8 (1.6X, n=5), day 10 (1.4X, n=7), and day 12 of pregnancy (1.5X, n=6) compared to UM. By day 15 of pregnancy (n=5) the number of proliferating CD8+ T cells returned to the UM level, however by this time the total number of splenic CD8+ T cells was 1.5 fold higher than UM (n=12 $p<0.001$). On gestational day 18, the number of proliferating CD8+ T cells declined further (0.3X, n=4), and the number of splenic CD8+T cells returned to the UM level (n=12, $p>0.05$). Compared to UM mice, there was no significant difference in the number of CD8+ T cells undergoing apoptosis at any gestational day examined (0.7-0.9X, $p>0.05$).

Conclusions: In normal murine pregnancy, the number of CD8+ T cells is increased in late gestation, and then returns to baseline at the end of pregnancy. This is due to an early increase then gradual decline in CD8+ T cell proliferation, accompanied by a steady rate of apoptosis. This argues that the maternal immune system undergoes dynamic homeostatic changes, and is not globally suppressed. *Supported by NIHRO1HD043-185& NIHT32AI055402.*

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Effect of Glucocorticoids (GC) on Glucocorticoid Receptor (GR) and Insulin-Like Growth Factor I (IGF-I) in the Fetal Baboon Thymus. Arturo Cerbulo-Vazquez,[1] Cun Li,[1,2] Gene Hubbard,[2] Natalia E Schlabritz-Loutsevitch,[1,2] Peter W Nathanielsz.[1,2] *[1]Obststerics and Gynecology, University of Texas Health Science Center, San Antonio, TX, USA; [2]SNPRC, Southwest Foundation for Biomedical Research, San Antonio, TX, USA.*

Objectives: Early thymocyte (T) maturation occurs in the cortex while later stages occur in the medulla. Thymic epithelial cells (TEC) synthesize GC and T express GR. TEC may influence T cell maturation by regulating apoptosis-induced GC-GR interactions. IGF-1 (also synthesized by TEC) may support thymocyte prolifetarion. Human fetuses of mothers in premature labor are exposed to GC. GC administered to pregnant baboons at 0.6, 0.65, and 0.7 gestation (G) alters fetal lymphocyte populations at 0.95 (G) (J Repro Immunol, 2006, 69:149). We determined if fetal GC exposure alters thymic 1) structure; 2) GR and IGF-I protein.

Methods: Pregnant baboons received saline (control CTR; *n*=6) or betamethasone i.m. (175 µg/kg daily for two days at 0.6, 0.65 and 0.7G; GC group; *n*=6), C-sectioned at at 0.9G under general anesthesia and thymic GR and IGF-I evaluated by immunohistochemistry.

Results: GR localized to medulla and a few cortical cells. IGF-I localized to cortex with little medullary expression. Medullary necrosis was greater in CTR than GC fetuses. T GR was located in cytoplasm. No gross differences were observed between CTR or GC fetuses for either IGF-I or GR.

Conclusions: A) early thymocyte maturation may be supported by IGF-1, b) later differentiation involves GR, and c) after exposure to GC doses equivalent to human therapy, no gross effects were detected on GR or IGF, but d) natural thymic necrosis was inhibited.

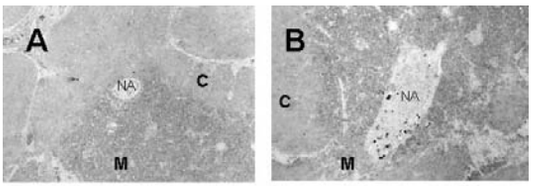

Fig. 1 GR expression in fetal baboon thymus (175 dGA). Thymus showing medulla (M) and cortex (C) areas from exposed to betamethasone (A), and saline control (B). Necrosis Area=NA. Magnification (x10)

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Susceptibility to LPS-Induced Preterm Delivery in T and B Cell Deficient Mice Does Not Correlate with the Early Presence of Uterine Macrophages. Lindsay S Christensen, Peyman Bizargity, Elizabeth A Bonney. *OB/GYN, University of Vermont, Burlington, VT, USA.*

Background: The exact mechanism by which bacterial products trigger preterm delivery and the immune cellular circuits involved remain unclear. Our recent data in normal C57BL/6 (B6) or recombinase deficient C57BL/6 mice (RAG-KO) indicates that T and B cells are not critical for LPS-induced preterm delivery and stresses the importance of related innate mechanisms. RAG-KO mice are more susceptible to LPS, suggesting that T or B cells may control the innate response. Macrophages are vital to innate immunity and produce proinflammatory cytokines that can activate prostaglandin synthesis and myometrial contraction. We questioned whether differences in susceptibility between the strains are due to differences in the uterine macrophage response to LPS and thus examined macrophages at the maternal-fetal interface early after injection.

Objective: To compare uterine macrophages levels at 2 and 6 hours after LPS injection in pregnant B6 and RAG-KO mice.

Methods: B6 and RAG-KO mice were mated and on gestation day 15, females were injected intraperitoneally with 3µg LPS in 200 µl Saline (PBS) or 200 µl PBS alone. Euthanasia and uterine harvest occurred 2 or 6 hours after injection. Frozen uterine sections were stained with the macrophage marker F4/80 or an isotype-matched control followed by an AlexaFluor 546- conjugated secondary and a nuclear stain (DAPI). Sections were visualized by fluorescence microscopy. For each mouse, F4/80+ DAPI+ and total DAPI + cells were counted in 3 areas of 1 representative section and the percentage of F4/80+ DAPI+ cells was calculated. The mean percentage for at least 6 representative areas per experimental group was analyzed by ANOVA.

Results: Two hours post-injection, macrophages levels were similar in B6 and RAG-KO mice injected with PBS (B6, n=3, 2.9±1.6; RAG-KO, n=3, 2.7 ± 1.2 % + per area). LPS injection increased macrophages ($p<0.05$) in both strains (B6, n=3, 4.4±2.9; RAG-KO, n=3, 5.5 ± 1.8,); no difference was evident between strains. The percentage of F4/80+ cells was similar 6 hours post-injection (B6, n=3, 3.4± 1.9 v. RAG-KO, n=3, 3.6± 2.8), and not elevated relative to the 2 hour time point.

Conclusion: Increased LPS-induced preterm delivery in RAG-KO mouse is not explained by early macrophage presence in the uterus. Differences may be due to late macrophage migration or to altered function. *Supported by the March of Dimes.*

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PTEN and PI3K-Akt Pathway Are Involved in the Regulation of Complement Regulatory Protein CD55 in Human Endometrial and Cervical Epithelial Cell Lines. Pawel Goluszko, Sayanti Bhattacharya, Chandra Yallampalli. *Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Objective. Decay-accelerating factor (CD55), is expressed in the plasma membranes and protects mammalian cells against the lytic action of serum complement. Phosphoinositide 3-kinases (PI3Ks) are involved in the regulation of cell functions by synthesizing a second messenger molecule PtdIns (3,4,5) P3. Akt, a serine-threonine kinase acts downstream of PI3K and regulates cell survival, growth and proliferation. The PI3K-Akt activity is controlled by tumor suppressor gene *PTEN*. In this study we assessed whether the PI3K-Akt activity affects the expression of CD55 in human endometrial and cervical cells.

Methods. Endometrial and cervical cell lines which differ in the constitutive PI3K activity were used in this study. Ishikawa and RL95-2 endometrial cell lines harbor PTEN mutation and have high levels of phosphorylated Akt (p-Akt). HEC-1-A and KLE endometrial cell lines and HeLa cells express wild-type PTEN and have minimal or no demonstrable levels of p-Akt. The expression of CD55 was evaluated by RT-PCR, immunoblotting and flow cytometry. The PI3K activity was assessed by immunoblotting with anti-p-Akt

antibodies. The effect of inhibition of PI3K-Akt pathway on CD55 expression was evaluated in cells treated with wortmannin (400 nM), LY294002 (25 mM), or with Akt inhibitor SH5 (5 mM).

Results. Immunoblotting densitometry and measurements of mean fluorescence intensities showed that the level of CD55 expression correlates with the status of PI3K-Akt pathway. The CD55 expression was lowest in HEC-1-A, Ishikawa and RL95-2 cells which constitutively express p-Akt. Higher CD55 expression was found in HeLa cells and KLE cells which express wild-type *PTEN* product and has no detectable phospho-Akt. Mean fluorescence intensities were 3.4-fold higher for KLE cells and 12-fold higher for HeLa cells compared to HEC-1-A cells. Treatment of cells with Akt inhibitor led to 1.1-1.4-fold increase in CD55 expression. The 1.1-5.7-fold increase following treatment with PI3K inhibitor wortmannin was found in Ishikawa cells, RL95-2 and KLE cells.

Conclusions. Human endometrial cell lines with elevated PI3K-Akt activity express lower level of CD55 compared to cell lines with intact PTEN gene function. These findings may indicate that structural alteration at the DNA level and resultant overexpression of PI3K-Akt pathway are involved in the downregulation of CD55.

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Serine-Theronine Kinase Akt Colocalizes with Cytokeratins in Human Endometrial and Cervical Cells. Pawel Goluszko, Chandra Yallampalli. *Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Objective.

Cell shape is determined by the cytoskeleton, which provides the mechanical support and is involved in cellular signaling. Apoptotic cells undergo major morphological changes such as rounding and contraction, a process regulated by caspases, the cysteine proteases responsible for events controlling the cell disassembly. The motifs in certain cytokeratins make them substrates for caspase degradation.

Anti-apoptotic serine/threonine kinase Akt provides a survival signal by phosphorylating downstream effector molecules including caspase-9. While studying the Akt distribution in human endometrial cell line we found that Akt shows filamentous pattern of staining resembling cytoskeleton organization. In this study we evaluated whether Akt staining correlates with the microfilaments (MF), microtubules (MT) or intermediate filaments.

Methods.

Endometrial Ishikawa, RL95-2, HEC-1-A, KLE and cervical HeLa cell lines were used in the study. Incubation with cytochalasin D, (1ug/ml) or nocodazole (1mg/ml) and labeling with BODIPY FL Phallacidin, anti α-tubulin or anti-Akt antibody were used to assess whether cytoskeleton disruptors affect Akt distribution and MF and MT organization. For colocalization, cells were stained with anti-cytokeratin 18 mouse antibody followed by anti-mouse Alexa 488 conjugate, and then stained with anti-Akt rabbit antibody and anti-rabbit Alexa 549 conjugate. The scans collected with laser scanning confocal microscope from channels filtered for Alexa 488 and Alexa 459 were combined digitally and evaluated with Imaris colocalization analysis software.

Results.

Filamentous pattern of Akt staining was most pronounced in Ishikawa and less obvious in HEC-1-A cells. Treatment with cytochalasin D or nocodazole resulted in disruption of MF and MT but had no effect on cytokeratin organization and Akt distribution. Double staining with anti-cytokeratin-18 and anti-Akt antibody showed overlapping staining for cytokeratin and Akt. Analysis of digitally acquired images showed highest correlation for colocalized channels in RL95-2 cells (0.87) followed by Ishikawa (0.79) and HeLa cells (0.72). Lowest correlation was found for KLE (0.65) and HEC-1-A cells (0.59)

Conclusions. This study indicates a strong colocalization pattern of serine/threonine kinase Akt with cytokeratins, and suggests a mechanism by which cytokeratins might be protected against cleavage by caspase-9 and caspase-3 in the early apoptotic stages.

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The Presence of Immune Suppressive CD4+ CD25^{high} Regulatory T-Cell Subset Expressing *Foxp3* in Blood and Decidua of Human Pregnancy. Laura Dussable,[1,2] David M Sansom,[1] Barbara Innes,[3] Judith N Bulmer,[3] Mark D Kilby.[2] *[1]Division of Immunity and Infection, University of Birmingham, Edgbaston, West Midlands, United Kingdom; [2]Division of Reproduction and Child Health, University of Birmingham, Birmingham, West Midlands, United Kingdom; [3]School of Surgical and Reproductive Sciences (Obstetrics and Gynaecology)., Newcastle University, Newcastle-upon-Tyne, North East, United Kingdom.*

Background: CD4+ CD25+ T regulatory cells (T-Reg), express *Foxp3*,suppress antigen-specific immune responses and are important for allograft tolerance. During pregnancy the mother tolerates an allograft expressing paternal antigens (the fetus), requiring substantial changes in immune regulation over a programmed period of time.

Methods: The presence of T-Reg cells (CD4+ CD25high*Foxp3*+) was assessed in the peripheral venous blood of 25 non-pregnant, 63 pregnant and seven postnatal healthy women by flow cytometry. Human decidua was obtained by surgical termination of pregnancy in the first (n=26), second (n=16) and third (n=5) trimester of human pregnancy. Paraffin sections were immunostained for Foxp3 and CD25. *Foxp3* +cells were quantified in 5 x400 fields and results compared between first, second and third trimester samples and according to the presence of extravillous trophoblast.

Results: Fluorometric studies of blood samples indicate an increase % of circulating CD4+ CD25high*Foxp3* T-cells in pregnant (3.06% [range 1.21-4.8%]) vs. non-pregnant controls (2.58% [range 1.1 - 5.03%]; $P<0.05$). A progression from 1st, 2nd and 3rd trimesters indicated the % of CD4+ CD25high*Foxp3* T-cells was 3.01%, 2.88% and 3.14%, respectively. Low numbers of *Foxp3*+ cells were detected in all decidua samples and their distribution mirrored that of CD25+ cells. In 1st trimester samples, *Foxp3*+ cells were often detected in lymphoid aggregates adjacent to endometrial glands. Increased numbers of *Foxp3*+ cells were detected in 1st (21.9±4.3) compared with 2nd trimester decidua (9.9±3.2; $P<0.05$) but there was no difference between 1st and 3rd trimester (12.0±3.1), nor between 2nd and 3rd trimester decidua. In 1st trimester decidua, numbers of *Foxp3*+ cells were increased in areas without extravillous trophoblast.

Conclusion: Normal human pregnancy is associated with an increase in the number of circulating CD4+ CD25high*Foxp3* T-cells. The presence of *Foxp3*+ cells in early gestation human decidua may be important in the initiation of materno-fetal tolerance at an autocrine level. (Supported by MRC).

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β-Defensin 2 Release by Human Keratinocytes Following Low Molecular Weight Hyaluronan Fragments Treatment. Silvia Gariboldi,[1] Franco Vicariotto,[2] Marco Mastrodonato,[3] Cristiano Rumio.[1] *[1]Department of Human Morphology, Università degli Studi di Milano, Milano, Italy; [2]European Society for Infectious Diseases in Obstetrics and Gynaecology, Trieste, Italy; [3]Sinclair Pharmaceuticals Ltd, Godalming, Surrey, United Kingdom.*

Aims: β-defensins are small cationic peptides with antibiotic and antimicotic activity. Hyaluronan and its degradation products have been described as endogenous ligands for TLR2 and TLR4, whose involvement in β-defensin expression has been reported in different epithelial tissues and cell lines. We aim to investigate weather low molecular weight hyaluronic acid induces β-defensin 2 release by keratinocytes, via TLR2 and TLR4.

Methods: The induction of β-defensin 2 production following in vitro treatment of human keratinocytes with a low molecular weight hyaluronic acid solution was evaluated by PCR-analysis and ELISA techniques. Studies on the involvement of TLR2 and TLR4 in β-defensin 2 production have been performed using specific blocking antibodies.

Results: PCR and ELISA revealed an intense β-defensin 2 production following hyaluronic acid treatment in human keratinocytes. The β-defensin 2 production induced by hyaluronan was abolished following block of TLR2 and TLR4 by specific antibodies, demonstrating the involvement of these receptors. The same hyaluronic acid treatment did not induce activation of inflammatory genes, such as IL-8, TNF-α, IL-1 and IL-6.

Conclusion: Our data show that hyaluronic acid is an efficient inducer of β-defensin 2 production in keratinocytes, via TLR2 and TLR4. This observation might be important to open new perspectives in the development of possible topical products containing hyaluronic acid, to improve the release of β-defensins by keratinocytes, ameliorating the self-defence of the skin in case of skin infections. Therefore, one of the possible applications for this kind of topical products might be the treatment of infective vulvitis, one of the most distressing gynaecological diseases for adult women.

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Recurrent Miscarriage Associated with the Antiphospholipid Syndrome (RM/APS): Does Gender of a Prior Pregnancy Impact Subsequent Pregnancy Outcome? Kiera von Besser,[1] Serena Wu,[1] Mary D Stephenson.[1,2]
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Objective: To investigate whether gender of an ongoing pregnancy impacts the probability of a successful outcome, and, to ascertain whether the gender of prior live birth(s) impacts subsequent pregnancy outcome, among women with RM/APS.

Materials and Method: Cohort-control study. RM subjects were evaluated by MDS between 1992-2004 (Stephenson, 1996). Couples who met APS criteria (Wilson et al, 1999), restricted to RM only, were followed prospectively. Cohort data was compared to live birth data from the Vital Statistics Agency of British Columbia from 1999-2004. Secondary sex ratios (SSRs) among successful pregnancy outcomes were calculated by dividing the number of male live births by female. Sex ratios were calculated for all pregnancies 24 weeks, regardless if they ended in success or demise. Pearsons[2] test with Yates continuity correction was applied.

Results: 388 subjects were identified. 226 subjects had 319 prior live births of known gender (174 male/145 female), giving a SSR of 1.20. There were also 25 prior fetal demises 24 wks of known gender (13/12) giving a sex ratio for all prior pregnancies at 24 wks of 1.19.

252 subjects delivered 301 subsequent live births of known gender (158/143), giving a SSR of 1.08. There were also 6 subsequent fetal demises (3/3) giving a sex ratio for all subsequent pregnancies of 1.10.

112 subjects delivered both prior and subsequent live births. The SSR was 1.36 (83/61) among their prior and 1.24 (68/55) among their subsequent live births. Including fetal demises, 119 subjects had ongoing prior and subsequent pregnancies. The sex ratio was 1.32 among their prior pregnancies and 1.10 among their subsequent.

As the control, a SSR of 1.06 (124,982/118,093) was calculated from Vital Statistics data. When the prior and subsequent SSRs of the cohort were compared to each other, as well as to the control, there were no statistically significant differences.

Conclusions: Our findings, from the largest study of its kind to date, suggest that, in patients with RM/APS, the gender of an ongoing pregnancy does not significantly affect the probability of a successful outcome, to any greater degree than it does in the general population. Also, the gender of a prior ongoing pregnancy does not significantly impact the likelihood of developing RM/APS.

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Characterization of the Embryonic Poly(A)-Binding Protein during Oocyte Maturation. JK Friend, FB Bezirci, E Seli. *Ob & Gyn, Yale U., New Haven, CT, USA.*

Introduction: Oocyte maturation is associated with repression of transcription. During oocyte maturation, fertilization, and early embryo development until the onset of zygotic gene expression, proteins are synthesized from maternally-derived mRNAs. The regulation of protein expression from these maternal mRNAs is post-transcriptional, and occurs mainly via poly(A)-tail elongation. The embryonic poly(A)-binding protein (ePAB) is the predominant poly(A)-binding protein before the activation of the zygotic genome, and plays a critical role in the activation of certain maternal mRNAs, those bound by CPEB and probably Pumilio. We are characterizing additional functions of ePAB during the process of oocyte maturation.

Methods and Results: Our model system is the Xenopus laevis oocyte where oocyte maturation is induced by the addition of progesterone. Our preliminary findings indicate that ePAB is phosphorylated, and that levels of phosphorylated ePAB increase upon progesterone-induced oocyte maturation. Moreover, glycerol gradient centrifugation revealed that nonphosphorylated and phosphorylated ePAB are contained in distinct complexes that change mobility upon oocyte maturation. Furthermore, oligo-dT selection for poly(A)-containing mRNAs strongly suggests that these mRNAs are bound exclusively by phosphorylated ePAB. Using affinity purification, we have determined that nonphosphorylated ePAB exists primarily in a large protein complex prior to oocyte maturation that is later disassembled after the addition of progesterone.

Conclusions: Based on these preliminary findings, we conclude that prior to oocyte maturation, the bulk of ePAB is nonphosphorylated and is found in a protein-rich complex separate from poly(A)-containing mRNAs. Upon oocyte maturation (when certain maternally-derived mRNAs are activated for translation), the majority of ePAB becomes phosphorylated, and this phosphorylated form of ePAB is likely bound to translationally-active mRNAs. We are currently investigating what kinase phosphorylates ePAB and whether this phosphorylation plays a role in translational up-regulation of ePAB-bound mRNAs.

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Role of NADPH Oxidase Isoforms in Control of Cellular Fate in Human Uterine Smooth Muscle. Xiao-Lan Cui, Baojun Chang, Leslie Myatt. *Dept. of Ob/Gyn, University of Cincinnati College of Medicine, Cincinnati, OH, USA.*

Introduction: Reactive oxygen species (ROS) play important roles in all aspects of cellular fate. NADPH oxidase isoforms, a family of genetically preserved enzyme complexes, have been shown to be the main sources of ROS in various cell types. However, the role of NADPH oxidase isoforms in human myometrium proliferation and differentiation has not been defined. In the myometrium, different smooth muscle phenotypes maybe associated with specific physiologic functions. We have shown that Angiotensin II (AngII) stimulates hypertrophy but not cell proliferation in ULTR cells, an in vitro model of human myometrium. ULTR cells at greater than 30 passages display a replicative senescent phenotype. By introducing human telomerase reverse transcriptase (hTERT) gene, we have obtained a stable cell line (ULTR-hT) which has a significantly increased division rate and distinct cellular morphology than the original ULTR cells.

Objective: To determine the relationship of expression of NADPH oxidase to ULTR cellular fate.

Methods: Early and late passages (p26-34) of ULTR and ULTR-hT (p31-38) cells were grown on either plastic or collagen IV (CN4)-coated surfaces. ULTR-hT cells were further stimulated with AngII (0.1 uM) for 24 hrs. Expression of NADPH oxidase core (Nox1-5 and Duox1/2) and associated subunits (p22phox, p47phox, NoxO1, p67phox, NoxA1, p40phox, and Rac1/2), and AngII receptors AT1/2 was identified by RT-PCR from cellular total RNA. Fluorescent immunohistochemistry (IHC) was employed to determine protein expression and localization.

Results: The mRNA level of house keeping gene β-actin was unchanged by any cellular manipulation. The senescent phenotype of ULTR cells was accompanied by an apparent down-regulation of Nox1, p22phox, and NoxA1 genes, and an up-regulation of AT1/2. Overexpression of hTERT did not reverse Nox1, p22phox and NoxA1 expression while cell division rate was increased. However, there was a down-regulation of Nox4, AT1/2 and rac2. Plating ULTR-hT cells on CN4 induced Nox4/5 down-regulation and up-regulation of Duox1/2, with no apparent change of AT1/2. However, exposure to CN4 re-directed cellular response to AngII such that only Nox5 was induced by AngII stimulation.

Conclusion: Expression of NADPH oxidase isoforms Nox1, 4, 5, and Duox1/2 are correlated with ULTR cell differentiation and cell fate control. Data also suggests that Ang II-induced myometrial hypertrophy involves Nox5 mediated ROS generation.

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Concentrations of AGEs, RAGE and VEGF in Plasma and in Follicular Fluids from Reproductive Women – The Influence of Aging. Eriko Y Fujii,[1] Masahiro Nakayama.[2] [1]*Women's Health, National Center for Child Health and Development, Tokyo, Japan;* [2]*ASKA Reproductive Clinic, Nara, Japan.*

[Introduction] Receptor for Advanced Glycation End products (RAGE) is a multiligand type glycoprotein, and is characterized based on its ability to bind AGEs, adducts formed by non-enzymatic glycation and oxidation of protein and lipids. This process occurs during normal course of aging. AGEs/RAGE interaction regulates various physiological function, such as inflammation, angiogenesis through VEGF inducement. A soluble form of RAGE (sRAGE) works as a decoy in the body and inhibits intracellular signaling.

[Objectives] The balance of these factors may contribute to reproductive dysfunction by aging. We aimed to measure the AGEs, sRAGE and VEGF concentrations in plasma and follicular fluids from reproductive women, and examined the differences of those factors between young group and old group.

[Material and Methods] Patients' plasma and follicular fluid were collected with consent based on regulations of the ethical committee, and we measured AGEs (pentosidine, CML), sRAGE and VEGF in duplicate using commercially available ELISA kits (Fushimi Co, R&D and Cyelex). Concentrations were calculated from each standard curve, and compared between the young group

under 34 years old, and the old group over 35 y.o. Data were evaluated for the difference in two groups by Student's t test , and the significance was determined by $p<0.05$.

[Results] 1) sRAGE in plasma, 1517±642 pg/ml (mean±SD), n=40 in the young group was significantly higher than 1268±530 pg/ml, n=51 in the old group. There was no significant difference in plasma VEGF. 2) VEGF in follicular fluid was 45±22 pg /mg protein, n=37 in the young, and 56±27 pg /mg protein, n=63 in the old was increased significantly. 3) We could not see statistical difference of pentosidine nor CML concentrations between two groups in plasma and follicular fluid samples.

[Conclusions] It has been reported that higher concentration of VEGF in follicular fluid may relate to worse pregnancy rate in ART. There was a significant decrease of plasma sRAGE in older group in our result, and because of this decrease of 'decoy', focal AGEs-RAGE-VEGF signaling might be activated in older women. Our results showed the possibility that AGEs/RAGE and VEGF regulation may contribute to the reproductive dysfunction by aging.

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Regulation and Function Analysis of mir-21 in Leiomyoma and Myometrium Smooth Muscle Cells as Well as in Transformed Leiomyoma and Leiomyosarcoma Cells. Qun Pan, Xiaoping Luo, Nasser Chegini. *OB/GYN, University of Florida, Gainesville, FL, USA.*

As a part of a novel endogenous RNA silencing machinery, a noncoding short RNA strand referred to as "microRNA" (miRNA) has been identified to regulate the stability of the target gene expression through mRNA degradation and repression. We have identified the expression of many of these miRNAs in leiomyoma, myometrium, their isolated smooth muscle cells (LSMC and MSMC), transformed LSMC (T-LSMC) and SKLM (leiomyosarcoma cell line), including miR-21 which is predicted to target the expression of many genes, including TGF-b and TGF-b type II receptor (TGF-bRII). However, the biological significance of these miRNAs in various cellular processes remains to be established. As such in the present study we examined the expression, regulation and function of miR-21 in LSMC, MSMC, T-LSMC and SKLM. We found that miR-21 is expressed and regulated by 17b-estridiol and medroxyprogesterone acetate (10^{-8} M) in these cells ($P<0.05$). We further assessed the regulatory function of gain of and loss of function of miR-21 on the expression of TGF-bRII. Transfection of LSMC, MSMC, T-LSMC and SKLM with pre-mir- and anti-mir-21 oligonueclotides resulted in a significant increase and/or inhibition of miR-21 expression in these cells, respectively as determined by realtime PCR ($P<0.05$). Over-expression of miR-21 resulted in a significant reduction, while transfection with antimir-21 increased the expression of TGFbRII mRNA and protein in these cells as compared to controls ($P<0.05$). We concluded that miR-21 is expressed in leiomyoma and myometrial cells, its expression is regulated by the ovarian steroids and it functions by targeting the expression of TGFbRII and possibly other genes with key regulatory action on cell growth, angiogenesis, transcription regulation, ECM turnover and apoptosis that results in leiomyomas growth and regression. (Supported by NIH grant HD37432).

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Opposing Changes in CRF and Urocortin in Human Umbillical Cord Venous Smooth Muscle Layers at Term. Jayaraman Lakshmanan,[1] Avish Arora,[1] Lilit Baldjyan,[2] Olga Miadel,[2] Sharon K Sugano,[1] Michael G Ross, Calvin J Hobel.[1] *[1]Ob-Gyn, Harbor-UCLA Medical Center, Torrance, CA, USA; [2]Ob-Gyn, Cedars-Sinai Medical Center, Los Angeles, CA, USA.*

Objective: Placenta and a number of gestational tissues are well recognized to express corticotrophin releasing factor (CRF), urocortin 1 (UCN1), UCN2, UCN3 and CRF –R1 and CRF-R2 receptor subtypes together with CRF-binding proteins locally. UCN2 and UCN3 are implicated in the reversal of stress responses initiated by CRF. In the present investigation, we evaluated functions of CRF and UCNs by quantifying their contents in venous smooth muscle layers using Image Pro 4.01 in human umbilical cords collected at preterm and term gestation

Methods: Umbilical cord specimens (3-4mm thickness, 6 pieces per umbilical cord) collected at preterm and term (n=6 each) at delivery were fixed in Bouin's solution and paraffin embedded. Sections were subjected to immunohistochemical analyses with polyclonal antibodies to CRF (1:300), UCN1 (1:250), UCN2 (1:250) and UCN3 (1:250) (Peninsula Laboratory, PA and Sigma Aldridge, MS) by standard ABC technique. Immunoreactive materials on the sections were identified using 3, 3'-diaminobenzidine as a chromagen. Immunostaining intensities (OD/area) on UC-sections were quantified by Image Pro 4.01 software and expressed as arbitrary units (AU). All values were expressed as mean ± SEM. Differences between groups were evaluated by ANOVA, followed by the post-hoc Tukey test for multiple comparison.

Results: Antibodies to CRF and UCNs elicited positive immunostaining of variable intensity in venous smooth muscle layers in UC-sections of preterm and term gestations**.** Immunostaining intensity (AU) of venous smooth muscle layers at preterm (PT) and term (T) are as follows**:** CRF-PT= 0.0451±0.0043 vs CRF-T= 0.1027± 0.0242 ($P<0.5$); UCN1-PT=0.1736 ± 0.0108 vs UCN1-T= 0.0959± 0.0116 ($P<0.01$); UCN2-PT= 0.0964± 0.0063 vs UCN2-T= 0.0724 ± 0.1632 (p=NS); UCN3-PT= 0.1214 ± 0.0034 vs 0.0732± 0.0050 ($P<0.05$).

Conclusion: CRF content in venous smooth muscle layer markedly increased at term, while UCN1 and UCN3 contents significantly decreased and no significant change occurred in UCN2 content**.** Based on the opposing changes in CRF vs UCN1 and UCN3 immunostaining, we speculate that CRF, but not UCN1 or UCN3, is the major key player of vasodilation and function locally at the level of venous smooth muscle cells at term. UCN1 and UCN3 may perform cytoprotective functions at preterm.

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Extension of Fertile Lifespan in Female Mice by Adult-Onset Caloric Restriction. Kaisa Selesniemi, Ho-Joon Lee, Jonathan L Tilly. *OB/GYN, Vincent Center for Reproductive Biology, Massachusetts General Hospital/ Harvard Medical School, Boston, MA, USA.*

Introduction: A beneficial effect of caloric restriction (CR) on mammalian female reproductive function has been documented since the 1940s (Am J Physiol. 1947; 150: 511-519, Am J Physiol. 1952; 170:72-76). These studies showed that when ad libitum (AL) feeding was resumed in CR females, fertility was sustained well beyond the age at which AL-fed females became infertile. However, much of what is known on the effects of CR on fertility derives from models in which CR was initiated at weaning. Further, there is large variation in how the experiments were conducted. **Objective:** Herein we tested if adult-onset CR could delay age-related infertility in females. **Methods:** CR (40%, NIA protocol as described in *J Geront.* 1999 54A:B492-B501) was initiated in C57BL/6 female mice at 4 months and continued until 15.5 months of age, at which time AL feeding was resumed. Matings were initiated at 10 months of age. For mating during CR, a male mouse was housed overnight in a cage with a female and removed the next morning, so that the female mice could be fed their dietary food ration. AL-fed and CR females followed the same mating regimen. The number of offspring born and that survived to weaning (day 21) were recorded. **Results:** Fertility was lost in 3 of 11 AL-fed females by 10 months of age and continued to decline through 15.5 months of age. Age-matched females maintained on CR during the same period exhibited poor fertility, with a total of 9 pregnancies achieved out of 12 females. Although CR females showed poor fertility while on CR, their fertility improved dramatically after the reinitiation of AL feeding at 15.5 months of age. While only 1 out of 8, AL-fed females achieved a pregnancy between 15.5-18.5 months of age, 6 out of 10 age-matched CR-then-AL-fed females achieved a total of 10 pregnancies in this 3-month time. Notably, 79% of the pups born to CR-then-AL-fed females between 15.5-18.5 months of age survived and developed to weaning without complications. **Conclusions:** Adult-onset CR allows maintenance of female fertility into advanced maternal age after the reinitiation of AL feeding. How long fertility can be maintained and the minimum time needed for the beneficial effects of CR to be realized remain to be investigated. Nonetheless, these observations suggest that there may be ways to safely extend fertility in females at ages when reproductive function is suboptimal (NIH R37-AG012279).

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Bile Acids in Human Ovarian Follicular Fluid. Laura P Smith,[1,2] Kaila Deiorio-Haggar,[1] Jason Reindollar,[3] Alan S Penzias,[1,2] Anny Usheva-Simidjiyska.[3] *[1]Ob/Gyn Reproductive Endocrinology & Infertility, BIDMC, Boston, MA, USA; [2]Boston IVF, Boston, MA, USA; [3]Endocrinology, BIDMC, Boston, MA, USA.*

INTRODUCTION: Bile acids are known to serve important functions in the hepatobiliary and gastrointestinal systems. The presence of bile acids in the human ovary and relation with fertility potential have never been previously evaluated.

METHODS: Human follicular fluid (FF) from large follicles and small follicles was obtained at vaginal oocyte retrieval. Human serum was obtained before and 36 hours after human chorionic gonadotropin (h-CG). Follicular fluid and

serum samples were analyzed for total bile acids by spectrophotometry. Bile acid concentrations were correlated with age, number of retrieved and mature oocytes, number of fertilized oocytes, and pregnancy.
RESULTS: Bile acid concentrations were analyzed and compared to the normal human serum bile acid concentration which ranges from 0 to 15 micromoles/L. Bile acids are present in follicular fluid with a mean concentration of 15.28 micromoles/L in large follicles and 14.22 micromoles/L in small follicles (p=0.108). Pre and post h-CG serum bile acid concentrations differed significantly (13.88 micromoles/L vs. 7.85 micromoles/L, p=0.004). There was a trend toward higher bile acid concentration in large follicles of young patients < 35 years old compared to older patients ≥ 40 years old (16.22 ± 5.55 vs. 13.56 ± 2.26, p=0.051). There was also a trend toward higher pre h-CG serum bile acid concentrations in older patients (4.47 ± 1.92 vs. 2.90 ± 0.72, p=0.079). There was no correlation between serum and follicular fluid bile acid concentrations and number of oocytes retrieved or number of mature oocytes, but there did appear to be a positive correlation between pre h-CG serum bile acid concentration and number of fertilized oocytes (Spearman's Correlation Coefficient 0.678, p=0.005).
CONCLUSIONS: This is the first demonstration of the presence of bile acids within human ovarian follicular fluid. There may be a relationship between bile acid concentration and fertility potential. The precise function of bile acids in human ovarian follicular fluid is under investigation.

Bile Acid Concentrations

	Total Bile Acids	P value
Pre h-CG serum	13.88 ± 5.98	0.004
Post h-CG serum	7.85 ± 2.73	
Large FF	15.28 ± 4.41	0.108‡
Small FF	14.22 ± 3.33	

Concentration = Mean ± STD micromoles/L;. pre vs. post; ‡ large vs. small

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Does Blastomer Extraction for PGD Decrease the Implantation Potential of Embryos Obtained by IVF-ICSI? Adi Reches,[1] Dalit Ben-yosef,[2] Mira Malcov,[2] Ami Amit,[1,2] Joseph Lessing,[1] Yuval Yaron.[3] *[1]Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; [2]Sara Racine IVF Unit, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; [3]Prenatal Diagnosis Unit - Genetic Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.*
Objective: Preimplantation genetic diagnosis (PGD) is a procedure that allows carriers of inherited diseases to undergo genetic analysis of embryos obtained by *in vitro* fertilization (IVF) in order to select unaffected embryos for transfer to the uterus. It is still controversial whether biopsy of the 8-cell embryo decreases their implantation potential. To evaluate this question, we performed a retrospective comparison of treatment outcomes of all PGD cycles versus intractoplasmic sperm injection (ICSI)
Methods: The study included all PGD cycles preformed at the Tel Aviv Sourasky Medical Center during a 4-year period (2002-2006). Patient information cycle outcomes were obtained from patient files and computerized databases. The control group included all patients undergoing ICSI during the same period. These were chosen since in all PGD cycles, ICSI is performed routinely.
Results: see table
Conclusions: Our results demonstrate that clinical pregnancy rates and implantation rates are similar in both PGD and ICSI groups. This suggests that blastomer extraction for PGD does not adversely affect the implantation potential of IVF embryos.

Results

	ICSI	**PGD**	P-value
Patients	983	75	
Cycles	2392	221	
Patient age	34.3 ± 5.9	32 ± 5.0	P<0.001
Oocytes retrieved	9.15 ± 6.3	14.5 ± 8.2	P<0.001
Embryos transferred	2.5 ± 1.0	2.7 ± 1.0	NS
ET rate (n)	88.8% (2124)	84.2% (186)	P=0.005
Positive HCG rate per ET (n)	27.5% (585)	24.2% (45)	NS
Clinical pregnancy (rate per ET)	431 (20.3%)	35 (18.8%)	NS
Implantation per embryo (rate)	536/5226 (10.3%)	43/428 (10.1%)	NS
Take home baby, including ongoing/ET	16.1% (400)	16.6% (31)	NS

NS = not significant

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IVF Treatment Significantly Affects the Homocysteine Pathway in the Maturing Follicle. Jolanda C Boxmeer,[1] Regine PM Steegers-Theunissen,[1,2,3,4] Jan Lindemans,[5] Mark F Wildhagen,[1,6] Frank H de Jong,[7] Elena Martini,[1] Eric AP Steegers,[1] Nick S Macklon.[1,8] *[1]Obstetrics and Gynecology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Epidemiology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [3]Pediatric Cardiology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [4]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [5]Clinical Chemistry, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [6]Urology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [7]Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [8]Reproductive Medicine and Gynecology, University Medical Center, Utrecht, Netherlands.*
Objective To investigate the impact of ovarian hyperstimulation treatment on the biomarkers of the homocysteine pathway in blood and follicular fluid, and their association with the follicle diameter as a measure of follicular maturation.
Methods In 201 women undergoing IVF/ICSI treatment blood samples were collected on cycle day 2 and the day of hCG administration. During oocyte retrieval in each woman the diameter of two follicles was measured and the corresponding follicular fluids were collected. In blood and follicular fluid total homocysteine (tHcy), folate, cobalamin and pyridoxal'5'phosphate (PLP) concentrations were determined. Women with a serum folate ≥ 22.5 nmol/L were classified as folic acid supplemented.
Results Ovarian hyperstimulation significantly decreased tHcy and cobalamin blood levels (both $p \leq 0.001$). The blood and follicular fluid concentrations of tHcy, folate, cobalamin and PLP were significantly correlated (all $p \leq 0.001$). In the total group, a two-fold increase of tHcy in follicular fluid resulted in a 0.06 mm decrease of the follicular diameter ($p \leq 0.05$). In non-supplemented women this decrease was 1.64 mm ($p \leq 0.01$). In supplemented women a two-fold increase of follicular fluid folate resulted in a 0.74 mm decrease of the follicular diameter ($p \leq 0.05$).
Conclusions Ovarian hyperstimulation reduces tHcy blood levels independent of folic acid supplementation. However, high follicular fluid tHcy and folic acid supplementation may have detrimental effects on the maturation of the follicle.

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Ultrastructural Differences between *In Vivo* and *In Vitro* Derived Murine Blastocysts. Luisa Delle Piane, Tamara Zdravkovic, Olga Genbacev, Gnanaratnam Giritharan, Paolo Rinaudo. *Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA.*
Objective: In vitro culture of preimplantation embryos is known to results in blastocysts with reduced cell number, slower development and overall abnormal gene expression. However, it is unknown if embryo conceived *in vivo* and *in vitro* differ on a sub cellular level. In this study, we describe the EM appearance of blastocysts conceived in vivo and in vitro (IVF).
Methods: CF-1 female mice were injected with 5 IU PMSG and 42-46 h later with 5 IU hCG. The following morning, unfertilized oocytes were incubated in Whitten's Medium (WM) containing 15 mg/ml BSA and sperm obtained from cauda epididymis of male B6D2F1/J mice for 4 hrs. The resulting embryos were cultured in WM to the blastocyst stage under 20% CO2 in humidified air at 37° C. Control in vivo embryos were collected 4 days after fertilization. The blastocysts were fixed for 30 minutes in 2.5% w/v glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), post-fixed for 40 rain in 2% w/v osmium tetroxide in the same buffer, quickly dehydrated in a series of ethanol solutions, and embedded in Epon. Thin sections were stained with uranyl acetate followed by lead citrate and were observed at electron microscope.
Results: Ten blastocysts from each group were collected and analyzed. Compared to the in vivo embryos, blastocysts generated in vitro exhibited significant differences. The surface of their trophectoderm (TE) layer had a reduced number of microvilli, the number of the apoptotic cells in the inner cell mass (ICM) was higher and the presence of non functional mitochondria was elevated.
Conclusion: In this study we have, for the first time, compared the ultrastructure of the *in vivo* and *in vitro* conceived blastocysts. Taken together, these results suggest that both, the higher rate of apoptosis and the morphological alterations in the mitochondrial structure in IVF embryos, are associated with stress during *in vitro* embryo culture. Therefore, these parameters can be used, in the future, as markers for the assessment of the embryo wellbeing in the IVF settings.

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Nitric Oxide Sufficiency in Oocytes Prevents Apoptosis in the Developing Embryo. AP Goud,[1] PT Goud,[1] MP Diamond,[1] B Gonik,[2] HM Abu-Soud.[1] *[1]Div REI. Dept OB/Gyn, Wayne State University, Detroit, MI, USA; [2]Div. MFM, Dept OB/Gyn, Wayne State University, Detroit, MI, USA.*

Deteriorating oocyte quality is a critical hurdle in the management of infertility, especially one associated with advancing age.Here, we explore a newly discovered role of Nitric oxide (NO) in the sustenance of oocyte quality.

Methods: Sibling oocytes from superovulated mice were subjected to intracytoplasmic sperm injection (ICSI) with cauda-epididymal spermatozoa following exposure to either the NO donor, *S*-nitroso *N*-acetyl penicillamine (SNAP, 0.23 µM/min); a soluble guanylyl cyclase inhibitor, 1H-[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one (ODQ, 100 µM) or an NO synthase inhibitor, N^{w}-nitro-L-arginine methyl ester (L-NAME, 1 mM). Their sibling oocytes were subjected to ICSI either before (young) or after culture for the corresponding period (old). Outcomes of fertilization, cleavage and development to the morula and blastocyst stages were compared. Some embryos from each subgroup were also subjected to TUNEL assay for apoptosis.

Results: Oocytes deteriorated in their ability to undergo normal fertilization and development to morulae/blastocysts after aging in culture, as compared to their sibling cohorts subjected to ICSI immediately after ovulation (P<0.05). This deterioration was prevented after oocyte exposure to SNAP. While, exposure to L-NAME or ODQ resulted in a significant compromise in fertilization and development to the morulae/blastocysts (*P*<0.05) with detection of apoptosis, which was also noted in embryos derived from aged oocytes but not in those from young or SNAP exposed oocytes.

Conclusions: NO is essential to sustain oocyte fertilizability and developmental ability, and to prevent blastomere apoptosis.

Nitric Oxide Sufficiency and Oocyte Quality

Group, N=		Survived (%)	2 PN (%)	2-4 Cell (%)	Morulae (%)	Blastocysts (%)
Group A (13.5 h)	24	19 (79.1)	15 (78.9)	15 (100)	12 (80.0)	10 (66.7)
Group B (16.5 h)	28	20 (71.4)	10 (50.0)a	10 (100)	6 (60.0)b	3 (30.0)c
Group C (SNAP)	26	20 (76.9)	15 (75.0)	15 (100)	13 (86.7)	11 (73.3)
Group D (15.5 h)	25	18 (72.0)	10 (55.5)	9 (90)	5 (55.5)	2 (40.0)
Group E (ODQ)	28	20 (71.4)	4 (20.0)d	4 (100)	0e	0f
Group F (16.5 h)	23	15 (65.2)	7 (46.7)	7 (100)	3 (42.9)	1 (33.3)
Group G (L-NAME)	22	15 (68.2)	3 (25.0)g	2 (66.7)	0h	0i

a-iP<0.05

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Levels of the Biomarkers of the Homocysteine Pathway in Follicular Fluid Affect Oocyte Quality. Jolanda C Boxmeer,[1] Nick S Macklon,[1,2] Jan Lindemans,[3] Nicole GM Beckers,[1] Marinus JC Eijkemans,[4] Joop SE Laven,[1] Eric AP Steegers,[1] Regine PM Steegers-Theunissen.[1,5,6,7] *[1]Obstetrics and Gynecology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Reproductive Medicine and Gynecology, University Medical Center, Utrecht, Netherlands; [3]Clinical Chemistry, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [4]Public Health, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [5]Epidemiology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [6]Pediatric Cardiology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [7]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*

Objective To investigate the effects of the levels of the biomarkers of the homocysteine pathway on IVF outcome.

Methods From 201 women undergoing an IVF or ICSI procedures, two blood samples and two mono follicular fluid samples were collected for determination of folate, cobalamin, and total homocysteine (tHcy). Total protein was determined in follicular fluid to adjust the biomarker concentrations for follicular maturation. Primary endpoint of the study was oocyte quality measured by fertilization and embryo quality (range 1-5; with 1 being best quality). Secondary endpoint was the occurrence of pregnancy.

Results 67% of the included women used a folic acid supplement (serum folate ≥ 22.5 nmol/L). In non-supplemented women higher cobalamin levels in follicular fluid correlated with a better embryo quality (estimate -0.87; $p \leq 0.05$) and higher tHcy levels (median 7.1 µmol/L, range 4.0-47.0) correlated with a worse embryo quality (estimate 1.01; $p \leq 0.05$). In supplemented women higher follicular fluid tHcy (median 6.4 µmol/L, range 3.5-73.6) correlated with better embryo quality (estimate -0.58; $p \leq 0.05$). The follicular fluid folate level of oocytes that did not fertilize was 1.1-fold higher than in the fluids of a fertilized oocyte (95% CI 1.00 –1.18; $p \leq 0.05$). A two-fold increase of follicular folate corresponded with a 3.3 higher chance to achieve pregnancy (95% CI 1.09-9.71).

Conclusions Cobalamin levels in follicular fluid are correlated with embryo quality. Folic acid supplementation modifies the tHcy and folate levels in follicular fluid and thereby affects oocyte quality. The level of folate in follicular fluid is important in the fertilization process.

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Case/Control Study of Patients Who Have High-Level Oocyte Immaturity on Multiple Cycles during ICSI. Andrew K Moore, Margaret Arny, Kelly Lynch, Diana Atashroo, Yusoff Dawood, Daniel Grow. *Department of OB/GYN, Baystate Medical Center, Springfield, MA, USA.*

Background: Previous reports have described cases of *complete* oocyte immaturity and have hypothesized a genetic etiology.(Levran et al, Windt et al) We recently reported that increasing *relative* oocyte immaturity is associated with worsening outcome, and that cycles with many immature oocytes are more common in younger women. (Moore et al, ASRM annual meeting, 2007) To further investigate this trend, we conducted a case/control analysis of patients with repeated cycles of high-level oocyte immaturity (HLOI).

Methods: Oocyte maturity data was collected on all ICSI cycles starting in 2002. We defined a cycle with HLOI as having >50% immature oocytes (>2 SD's above the median). A case was defined as a patient with HLOI on more than one cycle. Control subjects were age-matched and defined as having </= 35% (1SD above median) immature oocytes on all cycles.

Results: From 450 subjects, we identified 8 cases of recurrent HLOI (comprising 19 ICSI cycles) and 240 control subjects. At baseline, cases had more oocytes retrieved per cycle than controls (22.5 vs. 16.3, P<.0001). All other baseline characteristics were similar. All case subjects were younger than 37. Outcomes are shown in Table 1.

Discussion: Clinical pregnancy and live births were reduced in the case group, but more than expected based on % immaturity. During 19 cycles, there were no live births among the case group. Patients with recurrent HLOI represent a previously undescribed group of young patients with a very poor prognosis during ICSI treatment. This study may suggest that the problem underlying the immaturity may relate to overproduction of oocytes during stimulation, a factor which may be modifiable. However, the additional trend (though not significant) that even the mature oocytes from the case patients tend to have poor outcomes suggests the possibility of a maturation defect and warrants further consideration. Data on response to gonadotropins, fert rates, and implantation rates will be presented.

Table 1

	Cases	Controls	OR	95% CI
Clinical Pregnancy Rate	4/19 (21%)	168/358 (47%)	0.19	0.09-0.92
Clinical Pregnancies per Mature Oocyte	4/165 (2.4%)	168/4915 (3.4%)	0.70	0.25-1.9
Live Birth/Cycle	0/19 (0%)	129/358 (36%)	0.05	0.003-0.75
Live Births per Mature Oocyte	0/165 (0%)	129/4915 (2.6%)	0.11	0.01-1.80

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***CTF18* Is Important for Mammalian Fertility and Meiotic Chromosome Cohesion.** Karen M Berkowitz,[1] P Jeremy Wang,[2] Fang Yang,[2] Thomas A Jongens,[3] Klaus H Kaestner.[3] *[1]Obstetrics and Gynecology and Center for Research on Reproduction and Women's Health, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; [2]Department of Animal Biology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA; [3]Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA.*

Objective: *CTF18/Cutlet* encodes an evolutionarily conserved protein that is necessary for faithful transmission of chromosomes in yeast and crucial for fertility in the fly. We generated a mouse model that lacks *CTF18* and recently demonstrated that *CTF18* plays an important role in mammalian germ cell development. The objective of our studies is to elucidate the function of *CTF18* in mammalian gametogenesis and fertility.

Methods: We utilized mouse embryonic stem cells and Cre/loxP technology to generate a mouse model that lacks *CTF18*, termed *Chtf18* in the mouse. The phenotypic consequences of *Chtf18* deletion were assessed by gross, histological, chromosomal, and immunofluorescence examination of *Chtf18*-null and wild-type gonads. TUNEL analysis was also performed, and epididymal sperm concentrations of *Chtf18*-null and wild-type males were determined. Fertility was assessed by breeding *Chtf18*-null males or *Chtf18*-null females with wild-type mice over a period of 5 months.

Results: Adult *Chtf18*-null male and female mouse gonads are smaller and contain fewer germ cells than those of their wild-type adult littermates. *Chtf18*-null males are oligospermic, and their semiferous tubules contain aberrant spermatogenic cells and apoptotic nuclei. In addition, homologous

chromosomes of *Chtf18*-null spermatocytes separate prematurely during meiotic prophase I. *Chtf18*-null female ovaries contain fewer follicles that appear to be degenerating compared to that of wild-type females. Both *Chtf18*-null male and female mice are subfertile, and matings with wild-type mice yield fewer progeny than control littermates.
Conclusion: *CTF18* plays a significant role in mammalian fertility and meiotic chromosome cohesion.

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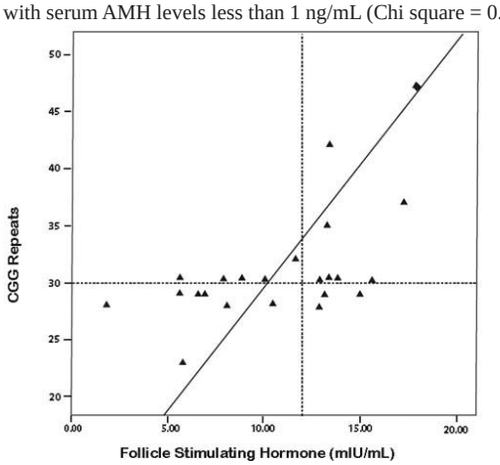
The Number of Triple Repeat Sequences in the FMR1 Gene Is Reflective of Ovarian Function. David H Barad,[1] Andrea Weghofer,[1,2] Norbert Gleicher.[1] *[1]Center for Human Reproduction, New York, NY, USA; [2]Department of Obstetrics and Gynecology, Allgemeines Krankenhaus of the University of Vienna Medical School, Vienna, Austria.*
Background: Full expansion and premutations in the FRM1 gene are associated with increased risk for premature ovarian failure in women. Whether ovarian function correlates to the number of triple repeats in general is, however, unknown.
Methods: Forty patients with fragile-X evaluations comprise the study group. Amongst those, 23 were performed for an initial diagnosis of premature ovarian aging (POA), 6 for the diagnosis of premature ovarian failure, and 11 for repeated pregnancy loss. In women under age 42, b-FSH levels over 12 mIU/mL and up to < 50 mIU/mL denoted a diagnosis of POA, while levels of ≥ 50mIU/mL were considered diagnostic for POF. All patients signed consent permitting review of medical records for clinical research purposes.
Results: Day 3 FSH levels ranged from 5.6 to 30.0 mIU/ml (mean 13.6 ± 7.2 mIU/ml). AMH levels ranged from < 0.1 to 2.7 ng/ml (mean 0.8 ± 0.8) and allele repeats ranged from 17 to 49, (mean for allele-1, 27.2 ± 4.3; allele-2, 32.0 ± 6.4). Mixed model analysis of variance for all patients, adjusted for age, race, and allele-1 revealed a statistically significant correlation between b-FSH levels and number of repeats in allele-2 (Figure 1; $p < 0.01$). We did not observe a significant linear relationship between serum AMH levels and CGG repeat counts, however CGG repeat counts above 32 were significantly associated with serum AMH levels less than 1 ng/mL (Chi square = 0.04).



Conclusions: Triple repeats above 30, a level currently considered well within normal, are associated with evidence of premature decline in ovarian function. The evaluation of triple repeats in FRM1 gene offers a first objective assessment of ovarian function and should be predictive of autologous reproductive life span in most women.

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Ovarian and Serum Levels of Mullerian Inhibiting Substance (MIS) after Ovarian Stimulation in Cisplatin Treated Rats. John Yeh, Beom Su Kim, Yuan Jing Liang, Jennifer Peresie. *Gynecology-Obstetrics, University at Buffalo, Buffalo, NY, USA.*
Objective: The levels of MIS in the serum and in the ovary have been demonstrated to decrease in response to increasing doses of cisplatin. We hypothesize that ovarian stimulation also will reveal dose related decreases in ovarian and serum MIS levels after cisplatin exposure, further demonstrating the follicular damage from chemotherapy.
Methods: Adult female Sprague-Dawley rats were treated with saline, 4.5 mg/kg cisplatin or 6.0 mg/kg cisplatin as two weekly injections. Five days following the last cisplatin injection, the rats were injected with pregnant mare serum gonadotropin (PMSG) and euthanized 54 hours following the PMSG injection. Serum was collected and both ovaries were obtained for protein analysis. Serum and ovarian levels of MIS were determined using an ELISA kit. Western blot analysis, immunohistochemical analysis, and TUNEL analysis were also performed on the ovarian proteins.
Results: Stimulated MIS levels declined in a linear fashion in response to increasing cisplatin doses ($P<0.001$). Ovarian protein levels measured by ELISA and western blot both indicated that stimulated ovarian MIS levels also decrease in a linear fashion ($P=0.001$ and $P<0.001$ respectively.) Immunohistochemical analysis revealed that while the total number of follicles remains the same, both the total number of MIS positive follicles, and the percentage of MIS positive follicles declines in a linear fashion ($P=0.001$ for both). TUNEL analysis reveals that there is no change in the incidence of apoptosis in the stimulated ovaries from any treatment group.
Conclusions: The administration of PMSG after treatment with cisplatin causes a dose dependent decrease in the levels of MIS in both the serum and in the ovary. Serum MIS levels following ovarian stimulation may be useful as a serum biomarker in this animal model to assess ovarian damage following the administration of cisplatin.

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Correlations of micro-RNAs 23a, 23b, 17-5Pp, 211 and 542-3p with Inflammatory and Steroidogenic Factors in Human Granulosa/Cumulus Cells. Tannaz Toloubeydokhti, Orhan Bukulmez, Kenneth C Drury, R Stan Williams, Nasser Chegini. *Obstetrics and Gynecology, University of Florida College of Medicine, Gaineville, FL, USA.*
As part of the novel endogenous RNA silencing machinery, noncoding short RNA strands, referred to as microRNAs (miRNAs), have been identified to regulate the stability of the target gene expression through degradation and repression. The expression of many of these miRNAs has been identified in human tissues however, their biological significance in various cellular processes remains to be elucidated. In this cross-sectional study of human granulosa cells, we aimed to study the potential correlations between five selected miRNAs, miR-23a, miR-23b, miR-542-3p, miR-211 and miR-17-5P, and mRNA expressions of some of their predicted target genes namely, cyclooxygenase (COX)-2, IL-1β, steroidogenic acute regulatory protein (StAR), aromatase and estrogen receptor (ER)β. Granulosa/cumulus cells were obtained from 26 women (age range 23-42; mean age 34.8 ± 0.5) undergoing oocyte retrieval for assisted reproduction. Total RNA was extracted from these cells and reversed-transcribed and real-time PCR was performed to measure steady-state levels of miRNAs and mRNA transcripts. We observed that the expression of miR-23a, miR-542-3p, miR-211 and miR-17-5P displayed a significant and positive correlation with the expression of COX-2. Moreover, miR-23b significantly correlated with StAR mRNA expression, but not with other genes tested ($P<0.05$). With advancing age, the expression of miR-17-5p and COX-2 was significantly decreased, with COX-2 expression displaying a positive correlation with aromatase, StAR and ERβ mRNA expressions ($P<0.05$). None of the factors tested showed any significant correlations with peak estradiol levels and number of oocytes retrieved. Receiver operating characteristic curve analysis revealed that female age cut-off value of 31.5 y best predicted whether miR-17-5p was below its mean arbitrary value. In conclusion, given the importance of miRNAs' regulatory function in gene expression, our results suggest that miR-23b may play an important role in gene expression related to granulosa cell luteinization, while variation in miR-17-5p expression with female age may suggest its potential role as a predictor of oocyte quality and granulosa cell function. Given the importance of miRNAs in gene regulation, the role of miRNAs in ovarian physiology and aging requires further investigation. Support: NIH grant 37432.

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Impaired Gremlin Expression in Cumulus Cells May Underlie Reproductive Dysfunction in Young Women with Diminished Ovarian Reserve (DOR). Keri Greenseid,[1] Sangita Jindal,[1] Joshua Hurwitz,[1,2] Nanette Santoro,[1] Lubna Pal.[1] *[1]Obstetrics & Gynecology and Women's Health, Albert Einsten College of Medicine, Bronx, NY, USA; [2]Reproductive Medicine Associates of Connecticut, Norwalk, CT, USA.*
Introduction: A symbiotic relationship between ovarian granulosa cells (GC) and the enclosed developing oocyte is critical to reproductive efficiency. Genetic modulations in GC's can lead to reproductive insufficiency, highlighting the critical role of GC's in reproductive competence. Defects in the oocyte-GC repertoire in women with DOR include lower E2 levels, luteal deficiency, poor fertilization rates, higher incidence of aneuploidy, lower pregnancy and higher miscarriage rates. Utilizing global gene expression analyses in cumulus GC's, we attempt to enhance our understanding of biological mechanisms

that may contribute to poor reproductive capacity in young women with DOR. Methods: 8 infertile women (<38 years old) undergoing IVF were prospectively enrolled into two groups based on ovarian reserve (normal reserve, FSH<10 & DOR, FSH ≥10mIU/ml). At egg retrieval, cumulus GC's were isolated, RNA extracted & transcribed into cDNA. Microarray targets were generated & cDNA hybridized to Affymetrix Human Genome U133 Plus 2.0 GeneChips. Microarray data were analyzed (Array Assist) and normalized (Robust Multichip Analysis). A difference in gene expression of > 2 fold was considered biologically relevant.
Results: Of the 8,846 genes identified to be differentially expressed in young women with DOR compared to normal reserve, 215 genes demonstrated consistency of expression across five different normalization schema; 94 were down regulated and 121 up-regulated. Expression of Gremlin, a member of the DAN family of genes known for its highly regulated expression pattern during folliculogenesis, was noted to be down-regulated 3-fold over two probe sets (-3.08) in women with DOR versus normal reserve; this down-regulation was confirmed by real-time PCR (-5.33).
Conclusions: This is the first demonstration linking differential expression of Gremlin with etiology of infertility in women. Gremlin is a downstream effector of oocyte-derived GDF-9 which facilitates cumulus cell expansion, a critical event in reproductive physiology. Our finding of a significant down-regulation of Gremlin expression in cumulus GC's associated with DOR may partly explain the physiology of poor reproductive performance.
NIH K24CD41978. NIH 5K12RR17672. Ferring Pharmaceuticals.

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Gonadotropin Releasing Hormone (GnRH) Antagonist Cetrorelix Acetate (CA) Inhibits Expression of Aromatase and Mullerian-Inhibiting Substance (MIS) in Human Ovarian Granulosa Cells. Nurit Winkler,[1] Orhan Bukulmez,[3] Daniel B Hardy,[2] Bruce R Carr,[1] Kevin J Doody,[1] Carole R Mendelson.[1,2] *[1]Obstetrics and Gynecology, UT Southwestern Medical Center at Dallas, Dallas, TX, USA; [2]Biochemistry, UT Southwestern Medical Center at Dallas, Dallas, TX, USA; [3]Obstetrics and Gynecology, University of Florida College of Medicine, Gainesville, FL, USA.*
GnRH antagonists have been widely used in assisted reproduction and in the treatment of other gynecological disorders. Unlike GnRH agonists, GnRH antagonists cause immediate suppression of gonadotropin secretion without an initial undesired flare effect. To date, GnRH antagonists are thought to exert their primary actions via inhibition of GnRH binding to the GnRH receptor I (GnRHRI) on pituitary gonadotropes. However, while previous studies have demonstrated the presence of GnRHRI in the ovary and in other tissues, including the breast, placenta and immune cells, little is known about the extra-pituitary actions of the GnRH analogs. Expression of aromatase, the key regulatory enzyme in estrogen biosynthesis, is a critical marker of follicular function, while MIS expression is an important index of ovarian reserve. The objective of the present study was to investigate the effects of the GnRH antagonist CA on expression of MIS and aromatase (CYP19) using luteinized human granulosa cells obtained during in-vitro fertilization cycles and an immortalized human granulosa cell line (HGL5). The granulosa cells were cultured +/- dibutyryl cAMP (1 mM) and incubated +/- the GnRH antagonist, CA, for 24-48 h. RNA was isolated, reversed-transcribed and real-time PCR was performed to measure mRNA transcripts for ovary-specific CYPIIa and MIS. We observed that cAMP markedly induced aromatase mRNA in both the primary and immortalized human granulosa cells. Interestingly, cAMP treatment of these cells also caused an upregulation of MIS mRNA. Co-treatment of the granulosa cells with the GnRH antagonist (1-2000 nM) caused a dose-dependent suppression of MIS and CYP19IIa mRNA. These findings suggest that GnRH antagonists, in addition to their central suppressive effects on the pituitary, have direct actions on ovarian granulosa cells to inhibit cAMP-induced expression of aromatase and MIS. Given the pivotal and possibly coordinated roles of aromatase and MIS in folliculogenesis and oocyte maturation, further studies are required to examine the ovarian actions of GnRH antagonists. Supported by NIH DK031206.

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Endocrine Disruptor Bisphenol A (BPA) Reduces FSH Stimulated cyp19 Expression and Downstream Estradiol Production in Human Granulosa KGN Cells. Jakub Kwintkiewicz, Linda C Giudice. *Obstetrics/Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA.*
Objectives:
Bisphenol A (BPA), a known endocrine disruptor, is a chemical used as a plasticizer in the manufacture of polycarbonate plastics and epoxy resins and is present in multitude products, including the interior coating of food cans, milk containers, and baby formula bottles. BPA can leach into foods during heat processing and is known to exert a variety of endocrine-like effects on different cell types acting as an estrogen because it contains two hydroxyl groups in its diphenyl structure.
In this study we focused on the effects of BPA on aromatase expression and estradiol production in the human granulosa KGN cell line. We also evaluated its effects on several transcription factors crucial in cyp19 expression.
Materials and Methods:
KGN cells were cultured in F-12DMEM and were starved for 48 h before experiments. Subsequently they were treated for 48 h with vehicle (control), FSH (100ng/ml), and/or BPA (20, 40, 60, 80, 100uM). Messenger RNA expression was quantified by real time PCR and estradiol secretion was measured in supernatants by ELISA.
Results:
FSH induced a 10-fold increase in aromatase expression. BPA induced a dose-dependent decrease in cyp19 production, with the greatest effect at 100uM ($p<0.001$), resulting in 91+/-3 % (mean+/-SEM) inhibition, compared to aromatase expression induced by FSH alone. BPA also reduced levels of estradiol secretion in a dose-dependent manner, with the greatest inhibition at 100uM ($p<0.01$) resulting in 90+/-5% decrease. We also evaluated expression of transcription factors known to be involved in regulating the activity of the ovary- specific aromatase proximal PII promoter. Interestingly factors known for induction of aromatase such us steroidogenic factor-1 and GATA-4, mimic the pattern of cyp19 expression after BPA treatment, whereas, other receptors previously reported to act as aromatase inhibitor, such as PPAR gamma were up-regulated by the addition of BPA. Moreover, expression of CREB remained virtually intact, suggesting that most likely mechanisms governing endocrine disruption by BPA are highly selective.
Conclusions:
BPA inhibited FSH stimulated aromatase expression and downstream estradiol secretion. We propose that constant exposure to this chemical may result in endocrine disruption which may contribute to reduced fertility and early ovarian senescence.

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Follicle Reassembly Using a Novel Substrate-Free Three-Dimensional Culture System. Jared C Robins,[1] Sandra A Carson,[1] Bala Bhagavath,[1] Jeffrey R Morgan.[2] *[1]Department of Obstetrics and Gynecology, Women and Infants Hospital/Warren Alpert School of Medicine at Brown University, Providence, RI, USA; [2]Department of Molecular Pharmacology, Physiology and Biotechnology, Brown University, Providence, RI, USA.*
Oocyte maturation occurs during folliculogenesis as a result of complex cell-to-cell communications between the granulosa cells and the oocyte. Maintaining the granulosa cells' spherical structure and network of gap junctions surrounding the oocyte is critical. This project tests the ability of a novel substrate-free three-dimensional culture system to form viable granulosa cell spheroids.
Methods: After IRB approval, freshly obtained follicular fluid from in vitro fertilization was obtained and granulosa cells were purified by percol gradient. Nonadhesive agarose hydrogels, containing 822 cylindrical round bottom recesses 200μm in diameter, were cast from micro-molds designed using computer-assisted rapid prototyping. Granulosa cells seeded at a density of 800,000 cells per gel were incubated for up to 10 days. Cellular viability was assessed with live:dead assay.
Results: After three days in culture, granulosa cells formed spheroids of densely packed cells that were difficult to disperse with multiple pipettings. The cells remained viable for at least 10 days.
Conclusions: Granulosa cells can be cultured in a novel substrate-free three-dimensional culture system. The cells form tightly adherent spheroids that remain viable for extended culture. The cohesiveness of the cells suggests the formation of gap junctions. This is under investigation with immunohistochemistry and electron microscopy. These experiments suggest that a substrate-free three-dimensional hydrogel culture system may be ideal to reassemble follicular structure important for future in vitro evidence testing and oocyte maturation.

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Acute-Phase Protein Serum Amyloid A (SAA) and Ovarian Function. Courtney A West,[1] Linda R Nelson,[1] Katherine F Roby.[2] *[1]Department of Obstetrics and Gynecology, University of Kansas School of Medicine, Kansas City, KS, USA; [2]Depratment of Anatomy an Cell Biology, University of Kansas School of Medicine, Kansas City, KS, USA.*
Objective: Serum Amyloid A (SAA) proteins are acute phase apolipoproteins

known to function as responders to pathogens, inflammation, and tissue injury. Previous studies in our laboratory demonstrated that SAA was produced in mouse granulosa and production was regulated by cytokines. Ovulation has long been considered an inflammatory reaction and patients with chronic inflammatory conditions often experience infertility. The present study was undertaken to explore the role of SAA in human ovarian function.
Methods: Ovarian granulosa and luteal cells were obtained from surgically removed specimens and mural and cumulus granulosa-luteal cells were obtained from IVF aspirates. RNA was extracted from fresh or cultured cells. Some cells were treated in vitro with TNFα or other cytokines for 24h. Expression of SAA was assessed by quantitative RT-PCR. In addition, serum levels of SAA were determined using a commercial ELISA in women undergoing ovulation induction (OI) and ART. SAA levels were measured at baseline, during OI, on the day of hCG administration and at the time of the pregnancy test.
Results: SAA mRNA was expressed in theca, granulosa, and granulosa-luteal cells. In granulosa-luteal cells both SAA1 and SAA2 mRNAs were expressed at higher levels in cumulus compared to mural granulosa. Expression of SAA1 and SAA2 in theca was increased following treatment with TNF in vitro. Serum levels indicated that patients with ovulatory dysfunction had increased levels of SAA at the time of hCG injection while patients without ovulatory dysfunction had lower SAA levels as compared to the baseline level. In addition, patients undergoing OI who achieved pregnancy exhibited increased levels of SAA at the time of the pregnancy test compared to baseline levels, whereas patients who did not become pregnant had lower post-cycle levels of SAA. Interestingly, SAA levels did not change in ART patients that became pregnant without undergoing OI (donor egg or frozen embryo transfer)
Conclusions: Human ovarian cells express SAA mRNAs which can be altered in vitro. Serum levels of SAA may correlate with the responsiveness of the ovary to gonadotropins as evidenced by altered levels in women with ovulatory dysfunction, and by an increase in pregnant patients following ovarian stimulation.

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Dose-Related Decreases in the Expression of Super-Oxide Dismutase 2 (SOD2) in Ovarian Structures Following Cisplatin Administration. John Yeh, Beom Su Kim, Felicia Hercules, Jennifer Peresie, Armando Arroyo. *Gynecology-Obstetrics, University at Buffalo, Buffalo, NY, USA.*
Objective: Cisplatin is a common chemotherapeutic agent given to women for treatment for a wide variety of cancers. We hypothesize that one mechanism by which cisplatin may cause damage to ovarian structures is by decreasing the amount of anti-oxidant activity in the ovary. We examined super-oxide dismutase 2 (SOD2), a critical anti-oxidant enzyme that has been shown to be affected by cisplatin in other tissues, in the ovaries of cisplatin treated animals.
Methods: Adult female Sprague Dawley rats were injected with saline, cisplatin 4.5 mg/kg or cisplatin 6.0 mg/kg as 2 weekly doses. Five days following the last injection, the rats were euthanized and both ovaries were excised. One ovary was processed for immunohistochemistry and the other was processed for protein analysis using western blot techniques for SOD2. The anti-SOD2 antibody was purchased from Santa Cruz. The immunohistochemical sections were scored using a semiquantitative H scoring method.
Results: Immunohistochemistry analysis of the expression pattern of SOD2 following cisplatin administration revealed that there was a significant linear decrease in a dose response pattern in the expression of SOD2 in antral follicles and in corpora lutea ($p<0.01$ for both). No change was found in the H score of SOD2 in other ovarian structures. Western blot analysis of SOD2 in the ovaries following increasing doses of cisplatin revealed no changes in the overall protein levels of SOD2 in the ovary.
Conclusions: This is the first report that administration of cisplatin causes changes in the expression pattern of SOD2 in antral follicles and in copora lutea. Cisplatin decreases the amount of SOD2 available in these structures, possibly leading to increased oxidative stress and free radical damage, thereby leading to ovarian damage found after cisplatin administration.

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Is There Evidence for Aromatase Activity in the Stroma of Postmenopausal Ovaries? MF Landay, RH Fogle, RB Allen, S Patel, FZ Stanczyk, RJ Paulson. *Ob/Gyn, USC, Keck School of Medicine, Los Angeles, CA, USA.*
Background: Following menopause, the ovaries continue to secrete androgens and estrogens. We have recently confirmed the production of androstenedione, testosterone and estradiol (E_2) up to ten years after menopause by measuring gradients from ovarian venous effluent and peripheral blood. Anti-müllerian hormone (AMH) and inhibin B have been shown to be markers of follicular activity. Peripheral levels of these hormones have previously been found to be undetectable in menopause, suggesting the absence of follicular activity in the postmenopausal ovary.
Objective: To investigate if the postmenopausal ovary continues to demonstrate evidence of follicular activity as the source of steroid production.
Design: Observational study
Materials and Methods: Eight subjects aged 53 ± 2.7 yr (range 48-56) were enrolled. Postmenopausal status was confirmed by preoperative FSH levels of more than 40 U/L and/or amenorrhea greater than 12 months. Serum was collected from the ovarian veins during total abdominal hysterectomy and bilateral oophorectomy. Peripheral blood was also collected pre-operatively, intraoperatively and postoperatively. All samples were analyzed for AMH and inhibin B using ELISAs with sensitivities of 0.05 ng/mL and 7 pg/mL, respectively. Androgen and estrogen levels in these samples have previously been reported, and documented a gradient between ovarian venous effluent and peripheral serum in all cases.
Results:
1) Six patients had no detectable follicular activity by AMH and inhibin B levels.
2) One patient demonstrated detectable inhibin B levels with an 7-fold gradient between ovarian venous effluent (226 pg/mL) and peripheral blood (31 pg/mL), however no AMH was detected.
3) In one patient, aged 52 and 120 months postmenopause, both AMH and inhibin B were detected. Peripheral inhibin B levels were high at 912 pg/mL. AMH was detectable at levels of 0.95 ng/mL.
Conclusions:
1) In the majority of patients, continued E_2 and androgen production in the ovary occurs in the absence of follicular activity as detected by AMH and inhibin B production.
2) Some patients have evidence of follicular function up to ten years after menopause.
3) Since E_2 is produced in post-menopausal ovaries in the absence of follicular activity, these data provide evidence for aromatase activity in the stroma of post-menopausal ovaries.

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Prostaglandin F2α Suppresses Cholesterol Uptake, Transport, and Processing Via Induction of Yin Yang 1 Protein and Recruitment of Histone Deacetylase in the Corpus Luteum. Mark P McLean,[1,2] Ricardo Bravo,[1] Oleg Kuzmenok,[1] Xiaohui Zhang,[1] Kathleen A Merkler,[1] Qiyuan Liu.[1] *[1]Department of Obstetrics & Gynecology, University of South Florida, Tampa, FL, USA; [2]Molecular Pharmacology & Physiology, University of South Florida, Tampa, FL, USA.*
To elucidate the process by which prostaglandin F2α (PGF2α) mediates luteal regression, this study examined the role that the transcription factor Yin Yang 1 (YY1) and histone deacetylase 1 (HDAC1) play in altering luteal cholesterol uptake by the scavenger receptor class B type I (SR-BI), intracellular cholesterol transport by steroidogenic acute regulatory protein (StAR), and cholesterol processing by P450 side chain cleavage enzyme (P450scc) expression. Rat luteal cells (10 days post-ovulation) were treated with PGF2α (24 hr) and Trichostatin A (TSA; 24 hr), a potent HDAC inhibitor. Protein expression was measured post-treatment by Western blot and cholesterol was localized via filipin staining. StAR and SR-BI promoter activation was also assessed in HEK 293T cells treated with YY1, mYY1, a deletion mutant lacking an essential region required for transcriptional repression, and TSA. PGF2α caused a significant 2-fold decline in StAR ($P<0.005$), and smaller declines in SR-BI and P450scc which occurred concomitantly with an increase in YY1 (4-fold, $P<0.001$) and intracellular lipid staining (20% increase). In contrast, 100 nM TSA treatment caused a dose dependent increase in SR-BI, StAR, and P450scc protein levels, 3.5-fold ($P<0.005$), 1.4-fold ($P<0.05$), 4.1-fold ($P<0.005$), respectively, and a 2.5-fold decline ($P<0.05$) in intracellular lipid levels. TSA prevented the PGF2α-induced decline in SR-BI, StAR, and P450scc expression. Promoter analysis demonstrated that wildtype YY1, but not mYY1, repressed SR-BI and StAR activation while the addition of TSA overcame the repressive effects. This study shows the critical role that YY1 plays in $PGF2_{\alpha}$ induced luteal regression by recruiting a histone deacetylase to block three essential processes in steroid production. In luteal cells YY1-mediated global repressive action prevents cholesterol metabolism by inhibiting cholesterol uptake, intracellular transport, and processing thus leading to functional and structural luteal demise. Supported by NIH HD35163 and NIH HL78817.

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Regulation of Intermedin Expression in Cycling Rat Ovary. Madhu Chauhan, Rebekah Elkins, Chandra Yallampalli. *Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

BACKGROUND: Intermedin (IMD) is a CT/CGRP family peptide involved in a variety of physiological functions, including vasodilatation and feto-placental growth. This peptide is expressed in a variety of tissues such as stomach, placenta, uterus, pituitary and ovary suggesting its different functions including in reproduction. IMD gene has an estrogen response element and we have shown that the plasma concentration of immuno-reactive IMD is elevated in rats with pregnancy. However, expression of IMD in the ovary and its regulation during estrous cycle is not known. We hypothesize that IMD is expressed in the ovary and its expression is hormonally regulated throughout the estrous cycle in rat.

OBJECTIVE: 1) To assess expression of IMD mRNA and its receptors components calcitonin receptor like receptor (CRLR), and receptor activity modifying proteins, RAMP1 and RAMP3 mRNA in rat ovary; on diestrus, proestrus and estrus stages of rat estrus cycle and ; 2) to demonstrate immunohistolocalization of IMD, CRLR, RAMP1, RAMP2 and RAMP3 in rat ovary.

METHODS: Groups of 4 Sprague-Dawley non-pregnant and pregnant rats on day 18 of gestation were used in this study. Non-pregnant rats were sacrificed on diestrus, proestrus and estrus stage. Ovaries were collected and total RNA was extracted using Trizol method. RNA was treated with DNase1 before performing the reverse transcriptase polymerase chain reaction (RT-PCR). Immunohistolocalization of IMD, RAMP1, RAMP2 and RAMP3 proteins were assessed in tissue sections of ovaries from pregnant rats sacrificed on day 18.

RESULTS: 1) IMD mRNA is regulated in rat estrus cycle and its expression is significantly downregulated in estrus stage compared to the diestrus and proestrus; 2)Expression of IMD receptor CRLR is highest in the diestrus stage, followed by a decline in proestrus which further declined during estrus; 3) expression RAMP1 mRNA is higher in proestrus compared to diestrus and estrus ($p<0.05$) but there is no significant change in the RAMP 3 expression during the estrus cycle and ; 4) IMD, RAMP1, RAMP2 and RAMP3 are immunolocalized in rat ovary in granulose cells of all follicles and granulosa cells of the corpus luteum.

CONCLUSION: IMD mRNA and protein are expressed in rat ovary suggesting a possible role for IMD in ovarian function.

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Defining and Defying Deterioration in Oocyte Quality with Advancing Chronological Age: Role of Nitric Oxide. PT Goud,[1] AP Goud,[1] MP Diamond,[1] B Gonik,[2] HM Abu-Soud.[1] *[1]Div Reprod Endocrinology, Dept Ob/Gyn, Wayne State University, Detroit, MI, USA; [2]Div Maternal and Fetal Medicine, Sinai Grace Hospital, Wayne State University, Detroit, MI, USA.*

Nitric oxide (NO) is a ubiquitous free radical essential for oocyte maturation, function and sustenance of oocyte quality post-ovulation. The current study investigates the role of NO insufficiency in the causation of oocyte quality deterioration with advancing chronological age.

Methods: In set 1, oocytes were retrieved from the following superovulated B6D2F1 mice: (a) young breeders (YB, n=109); (b) retired breeders (RB, n=40), and © old animals (OA, n=16), aged 6-12, 45-52, and 70-75 weeks respectively; and studied for zona pellucida dissolution time (ZPDT), spindle (α-tubulin fluorescence immunocytochemistry, Sigma) and chromosome morphology (DAPI, Vector), ooplasmic microtubule (MT) dynamics (OMD) in response to taxol [1], and cortical granule (CG) status (rhodamine conjugated lectin, Vector). In set 2 (n=56), oocytes from retired breeders were studied after exposure *in vitro* to an NO-donor, *S-nitroso acetyl penicillamine* (SNAP in M-16, ≈0.23μM NO/min , n=23, 3 h, 37°C, 5% CO_2), while their sibling control oocytes were cultured for corresponding period under identical conditions without SNAP.[1]. ZPDT, spindle and chromosome morphology, OMD and CG status were assessed with a confocal microscope and compared between the subgroups using ANOVA, chi square and/or Fisher's exacts test and appropriate post-hoc tests.

Results: In set 1, a significantly fewer oocytes were retrieved per animal (mean) in RB (10) and OA (4) compared to YB (27.3, $P<0.05$). Advancing age was also associated with a significant increase in ZPDT, OMD and CG loss in RB compared to YB ($P<0.01$). Furthermore, significantly fewer oocytes from RB than YB had normal spindle and chromosome morphology ($P<0.05$). Oocytes from OA had significant spindle and chromosome disarray, a near total CG loss and significantly harder ZP ($P<0.01$). These oocytes also exhibited diminished OMD in response to taxol although, metaphases were exquisitely sensitive to disruption with taxol. In set 2, exposure to SNAP in oocytes from RB resulted in a significantly lowerZPDT, OMD and CG loss, and significantly higher incidence of normal spindles (74.1%) compared to unexposed RB oocytes (27.6%, $P<0.05$).

Conclusion: Oocyte quality deterioration with advancing age may be related to NO insufficiency. [1.*Biochemistry* 2005; 44:11361-8].

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Functional GABA-A Receptors in Primary Cultures of Human Granulosa-Lutein Cells (GC). Li Hou,[1] Mary Charmaine Belcastro,[2] Tamer M Yalcinkaya.[1] *[1]Dept. of Ob/Gyn, Wake Forest University School of Medicine, Winston-Salem, NC, USA; [2]West Virginia University School of Medicine, Morgantown, WV, USA.*

The gamma-aminobutyric acid (GABA), its biosnthetic enzyme GAD and GABA-A receptors have been found in the oviduct and ovary. Objective: This study examined the expression of GABA-A receptor subunit genes and GAD and whether the GABA-A receptor could alter cytosolic Ca2+ in GC.

Methods: For qRT-PCR, both human cumulus and mural GC were obtained at the time of oocyte retrieval for IVF and cultured separately. Total RNA was isolated separately from each GC type and from human brain (positive control). The total RNA from 16 patients was pooled for each GC type and all RNA was treated with DNAse I. Two-step qRT –PCR was performed using gene-specific LUX™ primers for all 18 GABA-A receptor subunits, GAD65 and GAD67 plus GAPDH. Single and specific qRT-PCR products were verified by melting curve analysis, gel electrophoresis, and DNA sequencing. For Ca2+ study, GC were cultured on coverslips. GC were loaded with Fura-2-AM and changes in Ca2+ concentration of GC were studied using a dynamic digital Ca2+ imaging system. GABA-A agonist, muscimol, was used to study any dose-dependent effects on GC. GABA-A antagonist, 10 μM bicuculline, were perfused 1 min. prior to and during application of muscimol. The responses were represented as changes in the 340/380 nm fluorescence ratio over time. 50 μM ATP was used as positive control.

Results: The qRT-PCR results indicated that all GABA-A receptor subunits were expressed to various degrees in both types of GC, with the α5 expressed highest in both cell types. GABA-A receptor subunits showing the next highest expression in both cell types were β3, γ3 and ρ2. GAD67 isoenzyme was more abundantly expressed in cumulus and mural GC than GAD65. Ca2+ imaging showed that muscimol, had the ability to increase Ca2++ in GC, about 19% GC (n=6) cells responded to muscimol. Muscimol increased intracellular Ca2+ in a dose-dependent manner. The muscimol responsive cells was reduced by bicuculline, from 19% to 1 % (n=6, $p< 0.05$).

Conclusion: qRT-PCR indicates that GABA-A receptor subunit gene and GAD67 expression occurs in both cumulus and mural GC. The ability of bicuculline to inhibit the Ca2+ response to muscimol suggests the activation of GABA-A receptor. The current study confirms the presence of functional GABA-A in GC for the first time, and suggests that GABA may exert trophic effects in the ovary via GABA-A receptor.

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Effect of Nitric Oxide Synthase Inhibition on the Activation of p38 Mitogen-Activated Protein Kinase (p38 MAPK), Extracellular Signal-Regulated Kinase (ERK) and c-Jun N-Terminal Kinase (JNK) during Hypoxia in Cerebral Cortical Nuclei of Guinea Pig Fetus during Gestation. Qazi M Ashraf,[1] Dev Maulik,[2] Om P Mishra,[1] Maria Delivoria-Papadopoulos.[1] *[1]Department of Pediatrics, Drexel University College of Medicine, Philadelphia, PA, USA; [2]Department of Obstetrics and Gynecology, University of Missouri, Kansas City, MO, USA.*

The present study test the hypothesis that administration of L-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor, prior to hypoxia prevents the hypoxia-induced activation of p38 MAPK, ERK and JNK in the cerebral cortex of the guinea pig fetus during gestation. To test this hypothesis 34 guinea pig fetuses at 35 and 60 days gestation were assigned to normoxic (Nx, n=6), hypoxic (Hx, n=6) and hypoxic pretreated with NOS inhibitor (Hx+L-NAME, 30mg/kg i.p., n=5) groups. Hypoxia in the fetuses was induced by exposing the pregnant guinea pigs at both gestational ages to an FiO_2 of 0.07 for 60 min. Cerebral tissue hypoxia was documented biochemically by determining the tissue levels of ATP and phosphocreatine (PCr). Neuronal nuclei were isolated, purified and proteins separated by SDS-PAGE, and then probed with specific phosporylated ERK, JNK and p38 antibodies. In the 35days gestation group: Expression of p-p38 was 39.5±3.4 (Nx), 79.1±2.8 (Hx) 47.1±4.1 (Hx+L-NAME). p-ERK expression was 59.7±3.2 (Nx), 109.6±6.3 (Hx), 72.4±2.7 (Hx+L-NAME). p-JNK expression was 68.3±7.2 (Nx), 94.6±3.8 (Hx), 55.8±9.0 (Hx+L-NAME).

In the 60 days gestation group: Expression of p-p38 was 108.7±3.6 (Nx), 231.1±8.3 (Hx), 116.5±9.8 (Hx+L-NAME). p-ERK expression was 94.1±12.9 (Nx), 226.5±9.1 (Hx), 139.1±12.6 (Hx+L-NAME). p-JNK expression was 83.2±9.5(Nx), 218.2±6.9 (Hx) 105.3±10.8 (Hx+L-NAME). The data show that administration of L-NAME prior to hypoxia decreased the expression of phosporylated p38, ERK and JNK at both gestation ages however expression of phosporylation was higher at term as compared to preterm. Since a NOS inhibitor prevented the hypoxia-induced phosphorylation of p38, ERK and JNK in both gestational ages, we conclude that the hypoxia-induced activation of p38, ERK and JNK in the cerebral cortical nuclei of preterm and term guinea pig fetus is NO-mediated. We speculate that NO-mediated modification of cysteine residue leading to inhibition of MAP kinase phosphatases results in increased activation of p38, ERK and JNK in the guinea pig fetus. (NIH-HD 20337, NIH-HD 38079 and St. Christophers Foundation for Children).

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The Outcome of Pregnancies Complicated by Threatened Miscarriage Can Be Predicted by Plasma Anandamide Concentrations. Anthony H Taylor,[1] Osama MH Habayeb,[1] Mark D Evans,[2] Marcus S Cooke,[2] Stephen C Bell,[1] David J Taylor,[1] Justin C Konje.[1] *[1]Endocannabinoid Research Group, Reproductive Sciences Section, Department of CSMM, University of Leicester, Leicester, United Kingdom; [2]Radiation and Oxidative Stress Section, University of Leicester, United Kingdom.*

Background: The endocannabinoid, anandamide (AEA), is involved in the hormone-cytokine network that regulates implantation and early pregnancy maintenance with levels at the endometrial level considered a major checkpoint[1]. High levels (28nM) in culture are associated with embryo death[2] while plasma levels (>4nM) at 6 weeks in women undergoing IVF-ET are associated with failed pregnancy[1]. What is uncertain is whether systemic AEA levels after spontaneous conception in women presenting with threatened miscarriage are predictive of pregnancy outcome. Our aim was therefore to measure plasma AEA levels in women presenting with threatened miscarriage and to relate these to outcomes.

Methods: Plasma AEA levels were measured using a sensitive and previously validated HPLC-MS method[3] at 6-12 weeks gestation in 45 women (non-smokers, BMI <30Kg/m^2) presenting with threatened miscarriage and in whom a viable pregnancy was confirmed by ultrasound scan.

Results: Nine of the 45 women subsequently had a miscarriage. The plasma AEA levels in those women who had live births was ~2.85-fold lower than that in those who subsequently miscarried (1.21 ± 0.12nM versus 3.45 ± 0.31nM, Mean ± sem; respectively; P<0.0001 unpaired Student's t-test). The ROC analysis revealed an area under the curve of 0.972 ± 0.025 with a sensitivity of 100% (95%CI of 66.3% to 100.0%) and a specificity of 94.4% (95% CI of 81.34% to 99.3%). Using an AEA level of 2.0nM as the optimal cut-off point, a single plasma AEA measurement provided a sensitivity of 100% and a specificity of 94% with a negative predictive value of 100% and a positive predictive value of 78% for subsequent miscarriage.

Conclusion: These findings suggest a possible predictive role for plasma AEA in women presenting with threatened miscarriage. The data also indicate that systemic AEA levels may reflect local endometrial levels and therefore the local hormonal milieu in the early stages of normal pregnancies and in those complicated by threatened miscarriage.

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Subcellular Localization and Functional Capacity of Follicle Stimulating Hormone Receptor (FSHR) Splice Variants Detected in Patients with High and Low Response to FSH. Dimitrios Zattas, Tsilya Gerasimova, Emre Seli, Denny Sakkas, Maria D Lalioti. *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Introduction: Follicle stimulating hormone (FSH) mediates cyclic follicle growth and development and is widely used for controlled ovarian stimulation. The ovarian response of different women to FSH is variable, ranging from poor response to ovarian hyperstimulation and has been partly attributed to two common variants of the FSH receptor (FSHR). We have previously identified four abnormal FSHR splicing products (3 exon deletions and 1 intron insertion) in women with low and high response to FSH. Two of the splice variants, deletion of exon 2 and deletion of exon 6, showed a correlation with low and high response to FSH, respectively. In the current study, we evaluated the functional competence of the mutant FSHRs in vitro.

Methods: We established stable HEK293 cell lines expressing wild-type (wt) and splice-variant (del) FSHR under the control of the inducible Tet on/off system. The cells were transfected with a cAMP-responsive luciferase reporter plasmid and stimulated with FSH.

Results: The subcellular distribution of all splicing variants was the same as the controls and the protein localized mainly on the cell surface. All four splicing variants showed markedly decreased cAMP activation compared to controls when stimulated with FSH. However, all variants were able to form functional heterodimers with the wt receptor when co-expressed. Interestingly, the heterodimer containing the form of FSHR lacking exon 2, found in patients with decreased response to FSH, resulted in lower intracellular cAMP compared with the wild-type homodimer.

Conclusion: Our findings suggest that FSHR variants can contribute to abnormal response to stimulation in certain women undergoing IVF treatment. The variants require the presence of wild-type receptor in order to initiate signaling in response to FSH. Further analysis of this signaling cascade in granulosa cells is underway to estimate the final production of estrogen from these heterodimeric receptors.

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Proteomic Analysis of Pre-Receptive vs Receptive Human Endometrium. Francisco Dominguez,[1] Alicia Quinonero,[1] Juan Antonio Lopez,[2] Emilio Camafeita,[2] Enrique Calvo,[2] Marco Melo,[1] Jose Antonio Horcajadas,[1] Antonio Pellicer,[1] Carlos Simon.[1] *[1]Fundacion IVI., Instituto Universitario IVI. UNiversidad de Valencia, Valencia, Spain; [2]Proteomics, CNIC, Madrid, Spain.*

Objective: To compare the proteome of human endometrium in the pre-receptive versus the receptive phases of the menstrual cycle.

M&M: Endometrial biopsies were collected 2 and 7 days after urinary LH surge in the same menstrual cycle from three fertile women (n=3). Proteins were extracted using Sample Grinding Kit (GE Healthcare) and interfering substances removed using 2-D Clean-Up Kit (GE Healthcare). Labelling was performed with CyDye DIGE fluors (GE Healthcare) and proteins were separated using Difference Gel Electrophoresis (DIGE). For the isoelectric focusing, 24 cm IPG-strips in the nonlinear range of pH 3-11 were used. The second dimension was carried out using SDS-PAGE. Differentially expressed proteins were detected by image analysis using DeCyder v6.5 and the statistical module EDA (GE Healthcare). The spots of interest were subject to protein identification based on in-gel digestion, MALDI-TOF/TOF mass spectrometry and database searching. Western Blot analysis were performed in the same biopsies in order to validate some candidate proteins.

Results: Table 1 displays the differentially protein abundance between days LH+2 and LH+7 (> 2-fold change). Of these proteins, 10 were increased at LH+7 in comparison with LH+2, whereas only 2 proteins were decreased. Stathmin was found more than 2-fold decrease in LH+7 compared to LH+2 in the three patients studied in the validation studies performed by western Blot.

Conclusions: This study shows that the human endometrium has a differential protein repertoire during the window of implantation compared to the pre-receptive phase. The role of these proteins in the molecular events directed to embryo implantation is under research.

Differential protein abundance in human endometrium (receptive vs pre-receptive)

FOLD CHANGE	PROTEIN	GENE ONTOLOGY TERMS
- 2.38	Membrane-associated progesterone receptor component 1	Hormone receptor
- 2.22	Stathmin	Cytoskeletal protein binding
2.06	Anterior gradient protein 2 homolog precursor	
2.12	Annexin A2	Cytoskeletal protein binding
2.92	Myosin-9	Cytoskeletal protein binding
3.8	Isocitrate dehydrogenase [NADP] soluble	Oxidoreductase
4.31	Protein S100A10	Regulation of cellular process
5.02	Homogentisate 1,2-dioxygenase	Oxidoreductase

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ERK Expression and Activity in Human Myometrium and Leiomyoma. Tugba Altun,[1] William Murk,[1] Yesim H Uz,[2] Sinem Karipcin,[1] Umit A Kayisli,[1] Aydin Arici.[1] *[1]Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA; [2]Histology and Embryology, Trakya University School of Medicine, Edirne, Turkey.*

Background: The extracellular signal-regulated kinases 1 and 2 (ERK1/2) are a mitogen-activated protein kinase (MAPK) subfamily that act as key links in eukaryotic intracellular signaling transduction. Activated by phosphorylation in response to specific stimuli, ERK1/2 is known to play a role in the regulation of cellular proliferation and survival. The human myometrium is a tissue known to undergo cycle-dependent proliferative and apoptotic changes in response to sex steroids. We hypothesized that ERK1/2 activity is involved in mediating menstrual cycle-dependent changes in the myometrium.

Materials and Methods: Immunostaining for phospho-ERK and total-ERK was performed on myometrial tissues obtained from normal women (n=20) at different phases of the menstrual cycle. Staining intensities were evaluated by HSCORE. Myometrial smooth muscle cells were isolated and cultured from normal women and treated with vehicle, estrogen (10-8M), and progesterone (10-8M) for 5 and 15 minutes, and then subjected to Western blot analysis for P- and T-ERK. Statistical analysis was performed using one-way ANOVA.

Results: Tissue staining revealed that P-ERK was mostly nuclear in all tissues, while T-ERK was cytoplasmic and nuclear. P-ERK staining was significantly stronger in the secretory phase and strongest at the early secretory phase, compared to other phases ($p<0.05$). T-ERK staining intensity was moderate-high without variation across the menstrual cycle. In cultured myometrial cells, progesterone significantly increased P-ERK levels within 5 and 15 minutes ($p<0.05$) when compared to control.

Conclusions: Our results suggest that ERK activity in the human myometrium is regulated in a menstrual cycle-dependent manner. The increased phosphorylation in the secretory phase suggests the involvement of progesterone in ERK activation, as supported by our in vitro results. This increased ERK activity may play a role in regulating myometrial proliferation.

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Serum Advanced Glycation End-Products in Saudi Women with Polycystic Ovary Syndrome: A Prospective Study. Abdulrahim A Rouzi,[1] Mohamed M Ardawi.[2] *[1]Obstetrics and Gynecology, King Abdulaziz University, Jeddah, Western, Saudi Arabia; [2]Clinical Biochemistry, King Abdulaziz University, Jeddah, Wetern, Saudi Arabia.*

Objective: To determine the serum levels of advanced glycation end-products (AGE) in relation to specific receptors of AGE (RAGE) in women with and without polycystic ovary syndrome (PCOS).

Design: A prospective case control study in a tertiary referral university hospital.

Materials and Methods: One hundred Saudi women living in the Jeddah area were classified as follows: 50 with PCOS and 50 age-matched women without PCOS. Blood samples were collected from all women with or without PCOS between 8:00-11:00, after an overnight fast. The body mass index (BMI) (Kg/m^2), waist-to-hip-ratio (WHR) and serum levels of LH, FSH, TSH, FT_4, 17-OHP, 4-A, DHEAs, total T, free T, SHBG, insulin, free androgen index (FAI), and plasma levels of glucose were determined. Measures for insulin resistance (IR) including: fasting serum insulin, GIR and HOMA and measures of serum AGE and the expression of their RAGE in circulating monocytes were also determined.

Results: Women with PCOS exhibited increased levels of serum AGE proteins as compared with women without PCOS (10.35 0.20 vs 5.67 0.18; $P < 0.0001$), together with increased RAGE expression in monocytes: PCOS (31.63 8.22) vs without PCOS (6.58 2.47) ($P < 0.05$), respectively. Positive correlations were evident between AGE proteins and total T ($r = 0.68$, $P < 0.001$), FAI ($r = 0.55$, $P < 0.001$); WHR (0.35, $P < 0.05$); insulin ($r = 0.51$, $P < 0.01$); HOMA ($r = 0.48$, $P < 0.001$); and RAGE ($r = 0.63$, $P < 0.05$), respectively. A negative correlation was evident between AGE proteins and GIR ($r = -0.42$, $P < 0.05$). Using multiple regression analysis, total T was found to be the only independent predictor of AGE values ($P < 0.001$); $b = 0.065$) among various variables including: BMI, WHR, insulin, SHBG and HOMA (adjusted $R^2 = 0.63$, $P < 0.001$).

Conclusions: Women with PCOS exhibited significantly higher levels of serum AGE and RAGE monocyte-expression as compared with age-matched control without PCOS.

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The Role of Anti-Müllerian Hormone (AMH) in the Classification of Anovulation. Sharon Lie Fong,[1] Olivier Valkenburg,[1] Izaak Schipper,[1] Axel PN Themmen,[2] Frank H de Jong,[2] Joop SE Laven.[1] *[1]Division of Reproductive Medicine, Obstetrics and Gynaecology, Erasmus MC, University Medical Centre, Rotterdam, Zuid-Holland, Netherlands; [2]Internal Medicine, Erasmus MC, University Medical Centre, Rotterdam, Zuid-Holland, Netherlands.*

Context: Currently, serum gonadotrophin and oestradiol levels are being used for the classification of anovulation, according to the WHO criteria. In hypoestrogenic hypogonadotropic patients (WHO I anovulation), the number of follicles is normal. On the contrary, in normogonadotropic, normoestrogenic (WHO II) patients, the number of follicles is increased, whereas in hypoestrogenic hypergonadotropic patients (WHO III), the follicle pool is depleted. Since serum AMH levels correlate well with the number of early antral follicles, it was hypothesized that serum AMH levels will be normal in WHO I patients, increased in WHO II patients and decreased in WHO III patients. Hence, AMH might replace serum gonadotrophin and oestradiol assessments in the diagnosis of anovulation.

Objective: To asses the correlation of AMH levels with the WHO classes for anovulation.

Design: Patients with oligomenorrhea or secondary amenorrhea, up to the age of 40, were recruited. Standard endocrinological screening for anovulation was performed, including transvaginal ultrasound and serum AMH assessment.

Results: In WHO I patients, 83% had normal AMH levels and 17% had elevated AMH levels. Furthermore, only 50% of the WHO II patients had increased serum AMH levels. The majority (90%) of WHO III patients had AMH levels lower than controls, and 10% had normal AMH levels. Serum AMH concentrations were well correlated with the mean number of follicles ($r = +0.63$; $P < 0.001$). Increased AMH levels were moderately (sensitivity 79%) predictive for the presence of polycystic ovaries (PCO). Furthermore, in 54% of the patients diagnosed with polycystic ovary syndrome (PCOS), according to the Rotterdam 2003 consensus criteria, AMH levels were within the normal range. Therefore, based on AMH levels, the presence of PCOS could not be predicted (sensitivity 63.9%).

Conclusion: In anovulatory women, serum AMH did not seem to be a reliable substitute for the classification of anovulation. Although increased AMH levels were well correlated with the number of follicles, increased AMH levels were not predictive for PCO or PCOS.

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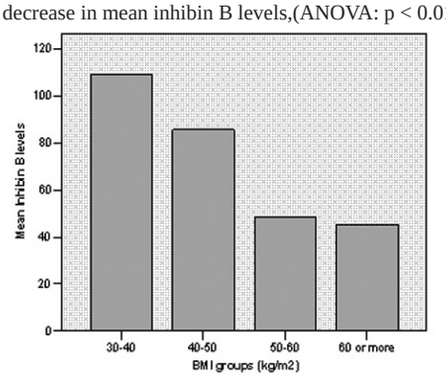
Determinants of Inhibin B Levels in Obese Males. Ahmad O Hammoud, Douglas T Carrell, A Wayne Meikle, Steven C Hunt, Ted D Adams, Mark Gibson. *University of Utah, Salt Lake City, UT, USA.*

Objectives: Obesity is associated with decreased inhibin B in the adult male and female. The purpose of this study was to measure inhibin B levels in obese men and to correlate these levels with various hormonal parameters known to be altered in obesity.

Methods: 64 men with BMI ≥ 35 kg/m2 were included in this study. At enrollment, study participants filled out multiple questionnaires, had detailed anthropometrics including BMI and percent body fat (bioelectrical impedance), and had comprehensive blood analyses including chemistry and various reproductive and non reproductive hormones: insulin, glucose, C-reactive protein, leptin, adiponectin, estradiol, FSH, LH, total and free testosterone and inhibin B levels. Inhibin B levels were measured using the *DSL Inhibin B ELISA kit.*

Results: In our cohort, increasing BMI was associated with a significant decrease in mean inhibin B levels,(ANOVA: $p < 0.016$),



Simple correlations showed that Inhibin B correlated negatively with FSH (r=-

0.31, p=0.029), estradiol (r=-0.35, p=0.013) and positively with Adiponectin (r=0.27, p=0.052). After correction for various parameters and exclusion of patients with diabetes (n=14), the linear regression showed that estrogen, FSH and adiponectin levels were useful in predicting inhibin B levels.

Table 1	Standardized Coefficients Beta	95% Confidence Interval for Beta		P value
(Constant)	92.7	-18.8	204.2	0.10
AGE	0.6	-0.8	2.0	0.38
HOMA-IR	-2.7	-6.6	1.2	0.17
%BODY FAT	-0.53	-2.4	1.4	0.58
C-REACTIVE PROTEIN	-12.2	-46.3	21.9	0.48
LEPTIN	0.32	-0.4	1.0	0.37
SEX HORMONE BINDING GLOBULIN	-0.015	-0.8	0.8	0.97
ESTRADIOL	-0.6	-1.1	-0.2	0.011*
ADIPONECTIN	4.44	0.05	8.8	0.047*
FOLLICLE STIMULATING HORMONE	-5.67	-10.1	-1.3	0.013*

When BMI was introduced to the model, BMI was negatively correlated with inhibin B independent from the effect of estrogen, FSH and adiponectin, denoting the presence of unknown factors associated with obesity that further depresses germ cell function.

Conclusion: Male obesity is associated with decreased inhibin B levels. Part of the reduction in inhibin is due to the elevation in estradiol levels; however, obesity appears to be associated with other factors that may reduce inhibin B synthesis.

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Inflammatory Markers in Women with Polycystic Ovary Syndrome. Mohamed S Ardawi,[1] Abdulrahim A Rouzi.[2] *[1]Clinical Biochemistry, King Abdulaziz University, Jeddah, Western, Saudi Arabia; [2]Obstetrics and Gynecology, King Abdulaziz University, Jeddah, Western, Saudi Arabia.*

Objective: To determine the levels of inflammatory and endothelial markers in relation to metabolic and hormonal profiles in women with polycystic ovary syndrome (PCOS).

Design: A Prospective Case Control Study.

Setting: Tertiary Referral University Hospital.

Subjects: One hundred Saudi women living in the Jeddah area were classified as follows: 50 with PCOS and 50 age-matched women without PCOS.

Interventions: Blood samples were collected from all women with or without PCOS between 8:00-11:00, after an overnight fast.

Main Outcome Measures: Measures of body mass index (BMI) (Kg/m^2), and that of serum levels of LH, FSH, TSH, FT_4, 17-OHP, Δ4-A, DHEAs, total T, free T, SHBG, insulin, HDL-c, triglycerides and plasma levels of glucose. Measures of insulin resistance (IR) including: fasting serum insulin, GIR and HOMA. Measures of plasma levels of endothelin-1 (ET-1), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and high sensitivity C-reactive proteins (hsCRP).

Results: Women with PCOS exhibited significantly higher levels of ET-1 ($P < 0.05$), sICAMP-1 ($P < 0.05$), sVCAM-1 ($P < 0.001$) and hsCRP ($P < 0.001$) as compared with age-matched controls, respectively. Positive correlations were evident between ET-1 and FAI ($r = 0.41$; $P < 0.01$) but ET-1 negatively correlated with SHBG ($r = -0.36$; $P < 0.05$). sVCAM-1 positively correlated with total T ($r = 0.62$; 0.001), hsCRP correlated with: BMI ($r = 0.73$; $P < 0.001$), and HOMA ($r = -0.39$; $P < 0.05$), respectively.

Conclusions: Women with PCOS exhibited abnormal levels of endothelial and inflammatory markers, which appear to be inter-related to hyperandrogenaemia.

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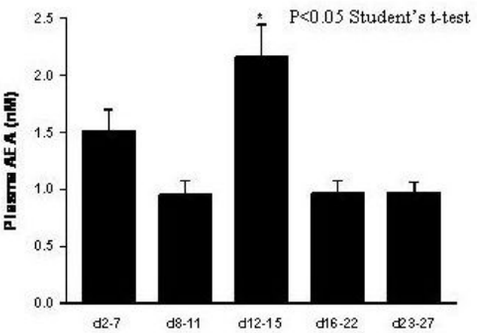
Variation of Plasma Anandamide Levels and Fatty Acid Amide Hydrolase Expression throughout the Menstrual Cycle. Mona R EL-TAlatini, Anthony H Taylor, Susan M Spurling, Justin C Konje. *Cancer Studies & Molecular Medicine, University of Leicester, Leicester, United Kingdom.*

Background: The hormonal variations associated with the menstrual cycle result in the generation of a fertilizable ovum and an endometrium receptive for successful implantation. Studies indicating a higher plasma anandamide (AEA) level in the early follicular phase compared to the late luteal phase (1), and a higher expression of fatty acid amide hydrolase (FAAH) in peripheral lymphocytes post-ovulation (2), suggest an involvement of the endocannabinoid system in menstrual cycle control. Our aims were to investigate changes in plasma AEA levels and in endometrial FAAH expression throughout the menstrual cycle.

Methods: Plasma AEA levels were measured using a HPLC MS/MS method from 47 women, median age 34yrs (range 20-45) and BMI $22Kg/m^2$ (range 19-27), with regular menstrual cycles, with no medical problem and not on any medication for the preceding 6 months. The menstrual cycle was divided to early follicular d2-7(n=11), late follicular d8-11(n=7), ovulatory d12-15(n=10), early luteal d16-22(n-10) and late luteal phases d23-28(n=9). Uterine biopsies were taken from hysterectomy specimens taken for benign conditions and subjected to immunohistochemistry for FAAH with polyclonal antibodies.

Results: AEA levels were significantly higher around ovulation in comparison to the pre-ovulatory or post-ovulatory phases as shown in the figure. FAAH expression in the endometrial stroma was unchanged throughout the follicular phase but increased during the mid to late luteal phase reaching a maximum in the late luteal phase.



Conclusion: A high AEA level in the early follicular phase and during ovulation suggests a role for AEA in ovulation. The lower levels of AEA in the luteal phase, essential for successful implantation, may be regulated by increased FAAH expression at the uterine level. The modulation of plasma AEA levels during the menstrual cycle strongly suggests that it is regulated by gonadal steroid hormones.

References:

(1). Habayeb OHM, *et al*., (2004) *J Clin Endocrinol Metab.* **89:** 5482-5487.
(2). Lazzarin, N. *et al.*, (2004) *Gynecol. Endocrinol*. **18:** 212-218.

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Characterization of Circulating IGFBPs and their Posttranslational Modification in Postnatal and Pregnant Rats. Qing Qiu,[1] Jin-Yi Jiang,[1] Benjamin K Tsang,[1,2] Andree Gruslin.[1,3] *[1]Hormones, Growth, and Development Program, Ottawa Health Research Institute, Ottawa, ON, Canada; [2]Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada; [3]Obstetrics and Gynecology, Ottawa Hospital, Ottawa, ON, Canada.*

Introduction: IGFs play an important role in the regulating placental and fetal growth. Biological activity is well regulated by IGFBPs either enhancing or inhibiting IGF biological function. Biding activity of IGFBP to IGF was further modulated by posttranslaitonal modification of IGFBPs. In this study, we systematically examine circulating IGFBP profiles in postnatal development and pregnancy through rat model and the effect of post-translational modifications on binding activity.

Method: Rat serum sample were collected at age day 1, 5, 10, 15, 20, 25, 50 and 120, as well as at pregnancy. Binding activities of IGFBP and their protein levels (IGFBP1,2,3,4,5,6) were assessed by Western ligand blot (WLB) and western blot (WB), respectively. The glyscosylation and phosphorylation status of IGFBPS were examined by deglycosylation treatment.

Results: Our results showed that both glycosylated and unglycosylated IGFBP2 elevate in fetal and newborn rat and graduately decline to a lower level at day 15 and kept lower constant level since then. Interestingly, biding activity of glycosylated IGFBP2 was not detected by WLB assay. The IGFBP-2 cleaved products were observed after rat day age day 15, which associated with a decrease in full length of IGFBP-2, suggesting endoproteolytic processing may involved in decreasing IGFBP content. IGFBP-3, with heterogeneous glycosylation, were appeared after age day 25 and disappeared during pregnancy, recurrence again postpartum. Glycosylation of IGFBP3 has no effect on its binding activity. Unglycosylated and glycosylated IGFBP were constant in life time. Physiologically constant IGFBP5 were detected by WB in protein, but not by WLB in binging activity.

Conclusion: Highly elevated circulation IGFBP2 suggest physiological role in new born and early postnatal development. Its binding activity are well regulated by its posttranslation modification, such as glycosylation of IGFBP2 in inactivating binding activity and possible involvement of active endoproteolytic processing in maintaining binding active IGFBP-2 at low

level. Reduction of IGFBP-3 during pregnancy may result in an increase in IGF-II bioavailability in regulating placental growth, a well known function of IGF-II.

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Hormone Changes in Women of Reproductive Age Associated with Tobacco Smoke Exposure Using Metabolomics. Offie P Soldin,[1] Steven J Soldin,[2] Habtom Ressom,[3] Helain J Landy.[4] *[1]Medicine, Oncology and Physiology and Center for Sex Differences, Georgetown University Medical Center for the Obstetric Pharmacology Research Unit (OPRU), Washington, DC, USA; [2]Departments of Medicine and Pharmacology and Bioanalytical Core Laboratory, Georgetown University Medical Center, Washington, DC, USA; [3]Department of Biostatistics, Bioinformatics and Biomathematics, Georgetown University Medical Center, Washington, DC, USA; [4]Department of Obstetrics and Gynecology, Georgetown Hospital, Georgetown University Medical Center, Washington, DC, USA.*

Background: Cigarette smoking affects hormone biosynthesis, storage, release, binding, transport and clearance, resulting in changes in circulating hormone levels. We used metabolomics to analyze the effects of cigarette smoking and second hand smoke (SHS) on changes in hormone levels in women of childbearing age.

Methods: This is a three arm study; women aged 18-44years who are active smokers, exposed to SHS (passive smokers), or non-exposed were recruited from the Washington D.C. area. All women completed a detailed staff-administered questionnaire probing their medical history, occupational, lifestyle factors and diet. Blood and urine samples were collected at the follicular phase. Hormone profiles were determined using metabolomics for serum thyroxine and triiodothyronine and 17 steroid hormones, as well as for cotinine using isotope-dilution tandem mass spectrometry (LC/MS/MS-API 5000). In addition, all samples were analyzed for serum LH, FSH, TSH and creatinine.

Results: The relationships of cigarette smoking, age, relative weight, and dietary intake to serum thyroxine, triiodothyronine, estradiol (E2), estrone (E1), Progesterone (P), 17-hydroxyprogesterone (17OHP),dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione, cortisol, 11-deoxycortisol, corticosterone, testosterone, aldosterone and vitamin D3 were analyzed using LC/MS/MS. Mean TSH in non-exposed, SHS-exposed and smokers: 1.32, 0.86, and 1.02mIU/mL respectively. Similarly, mean T4 9.8, 12.23 (23% increase) (p=0.05), 9.41µg/dL (-7%) in active smokers; (active vs. exposed p=0.03). SHS increased DHEA levels (33% higher, P = 0.02), DHEAS (23% higher, P = 0.07), cortisol (21% lower, P = 0.01), aldosterone (63% lower, P = 0.02) and androstenedione (20% higher, P = 0.01). These data suggest that active smoking and SHS can have a profound effect on serum T4, adrenal steroid and sex hormone concentrations in women of childbearing age.

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Prevalence and Predictors of the Metabolic Syndrome in Saudi Women with Polycystic Ovary Syndrome: A Prospective Study. Abdulrahim A Rouzi,[1] Mohamed S Ardawi.[2] *[1]Obstetrics and Gynecology, King Abdulaziz University, Jeddah, Western, Saudi Arabia; [2]Clinical Biochmistry, King Abdulaziz University, Jeddah, Western, Saudi Arabia.*

Objective: To determine the prevalence and predictors of the metabolic syndrome (MBS) among in Saudi women with polycystic ovary syndrome (PCOS) in comparison to women without PCOS and to assess the role of androgens and insulin resistance (IR) in MBS development.

Design: A prospective Case Control Study.

Setting: Tertiary Referral University Hospital.

Subjects: Six hundreds Saudi women living in the Jeddah area were classified as follows: 300 with PCOS and 300 age-matched women without PCOS.

Interventions: Blood samples were collected from all women with or without PCOS between 8:00-11:00, after an overnight fast.

Main Outcome Measures: Measures of abdominal obesity, blood pressure and that of serum levels of LH, FSH, TSH, FT_4, 17-OHP, Δ4-A, DHEAs, total T, free T, SHBG, insulin, HDL-c, triglycerides and plasma levels of glucose. Measures IR including: fasting serum insulin, GIR and HOMA.

Results: Age-adjusted prevalence of MBS was higher in women with PCOS (53.5%, 95% CI: 37.6-61.2%) as compared with women without PCOS (14.7%, 95% CI: 10.2-18.6%) (P<0.000). In the same age group, the risk of MBS in women with PCOS was greater than that for women without PCOS (P<0.001). Markers of IR were significantly abnormal in women with both PCOS and MBS in comparison to those without MBS (P<0.001). The most common abnormal components of MBS in women with both PCOS and MBS (after adjustment for age) were: decreased HDL-c (83.1 ± 10.5%); increased triglycerides (53.4 ± 7.7%); and increased BMI (38.2 ± 4.6%), respectively. The prevalence of MBS from lowest to highest tertile of free T level was 20.1, 33.7 and 54.2%, respectively; in women with both PCOS and MBS. In women with PCOS, 9% exhibited all 5 components of MBS; 14.1% had 4 components, and 40.5% had 3 components.

Conclusions: Women with PCOS exhibited significantly higher prevalence of MBS (3.6-fold) as compared with age-matched control without PCOS. IR is a possible common pathogenetic factor for both MBS and the PCOS. It is suggested that more intensive screening and/or therapy monitoring of MBS among women with PCOS should be part of the therapeutic modalities of the condition.

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Case of Sisters with Complete Androgen Insensitivity Syndrome and Discordant Mullerian Remnants. Jennifer L Nichols, Jennifer J Gell, Eric J Bieber. *Reproductive Endocrinology and Infertility, Geisinger Medical Center, Danville, PA, USA.*

Complete androgen insensitivity is an X-linked recessive disorder resulting in the abnormal expression of the androgen receptor. Affected individuals are most commonly phenotypically female but genotypically male. The prevalence of this disorder is 1 in 20,000 live male births. We present a case of complete androgen insensitivity in 2 members of the same family with differing residual Mullerian tissue.

Sister A presented at age 17 for evaluation of primary amenorrhea. A karyotype revealed 46,XY. An MRI of the pelvis showed a hypoplastic uterus but no ovaries. This patient underwent laparoscopic bilateral gonadectomy and hemihysterectomy. On examination under anesthesia, she was noted to have a normal vagina with no cervix noted. At laparoscopic evaluation, she was noted to have bilateral elongated gonads and what appeared to be a remnant of uterine tissue. Pathology revealed portions of immature testicles and fragments of smooth muscle in what grossly appeared to be the uterine remnant. The patient's total testosterone following surgery was noted to be elevated at 2.91 ng/ml. Other laboratory evaluation showed FSH 4.5 mIU/ml, LH 7.04 mIU/ml, free testosterone 3.9 pg/ml, and estradiol 24.6 pg/ml.

Approximately two years later sister B presented at age 17 for evaluation of primary amenorrhea. No uterus or ovaries were visualized on pelvic ultrasound. Again a karyotype revealed 46,XY. Laboratory evaluation demonstrated FSH 1.0 mIU/ml, LH 13.28 mIU/ml, estradiol 37.3 pg/ml, total testosterone 9.25 ng/ml, and free testosterone 11.1 pg/ml. She underwent a laparoscopic bilateral gonadectomy. No uterus, cervix or pelvic masses were identified on exam under anesthesia. At laparoscopy, both gonads were visualized and removed without difficulty but no uterus was visualized. Pathology reported two testicular and epididymal-like structures.

This case demonstrates the presentation and laparoscopic results of complete androgen insensitivity syndrome discovered in two siblings. Although both girls are genotypically male, they differ in the presence of vestigial Mullerian tissue. The case shows with complete androgen insensitivity, as an X-linked defect, one should consider apparent sisters of affected individuals, as well as offspring of unaffected individuals with a family member diagnosed.

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Comparison of a Novel Solid Phase Method for Extracting Anandamide from Plasma with a More Traditional Liquid Phase Method. Patricia M Lam, Tim H Marczylo, Anthony H Taylor, Justin C Konje. *Endocannabinoid Research Group, Reproductive Sciences Section, Department of CSMM, University of Leicester, Leicester, Leicestershire, United Kingdom.*

Background: Anandamide (*N*-arachidonoylethanolamine, AEA) is an endocannabinoid that binds to and activates the cannabinoid receptors, CB1 and CB2 and may have important roles in the regulation of human reproduction. The traditional lipid extraction methods used for AEA[1] are cumbersome, slow and of low efficiency. *The aim of this study was to determine whether the use of a solid phase (SPE) method of AEA extraction from human plasma would offer any advantages over the traditional liquid phase (LPE) method.*

Methods: Pooled human plasma was obtained from the local blood transfusion unit and aliquots stored at -20°C prior to extraction. An internal standard of 2.5pmol of deuterated AEA (AEA-d_8) was added to each plasma sample and AEA extracted from 5 aliquots on each occasion over three days using the LPE[1] and SPE methods. SPE was performed with Waters Oasis HLB 1cc/30mg cartridges on a Waters vacuum manifold. Cartridges were activated with methanol and water, the samples applied and washed with 40% methanol at

1ml/min. AEA was eluted with 1ml acetonitrile and the eluents dried under a stream of nitrogen before reconstitution in 80 ml acetonitrile. AEA levels were measured using UPLC-MS/MS against authentic standards.

Results: These are shown in the table.

Conclusion: The SPE method was ~3-fold more efficient at extracting AEA compared to the traditional LPE method. The intra-day and inter-day assay variability were similar for both techniques, although the SPE method was quicker, cheaper and required less plasma to generate data similar to that from the traditional LPE method, suggesting that the SPE method may be more efficient than the LPE method, and thus making it more suitable for routine analysis of multiple plasma AEA samples.

Reference:

1. Habayeb, O.M.H, *et al*., (2004) J. Clin. Endocrinol. Metab. **89:** 5482-7.

Comparison of plasma AEA extraction methods

Parameter	LPE	SPE
Extraction efficiency (%)	19	60*
Intra-day variability (%)	2.9	40 ns
Inter-day variability 3 days, n=5 (%)	12.6	11.6 ns
Inter-day AEA (mean±SD) (nM), n=15	1.18±0.15	1.18±0.14 ns
Processing time for 10 samples (hr)	~10	~0.4
Amount of plasma required/test (ml)	2	0.5

*P<0.05; ns not significantly different; Student's t-test

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Description of a Fast, Sensitive and Accurate UPLC-MS/MS Method for the Measurement of Anandamide. Patricia M Lam, Tim H Marczylo, Mona R El-Talatini, Mark Finney, Vijaianitha Nallendran, Anthony H Taylor, Justin C Konje. *Endocannabinoid Research Group, Reproductive Sciences Section, Department of CSMM, University of Leicester, Leicester, Leicestershire, United Kingdom.*

Background: The precise role of the endocannabinoid, anandamide (*N*-arachidonoylethanolamine, AEA) in reproduction has been hampered by difficulties in its accurate measurement. AEA levels have previously been measured by TLC, GC-MS and HPLC-MS but these are laborious. Our aim was to improve the analysis of AEA using UPLC and tandem MS/MS with a standard isotope-dilution method[1,2].

Methods: Authentic non-labelled and deuterium-labelled AEA (AEA-d8) diluted in acetonitrile were maintained at 4°C before analysis and separation by UPLC on a C_{18} (2.1 x 50mm) column maintained at 40°C using a gradient of 80% A, 0.5min: 80% A, 1.5min: 0% A, 2.5min: 80% A, 3.5min: 80% A with a flow rate of 0.7ml/min. The mobile phases were **A** (2mM ammonium acetate, 0.1% formic acid) and **B** (acetonitrile, 0.1% formic acid). The analytes were quantified using multiple-reaction monitoring in positive ion mode with a Quattro Premier mass spectrometer. Entry, collision and exit energies were -2, 17 and -17eV, respectively. Calibration curves were performed in triplicate with 2.5pmol AEA-d8 as the internal standard. Transitions employed were 348.3>62.3 and 356.3>63.3 for AEA and AEA-d8, respectively.

Results: Calibration curves (1.66 to 133fmol AEA on column; n=15) were linear, producing a mean (±SD) gradient of y = 2.48±0.14x, crossing the y-axis very close to the origin (0.004±0.04 units). Variability was limited, with an r^2 =0.999. Measurements were precise, 133fmol AEA produced a CV of only 3.7%, and the retention time was consistent at 1.67±0.0009min (n=20). The limit of quantification (Signal/Noise>10) was 0.22fmol on column and the limit of detection (LOD) was 0.055fmol on column (Signal/Noise=3). Accuracy for 3.33fmol, 6.65fmol and 133fmol AEA was 97.5±9.5%, 98.5±6.1% and 104.5±3.2%, respectively.

Conclusions: The method described is an improvement over other LC-MS/MS methods[1,2] with a lower LOD [0.055fmol *v.* 25fmol[1] or 43fmol[2]] and shorter run time [4min *v.* 15min[1] or 5.4min[2]]. Thus, an improvement in terms of speed, increased sensitivity and better reproducibility will allow for a more accurate assessment of AEA concentrations in a number of biological samples.

References:

(1) Richardson, D., *et al.*, (2007) *Anal. Biochem.* **360:**216-26
(2) Vogeser, M., *et al.*, (2006) *Clin. Chem. Lab. Med.* **44:**488-91

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GATA Transcripion Factors Stimulate Luteinizing Hormone β (LHβ) Gene Expression. Weiming Zheng, Lisa Halvorson. *Obstetrics & Gynecology, University of Texas Southwestern Medical Center, Dallas, TX, USA.*

Objectives: The GATA family of transcription factors consists of six zinc-finger proteins with a critical role in tissue-specific and developmentally-regulated gene expression. GATA factors exert their effects alone and through interactions with cofactors, such as friend of GATA (FOG), as well as with nuclear hormone receptors, including steroidogenic factor-1 (SF-1), a well-described activator of gonadotropin gene expression. The objective of these studies was to define the role of GATA family members in the gonadotrope. **Methods:** 1) Total RNA was extracted from the gonadotrope cell line, LβT2, and analyzed by standard RT-PCR analysis. 2) The CV-1 fibroblast cell line was transiently transfected by the calcium phosphate precipitation method with a rat -207/+5 LHβ promoter-reporter vector as well as CMV-driven expression vectors for GATA-2, GATA-3, dnGATA-3, FOG-2 and/or SF-1. **Results:** Gonadotrope LβT2 cells were found to express transcripts encoding GATA-2, GATA-3, and GATA-4 as well as FOG-1 and FOG-2. GATA-2 and GATA-3 stimulated LHβ gene promoter activity by approximately 5-fold ($p<0.05$) and synergistically increased the SF-1 response (40-fold versus 10-fold for SF-1 alone; $p<0.05$). The GATA-mediated increase in LHβ gene expression was nearly eliminated with co-transfection of FOG-2. Similarly, co-transfection with a GATA-3 dominant negative construct blunted wild-type GATA-3 effects in a dose-dependent fashion. **Conclusions:** These data demonstrate expression of both GATA and the functionally related FOG proteins in a well-characterized gonadotrope cell line. Furthermore, they demonstrate a functional role for these factors in regulation of gonadotrope function, specifically expression of the LHβ gene.

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Maternal Glucocorticoid Injections in Early Pregnancy Altered the Pituitary-Adrenal Axis in Sheep. T Braun,[1,2] L Shaofu,[3] SG Matthews,[1] DM Sloboda,[3,4,5] TJM Moss,[3,4] JP Newnham,[3,4] JRG Challis.[1] *[1]Department of Physiology and Obstetrics and Gynecology, University of Toronto; [2]Department of Obstetrics and Gynecology, Charité University; [3]School of Women's and Infants' Health, University of Western Australia; [4]Women and Infants Research Foundation, University of Western Australia; [5]The Liggins Institute, University of Auckland.*

Low-dose dexamethasone (DEX) therapy early in pregnancy is used in fetuses with suspected risk of congenital adrenal hyperplasia. Several adverse neurological events in prenatally treated children have been reported and the fetal hypothalamic-pituitary-adrenal (HPA) axis may be involved. **AIM:** To investigate the immediate and long-term effects of early maternal DEX administration on fetal growth and pituitary-adrenal activity in sheep. **METHOD:** Pregnant ewes carrying singleton fetuses (total n=119) were randomized to control (2ml saline/ewe) or DEX treatment (0.14mg/kg ewe weight) consisting of four intramuscular injections at 12-hourly intervals over 48 hours on 40-41 days of gestation (dG). At 50, 100, 125, and 140dG fetal weights were recorded. I^{125}-RIA, qRT-PCR and in-situ hybridisation were used to measure fetal plasma cortisol and ACTH levels and to analyse adrenal and pituitary mRNA expression. **RESULTS:** DEX-exposed fetuses were lighter than control animals at 100dG*, but not at other times; in general fetal organ weights were similar between treatment groups. Fetal plasma ACTH was unaffected by DEX and did not differ between genders. Similarly, POMC and PC-1 mRNA in pars distalis were unaltered after DEX. However, fetal plasma cortisol was reduced after DEX in both male and females at 50dG*, was similar at 100 and 125dG, then elevated at 140dG*. Plasma cortisol in female fetuses in control and after DEX was significantly higher than in males. The increases in cortisol after DEX at 140dG* were associated with increased fetal adrenal expression of P450C17 and 3bHSD mRNA in females, reduced expression of MC2R in males, but no difference in STAR mRNA. **CONCLUSION:** We conclude that in sheep, early DEX programs the developmental trajectory of the fetal HPA with increased activation directly of the adrenal, but not pars distalis function. In females this effect may be attributed to increased fetal adrenal steroidogenic activity. The effect of DEX in increasing cortisol in males, albeit at a significantly lower level than in females, appears to be independent of the enzymes that we have measured.*p<0.05.

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Synaptophysin and Gonadotropin-Releasing Hormone (GnRH) Are Colocalized in Rat Hypothalamus. Armando Arroyo,[1] Beom Su Kim,[1] Amanda Biehl,[1] Blenna CL Bett,[1,2] John Yeh.[1] *[1]Gynecology-Obstetrics, University of Buffalo, Buffalo, NY, USA; [2]Physiology and Biophysics, University at Buffalo, Buffalo, NY, USA.*

The cellular and molecular mechanisms that control gonadotropin-releasing hormone (GnRH) release are not completely understood. GnRH is stored in synaptic vesicles and released by exocytosis at GnRH nerve terminals. There are currently nine families of synaptic vesicle proteins that are involved in neurotransmitter release by exocytosis. Synaptophysin is one of the most common synaptic vesicle proteins present in synaptic vesicles in neurons. The hypothesis of this study is that synaptophysin is expressed in GnRH

neurons. We obtained sections from the hypothalamus of female Sprague Dawley rats. Double-label fluorescence immunohistochemistry was performed on free-floating sections. Sections were incubated with a mixture of mouse monoclonal antibody against GnRH (1:200- Chemicon International) and with a rabbit polyclonal antibody against synaptophysin (1:100- Santa Cruz Biotechnology) for 16 h. After incubation the sections were washed and incubated with a mixture of Alexa 488 conjugated goat antimouse and Alexa 594 conjugated goat antirabbit (1:1000; Molecular Probes) for 2 h. Slices were visualized with confocal microscopy (Zeiss LSM-510). Fifteen out of a total of fifteen GnRH cell bodies in the medial preoptic area and most GnRH neuron terminals in the median eminence showed intense immunostaining for synaptophysin. This is the first study to demonstrate that synaptophysin is expressed in rat GnRH neuron terminals. This suggests that synaptophysin is present in GnRH neuron vesicles. Thus, GnRH release by exocytosis may be mediated by synaptic vesicle proteins.

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Cortisol Responses in Young Sheep after Dexamethasone Exposure in Early Gestation. Shaofu Li,[1] Ilias Nitsos,[2] Graeme Polglase,[3] Thorsten Braun,[4] Timothy JM Moss,[5] John RG Challis,[6] John P Newnham.[7] *[1]School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia; [2]School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia; [3]School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia; [4]Dept of Obstetrics and Gynaecology, Charité University, Berlin, Germany; [5]School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia; [6]Depts of Physiology and Obstetrics and Gynecology, The University of Toronto, Toronto, Canada.*
Objective: Our aim was to identify the effects of early gestation GC exposure on fetal and postnatal HPA axis development and function in postnatal life. **Method:** Pregnant ewes carrying singleton fetuses were randomized to control (2 ml saline/ewe) or dex groups (0.14 mg/kg), consisting of four at 12 h intervals on days 40-41 of pregnancy. At 7 months postnatal age, catheters were implanted; a bolus injection of CRH (0.5 mg/body weight) and AVP (0.1 mg/body weight) were administered and arterial blood samples were taken at -30, -15, 0, 5, 10, 20, 30 60, 90, 120 and 180 min. Levels of hepatic CBG mRNA were determined by qPCR, expressed relative to 18s rRNA. Plasma cortisol and CBG levels were measured by radioimmunoassay. **Results:** Both total and free cortisol levels in the dex females (n=5) were lower than in dex males (n=7) from 30 to 90 min ($P \leq 0.05$) and lower than in control females (n=6) at 30 minutes ($P \leq 0.04$), also in the dex-M group were higher than in control males (n=5) at 60 min ($P \leq 0.04$). Plasma CBG levels and CBG mRNA expression were not altered by dexamethasone exposure or sex. **Conclusions:** These findings suggest that prenatal glucocorticoid exposure alters the development of the HPA axis differentially according to the sex of the exposed fetus.

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Hippocampal Corticosteroid Receptors Expression in Young Sheep: Effects of Glucocorticoid Exposure. Shaofu Li,[1] Ilias Nitsos,[2] Graeme Polglase,[3] Thorsten Braun,[4] Timothy JM Moss,[5] John RG Challis,[6] John P Newnham.[7] *[1]School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia; [2]School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia; [3]School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia; [4]Dept of Obstetrics and Gynaecology, Charite University, Berlin, Germany; [5]School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia; [6]Depts of Physiology and Obstetrics and Gynecology, The University of Toronto, Toronto, Canada; [7]School of Women's and Infants' Health, The University of Western Australia, Perth, WA, Australia.*
Objective: To determine maternal injections of synthetic glucocorticoids in early gestation can alter expression of hippocampal corticosteroid receptors at 7 months postnatal age. **Method:** Pregnant ewes carrying singleton fetuses were randomized to control (2 ml saline/ewe) or dexamethasone treatment (0.14 mg/kg) consisting of four injections at 12 h intervals on days 40-41. Hippocampal was collected from the offspring at 7 months postnatal age. Levels of mRNA of GR and MR were determined using qPCR and levels relatived to 18s rRNA. **Results:** Dexamethasone-treated male animals (n=7) had significantly higher levels of MR gene expression than both control males (n=5; P=0.028) and females (n=6; P=0.037). GR gene expression levels were higher in treated *vs.* control males (P=0.043), but in females levels in treated and control animals were similar. Total body, brain and hippocampus weights were similar. **Conclusions:** Maternal dexamethasone administration in early pregnancy resulted in gender-dependent changes in hippocampal gene expression when measured in the offspring seven months after birth.

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Stimulation & Pregnancy Outcomes in Women <35 with Diminished Ovarian Reserve. Susan BA Hudson, Phoebe M Hughes, Charles C Coddington, Amy L Weaver, Dean E Morbeck. *Obstetrics & Gynecology, Division of REI, Mayo Clinic, Rochester, MN, USA.*
Objective: Diminished ovarian reserve (DOR) affects many younger women. We analyzed superovulation with intrauterine insemination (SO) cycles & in vitro fertilization (IVF) cycles of women with DOR to determine if women <35 with DOR differ from their older counterparts.
Method: IRB approved retrospective review of SO & IVF cycles from 1/2005-6/2007 with follicle stimulating hormone (FSH) levels ≥10 based on age <35 or ≥ 35.
Results: 49 SO cycles were performed in 18 women. No differences were noted in clinical pregnancy. Women <35 were more likely to have inseminates with a lower mean TMS/Ins, & median TMS/Ins was 71.0 among pregnancy cycles compared to 28 for unsuccessful cycles. For IVF, mean total gonadotropin dosage was significantly lower in women <35, & mean number of follicles ≥15 mm & peak E2 were significantly higher in women <35. SO & IVF measures are in Tables 1 & 2, respectively.
Conclusions: Similar clinical pregnancy outcomes were seen despite age. In IVF, women <35 required less gonadotropins and generated more follicles but with no significant difference in number of mature oocytes or clinical pregnancies. Of note, pregnancy rates for SO in women <35 with DOR are substantially lower than expected in our clinical practice. As IVF yielded a substantially higher chance of pregnancy, consideration should be made to expedite progression to IVF.

Table 1

	Total (N=49)	Age<35 (N=24)	Age≥35 (N=25)	p-value
Method of SO				
Clomiphene	8 (16.3%)	3 (12.5%)	5 (20%)	
Minimal Stimulation	25 (51%)	12 (50%)	13 (52%)	
Gonadotropins	16 (32.7%)	9 (37.5%)	7 (28%)	
TMS/Ins				0.051
Mean (SD)	50.6 (54.1)	40.3 (53.4)	60.4 (54.1)	
Median	28	20.9	39.8	
FSH (IU/L)				
Mean (SD)	12.3 (2.9)	11.3 (1.2)	13.2 (3.6)	
Median	11.8	11	12	
Clinical Pregnancy	4 (8.2%)	2 (8.3%)	2 (8%)	0.95

Table 2

	Total (N=32)	Age<35 (N=16)	Age≥35 (N=16)	p-value
Gonadotropins (IU)				<0.001
Mean (SD)	4867.2 (1552.8)	3993.8 (1496.9)	5740.6 (1056)	
Median	5000	3787.5	5475	
# Follicles ≥15 mm				0.040
N	31	16	15	
Mean (SD)	8.1 (4.7)	9.7 (5)	6.4 (3.7)	
Median	7	9.5	5	
Peak E2 (pg/mL)				0.057
Mean (SD)	1362.4 (1232.3)	1775.1 (1474)	949 (776)	
Median	1124.5	1560	625.5	
# Mature Oocytes				0.34
Mean (SD)	7.5 (5.3)	8.9 (5.8)	6.1 (4.5)	
Median	6.5	8	5.5	
Clinical Pregnancy	10 (31.3%)	6 (37.5%)	4 (25%)	0.46

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Toward Gene Therapy of Premature Ovarian Failure: Intraovarian Injection of Adenovirus Expressing Human FSH Receptor Restores Folliculogenesis in FSHR (-/-) Mice. Mohsen Ghadami,[1,2] Ebtehal El-Demerdash,[1] Dong Zhang,[1,2] Memmy H Hassan,[1] Manubai Nagamani,[1] Xinlei Chen,[4] Jorge Chedress,[3] M Ram Sairam,[4] Ayman Al-Hendy.[1,2] *[1]Dep of Ob/Gyn, UTMB, Galveston, TX, USA; [2]Dep of Ob/Gyn, Meharry Med Col, Nashville, TN, USA; [3]Dep of Ob/Gyn, Uni of Saskatchewan, Saskatoon, SK, Canada; [4]Cli Res Inst of Montreal, Uni of Montreal, Montreal, QC, Canada.*

Introduction: The phenotype of FSHR (-/-) mice is reminiscent of human ROS, a heterogeneous disorder characterized by primary amenorrhea, normal karyotype, an elevated FSH and decrease in E2. We have recently demonstrated that a normal copy of hFSHR gene delivered via an adenovirus is able to correct the Finish C566T mutation and restore FSH responsiveness in JC410 and COS7 cells (Rep Sci Vol. 14, No.1 (S) Jan 2007(127A). **Objective:** To assess the effects of intraovarian injection of Ad-FSHR on the reproductive system of FSHR (-/-) mice. **Methods:** About 50 µl containing $3x10^9$ pfu of Ad-hFSHR were injected directly into each ovary of treated group and same amount of Ad-LacZ were injected into the ovaries of control animals. Vaginal smears were collected and body weight was measured daily. Four weeks after the injection animals were sacrificed and all organs were weighted and evaluated by H&E. FSH, E2 and P4 measured before and after treatment. **Results:** Ad-hFSHR-treated mice showed obvious estrogenic changes in vaginal smear while in control animals vaginal smear remained at diestrus stage. Significant increase in total body weight and estrogen dependent organs weight (uterus, ovary, vagina) was observed in treated animals compare to control group (P<0.02). No significant weight changes were observed in other organs. H&E evaluation of the ovaries showed significant increases in both the total number of follicles and the collective diameter of the follicles in treated animals compare to controls. On average 18 follicles/ovary were observed in Ad-hFSHR-treated group of which 4 follicles were at the antral stage while only 2 follicles observed in Ad-LacZ control group, with zero follicles at antral stage. A 2.5 to 3 folds increase in E2 and about 50% decrease of FSH observed in treated animals compared to control mice. There was no significant change in serum progesterone level between treated and control groups. **Conclusion:** Intraovarian injection of an adenovirus expressing human FSHR gene is able to restore folliculogenesis and resume estrogen hormone production in female FORKO mice.

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Non-Invasive Metabolomic Profiling of Human Embryo Culture Media Using ^{1}H NMR Correlates with Pregnancy Outcome. E Seli,[1] L Botros,[2] D Sakkas,[1] DA Burns.[2] *[1]Ob & Gyn, Yale U., New Haven, CT, USA; [2]Chemistry, McGill U., Montreal, QC, Canada.*

Objective: The sentinel issue surrounding multiple gestations following IVF is the inability to precisely estimate the reproductive potential of individual embryos with the currently used embryo grading systems based on embryo cleavage rate and morphology. Recently, metabolomic profiling of spent culture media using Raman and Near-infrared spectroscopy have been reported to predict reproductive potential of embryos. In this study we applied proton nuclear magnetic resonance (^{1}H NMR) spectroscopy to analyze metabolomic profile of embryo culture media and to identify components of the media that correlate with reproductive potential.

Methods: Eighteen spent media samples from embryos that failed to implant, and 16 samples from embryos that resulted in pregnancy and delivery were individually collected after embryo transfer on day 3, and evaluated using ^{1}H NMR. The spectra obtained were analyzed using a selective genetic algorithm (GA) to determine regions predictive of pregnancy outcome as determined by logistic regression. To avoid random correlations, a leave-one out cross-validation was used. Sensitivity and specificity of predicting pregnancy (described as implantation and delivery) were calculated.

Results: Using the GA, two areas in the ^{1}H NMR spectral region were identified as most discriminatory between the two groups. Viability indices calculated by ^{1}H NMR using these regions were significantly higher in culture media of embryos with proven reproductive potential (0.66±0.13) compared to those that failed to implant (0.33±0.16) (P<0.001). Compiled outcomes from the leave-one-out cross-validation of the logistic regression using the ^{1}H NMR measurements resulted in a sensitivity of 96% and a specificity of 77.8%. Quantification by integration showed significantly decreased glutamate levels (p=0.002) and a trend toward an increase in pyruvate levels (p=0.1) in culture media of embryos that did not cause pregnancy.

Conclusion: Metabolomic profile of spent embryo culture media using ^{1}H NMR correlates with the reproductive potential of embryos. The lower glutamate levels detected in culture media of embryos that failed to implant could potentially be due to the toxicity associated with increased embryonic glutamate uptake. Additional studies using complementary approaches are needed to further delineate molecular components associated with reproductive potential.

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Beta-Carotene Levels in Follicular Fluid Correlate with Successful In Vitro Fertilization of Human Oocytes. Wendy B Shelly,[1] Richard W Browne,[2] Heather G Huddleston,[1] Julia R Sandler,[1] Victor Y Fujimoto.[1] *[1]Department of Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA; [2]Department of Biotechnical and Clinical Laboratory Sciences, University at Buffalo, Buffalo, NY, USA.*

Objective: Beta-carotene and other carotenoids are known antioxidants previously identified in human follicular fluid (FF). In addition to their inherent antioxidant properties, carotenoids have been identified as precursors of the antioxidant retinol in bovine FF. High retinol levels in bovine FF are associated with non-atretic follicles. This would suggest a possible role for retinol and its carotenoid precursors in follicular heath and the general oxidative state of the follicle. We sought to measure carotenoids and retinol in the FF of women undergoing IVF and correlate these levels with normal fertilization as a marker of follicular/oocyte health.

Design: Prospective Cohort Study

Materials and Methods: FF from a single 18-20 mm follicle was obtained from 24 women (age 29-43) undergoing IVF and intracytoplasmic sperm injection (ICSI). Serum was also obtained at the time of oocyte retrieval. Retinol, vitamin E (α, γ, and δ tocopherol) and carotenoids (β-carotene, β-cryptoxanthin, lycopene and lutein/zeaxanthin) were measured using HPLC. We correlated FF carotenoid and retinol levels with oocyte fertilization status following ICSI.

Results: As previously reported, retinol, vitamin E and carotenoids were all identified in the FF. Each fat-soluble vitamin level was significantly lower in FF compared to serum (p<0.001 for all analytes) and the levels were strongly correlated (r2>0.45, p<0.003 for all analytes). Mean levels of FF β-carotene were significantly higher in those follicles that resulted in a fertilized oocyte (0.08 +/- 0.04 µg/mL vs. 0.01 +/- 0.01 µg/mL, P=0.015). Other carotenoids, retinol, and vitamin E levels did not correlate with fertilization outcomes.

Conclusions: Our finding of a strong association between FF β-carotene concentration and subsequent normal fertilization of the oocytes suggests an important antioxidant role for β-carotene in the health of the human ovarian follicle/oocyte. The lack of correlation with other carotenoids, retinol and vitamin E suggests that the antioxidant properties of β-carotene act by preventing singlet oxygen and scavenging the peroxyl radical and may directly influence oocyte competence.

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FSH/IUI Pregnancy Outcomes for over 5200 Cycles. Rita M Sneeringer,[1,2] Alan Penzias.[1,2] [1]*REI, Beth Israel Deaconess, Boston, MA;* [2]*Boston IVF, Waltham, MA.*

INTRODUCTION: Follicle Stimulating Hormone combined with intrauterine insemination (FSH/IUI) is a frequently utilized treatment for infertile couples. Efficacy rates published in the literature are based on small groups. This study represents the largest series to date of pregnancy outcomes for FSH/IUI.

MATERIALS/METHODS: A data analysis of all infertile patients treated with FSH/IUI from September 2002 until October 2007 was performed. Demographic and cycle parameters were summarized. The per cycle pregnancy outcomes for 5,167 FSH/IUI cycles among 2,166 patients were evaluated.

RESULTS: Pregnancy rate per cycle decreased dramatically with advancing age. Per cycle pregnancy ranged from 13% per cycle in the <35 years group to 3.2% in the >42 years group with intermediate rates in the intervening groups (Table 1). The rate of cycle cancellation was similar across age groups. IVF conversions were rare, accounting for less than 1% of cycles for all age groups. The mean number of follicles for each group were similar ranging from 3-4 follicles with standard deviation of 2-3 for all groups. Thus, other than age, the comparison groups were similar in cycle response and completion.

CONCLUSIONS: When counseling patients on the efficacy of FSH/IUI treatments, age is the primary factor that should be considered when providing accurate estimates of per cycle success rates. A significant decline in pregnancy rate is noted in this study with advancing age. Previously published pregnancy rates are higher than we observed. This is likely secondary to age restricted groups and smaller sample size in these prior studies.

FSH/IUI Pregnancy Outcomes per Cycle by Age

	<35	35-37	38-40	41-42	>42
Number of Cycles	2038	1188	1112	480	349
Pregnant	264 (13.0)	114 (9.6)	78 (7.0)	29 (6.0)	11 (3.2)
Not Pregnant	1466 (71.9)	917 (77.2)	884 (79.5)	376 (78.3)	293 (84)
Pending Results	85 (4.2)	45 (3.8)	54 (4.9)	28 (5.8)	7 (2.0)
Results Not Recorded	15 (0.7)	11 (0.9)	9 (0.8)	6 (1.3)	2 (0.6)
Cancelled	204 (10.0)	71 (8.9)	49 (8.2)	38 (9.6)	36 (10.3)
IVF conversion	18 (0.9)	6 (0.5)	5 (0.4)	1 (0.2)	0

N (%) per cycle

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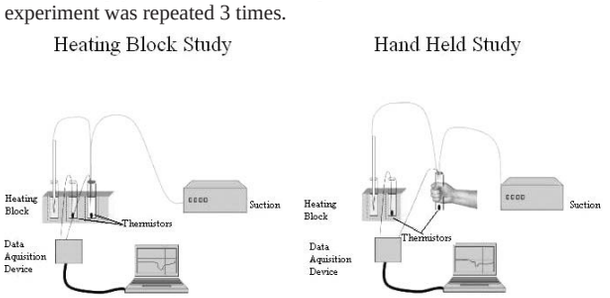
Fluid Temperature during Follicle Aspiration: Do Collection Tubes Need To Be in a Heating Block? MJ Kalan,[1] DE Lewis,[2] RB Allen,[1] MF Landay,[1] RJ Paulson.[1] [1]*OB/GYN, USC, Keck School of Medicine, L.A., CA, USA;* [2]*Biomedical Engeneering, LAC+USC Med Ctr, L.A., CA, USA.*

Mature oocytes obtained during egg retrieval have been shown to undergo spindle depolymerization as a result of cooling. For this reason, IVF programs strive to maintain constant temperature during follicle aspiration. We sought to elucidate if there was a difference in temperature of fluid aspirated into a hand held (HH) or heating block (HB) encased collection tube.

The experiment was performed in an ambient temperature of 23°C. Thermistors, pinhead sized sensors with an accuracy of 1%, were used to monitor temperature differences between control fluid (CF) (sterile water 37ºC) and aspirated fluid. Data was recorded using an 8-channel data acquisition system from DATAQ Instruments.

One thermistor was placed into the CF, the other was placed into an empty collection tube set in the heating block or held in a gloved hand. A 33cm, 17 gauge, single lumen needle connected to 70 cm of tubing was used to aspirate into the empty collection tube. Temperature was recorded 2x/second, each experiment was repeated 3 times.



Baseline empty collection tube temperature was significantly cooler in the HH vs the HB group (32.99±1.39°C vs 36.61±.16°C, p=.016). Two seconds after aspiration, the lowest aspirate temperature was observed, (HH 30.34±.14°C, HB 30.48±.23°C, p>.05) no difference between groups. In both groups, temperature quickly increased as aspiration progressed (HH 34.57±.38°C, HB 34.86±.33°C, p>.05). The HB group took an average of 5.28 min to return to baseline (37°C), the HH group never returned to baseline.

Substantial cooling of aspirated fluid occurs during oocyte retrieval, with a mean temperature decrease of 6.59±.19°C corresponding to 30.41°C. Considering this dramatic decrease, the difference between temperatures in the HH vs. the HB group is negligible. Current aspiration systems poorly regulate temperature, thus the choice of aspirating into a test tube warmed by hand or by heating block is inconsequential.

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Clinical Management of Twin Gestations with Recurrent Preterm Labor Symptoms. Lesley de la Torre,[1] Luis Roca,[1] Niki B Istwan,[2] Debbie J Rhea,[2] Gary J Stanziano,[2] Victor Hugo Gonzalez-Quintero.[1] [1]*Obstetrics and Gynecology, University of Miami Medical Center, Miami, FL, USA;* [2]*Clinical Research, Matria Healthcare, Marietta, GA, USA.*

OBJECTIVE To examine pregnancy outcomes in women with twin pregnancies receiving nifedipine tocolysis (NT) who experienced recurrent preterm labor symptoms (RPTLsx).

STUDY DESIGN Twin pregnancies enrolled for outpatient preterm labor (PTL) surveillance services prescribed NT following an initial episode of PTL were identified from a database (n=1421). Eligible for inclusion were patients later hospitalized with acute RPTLsx (n=862). Included were those <35 weeks' gestational age (GA), with intact membranes, remaining undelivered for >48 hours after RPTLsx . Pregnancy outcomes of women resuming NT (RNT group, n=418) following hospitalization were compared to those having an alteration in treatment (AltTx group, n=238) to continuous subcutaneous terbutaline. Per normality assumptions, either independent Student´s *t* or Mann-Whitney U test statistics were used for continuous variables; Pearson´s chi-square for categoric. All *p*-values presented as two-sided, significant at <0.05.

RESULTS Overall, 862 (60.7%) of twin pregnancies prescribed NT experienced RPTLsx; 206 (23.9%) were not eligible for continued tocolysis. Pregnancy outcomes are presented in table.

	RNT group n=418	AltTx group n=238	*p*-value	OR (95% CI)
GA at RPTLsx	30.2 ± 2.9 30.7 (22.4, 34.9)	29.9 ± 2.8 30.4 (21.4, 34.9)	0.123	---
Prolongation after RPTLsx (days)	27.5 ± 19.9 23 (3, 103)	34.7 ± 18.8 32 (3, 90)	<0.001	---
GA at Delivery				
<35 weeks	55.5%	42.4%	0.001	0.59 (0.43,0.81)
<32 weeks	17.7%	9.2%	0.003	0.47 (0.29,0.78)
VLBW(<1500gms)	15.0%	6.5%	<0.001	0.40 (0.26,0.60)
NICU admission	52.9%	44.7%	0.005	0.72 (0.58,0.90)

CONCLUSION RPTLsx are common. In twin pregnancies receiving NT, alteration of tocolytic treatment following RPTLsx had a positive impact on pregnancy prolongation and neonatal outcomes.

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Routine Cervical Dilatation during Elective Cesarean Section – Is It Really Necessary? Arie Koifman, Avi Harlev, Eyal Sheiner, Fernanda Press, Arnon Wiznitzer. *Obstetrics and Gynecology, Soroka University Medical Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel.*

Objective: The purpose of this study was to examine the necessity of routine cervical dilatation during elective cesarean section.

Material and Methods A retrospective cohort study was performed, including all cases of elective cesarean sections performed at a tertiary Medical Center during 2005. Stratified analysis, using the Mantel-Haenszel technique, was done to control for confounders.

Results Out of a total of 666 elective cesarean deliveries (CD), 348 underwent routine cervical dilatation. The overall rate of febrile morbidity was 4.2%. No significant differences in postpartum febrile morbidity were noted between the groups (5.1 and 3.1%; P=0.071). In addition, hospitalization duration did not differ between the groups (4.1±1.4 and 4.1±2.0 days P=0.95). About 31 % of all elective operations were repeated CD. There was no difference in febrile morbidity between the groups even in that subgroup of the elective CD. Likewise, there was no difference in anemia rate between the two groups (hemoglobin 9.50 ±0.73 mg and 9.54±0.65mg P= 0.91). Controlling for a previous vaginal delivery, using the Mantel-Haenszel technique, no significant association was noted between cervical dilatation and fever (weighted OR=2.2; 95% CI 0.8-6.4; P=0.161). Nevertheless, in a subgroup of patients following a previous vaginal delivery, cervical dilatation was significantly associated with post-operative fever (OR=5.8; 95%CI 1.2-38.0; P=0.021).

Conclusion Routine cervical dilatation seems to have no role in reducing

puerperal febrile morbidity and other complications. Moreover, in a sub-group of patients with a previous vaginal delivery, it might pose an additional risk for febrile morbidity.

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Vaginal Delivery in Women with an Unfavorable Cervix Undergoing Labor Induction: How Long Is Too Long for the First Stage of Labor? Shani Delaney, Anjali Kaimal, Yvonne Cheng, Brian Shaffer, Teresa Jones, Aaron Caughey. *Obstetrics and Gynecology, University of California, San Francisco, San Francisco, CA, USA.*

OBJECTIVE: To examine the probability of vaginal delivery in patients undergoing induction of labor (IOL) with a prolonged first stage of labor.

STUDY DESIGN: A retrospective cohort of 1002 women with term, singleton pregnancies undergoing IOL with an unfavorable cervix was examined. The first stage of labor (FSL) was divided into 12-hour intervals and the percentage of patients achieving vaginal delivery (VD) in each group was calculated. Further, we observed the effect of parity on the probability of VD with progressively increasing FSL.

RESULTS: Overall, the rate of successful VD decreased with increasing length of FSL, but even beyond 48 hours of induction, at least 50% of women achieved VD. Considering parity, the chance of vaginal delivery decreases and plateaus at FSL >24 hours in nulliparas, while for multiparas, we observed a significant drop in the rate of VD after a FSL of >36 hours.

Rate of vaginal delivery stratified by length of first stage of labor (hours)

	<12	12-24	24-36	36-48	>48	p-value
All patients (n=1002)	83%	72%	61%	57%	52%	<0.001
Nulliparas (n=725)	80%	67%	55%	58%	52%	<0.001
Multiparas (277)	91%	90%	80%	50%	50%	0.006

CONCLUSION: The majority of women with an unfavorable cervix undergoing IOL at term deliver vaginally, even with a prolonged first stage of labor. This is important information to discuss with women prior to IOL when establishing labor expectations. Providers should consider the ongoing success of labor induction when contemplating a diagnosis of "failed induction".

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Changes of Lipid Peroxidation and Protein Carbonyls Formation by Antibiotic Therapy in the Maternal Venous Plasma of Preterm Premature Rupture of Membranes. Yoon Ha Kim,[1] Tae-Bok Song,[1] Cheol Hong Kim,[1] Jong Woon Kim,[1] Moon Kyoung Cho,[1] Sung Yeul Yang,[2] Bong Whan Ahn.[2] *[1]Obstetrics & Gynecology, Chonnam National University Medical School, Gwangju, Korea; [2]Biochemistry, Chonnam National University Medical School, Gwangju, Korea.*

Objective: This study was performed to investigate and compare changes of the lipid peroxide levels and the protein carbonyls formation in the maternal venous plasma of preterm premature rupture of membrane (PPROM) during antibiotics administration.

Materials and methods: Thirty-six pregnant women with PPROM between 25 and 32 weeks of gestation were chosen for this study. Eighteen patients (group 1) were treated with amoxicillin and erythromycin for 7 day period while the other 18 patients (group 2) were treated with 3rd generation cephalosporin and erythromycin for the same period. Samples of maternal blood were obtained from the two groups at before the antibiotics administration, day 3, and day 7 after the antibiotics administration. Lipid peroxide levels were measured by thiobarbituric acid reaction and protein carbonyl contents were determined by the 2,4-dinitrophenylhydrazine method.

Results: 1. The lipid peroxide levels and protein carbonyls formation in the maternal venous plasma of PPROM was significantly higher than that of normal pregnancy (4.77±0.36 vs 7.11±0.41 nmol/mg protein, p<0.01), (3.55±0.22 vs 5.69±0.30 nmol/mg protein, p<0.01). 2. There were no significant differences in the lipid peroxide levels and protein carbonyls formation of the maternal venous plasma with PPROM mixed and incubated by amoxicillin, cefodizime, and erythromycin (in vitro). 3. There were no significant differences in the lipid peroxide levels and protein carbonyls formation of the venous plasma of group 1 among before the antibiotics administration, day 3, and day 7 after the antibiotics administration. 4. The protein carbonyls formation in the venous plasma of group 2 was significantly decreased at day 3 and day 7 after the antibiotics administration than that of before the antibiotics administration (6.04 ±0.44, 5.53±0.37, 7.04±0.51 nmol/mg protein, p<0.01).

Conclusion: In the maternal venous plasma of PPROM, the lipid peroxide levels and protein carbonyls formation were increased. The results suggest that reactive oxygen species formation by inflammatory reaction is suppressed by combined treatment of 3rd generation cephalosporin and erythromycin.

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The Evaluation of Fetal Middle Cerebral Artery Doppler Correlates with the Onset of Labor. Filiberto M Severi,[1] Carlotta Boni,[1] Alberto Imperatore,[1] Luca Bruni,[1] Caterina Bocchi,[1] Fernando M Reis,[2] Felice Petraglia.[1] *[1]Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Siena, Italy; [2]Obstetrics and Gynecology, UFMG, Belo Horizonte, Brazil.*

OBJECTIVE: The aim of the present prospective cohort study was to evaluate the correlation between blood flow Pulsatility Index in fetal Middle Cerebral Artery (MCA) and the onset of spontaneous labor.

STUDY DESIGN: Doppler evaluation of fetal MCA were performed between 24 and 41 weeks gestation in 664 consecutive pregnant women with a singleton pregnancy and known gestational age. The study population was divided according to the MCA PI (< 0.74 or >/= 0.74 MoM) and, using survival analysis and Cox regression, the two subgroups obtained were compared. In these analyses, the event was delivery (following spontaneous labor) and the time variable was time elapsed since Doppler exam.

RESULTS: The median time elapsed between Doppler evaluation and spontaneous labor was significantly shorter in the women with MCA PI lower than 0.74 MoM (5.5 days, interquartile range 2-10) in comparison with the women with MCA PI higher or equal than 0.74 MoM (22.5 days, interquartile 5-37.5 days (P<0.001, Mann-Whitney test). After correction for birth weight and umbilical artery PI, survival analysis and Cox regression confirmed that MCA PI was independently associated with the number of days elapsed from Doppler to spontaneous labor and delivery (p< 0.001, Exp(B) 2.77, CI 95% 1.95-3.90).

CONCLUSIONS: The present data show that, at term of pregnancy, fetal cerebral resistance reduction anticipates the onset of spontaneous labor.

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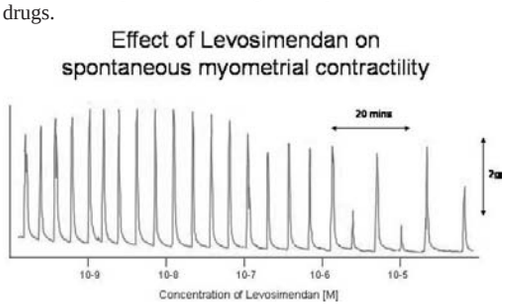
Novel Calcium Sensitizers and Human Uterine Contractility. Mark P Hehir, Audrey T Moynihan, Terry J Smith, John J Morrison. *Department of Obstetrics and Gynaecology, National University of Ireland, Galway, Ireland.*

Objective: The factors regulating contractility of uterine smooth muscle are central to the occurrence of preterm labour and delivery. Calcium sensitizers are a novel class of drugs with unique molecular actions. Levosimendan, the best characterized of these compounds, is used in the treatment of acute and chronic heart failure and is a compound which exerts a number of effects on smooth muscle. It can exert an inotropic effect via sensitization of myofilaments to calcium and also exerts a relaxant effect by opening ATP-dependent potassium channels. For these reasons we hypothesized that Levosimendan may have an effect on myometrial contractility and investigated its action on both spontaneous and agonist induced contractions.

Method: Biopsies of human myometrium were obtained at elective caesarean section (n=16). Dissected myometrial strips suspended under isometric conditions, undergoing spontaneous and oxytocin-induced contractions, were exposed to cumulative additions of levosimendan in the concentration range of 1 nmol/L to 100 mmol/L. Two sets of control experiments were performed simultaneously as follows: 1. Strips exposed to either physiological salt solution (PSS) only, for spontaneous contractions, or 0.5 nmol/L oxytocin; 2. Strips exposed to PSS/oxytocin and vehicle for levosimendan.

Results: Levosimendan exerted an inhibitory effect on spontaneous and agonist induced contractions, compared to control strips. The mean maximal inhibition values were as follows: 45.34 ± 5.92% for spontaneous contractions (n=6; P< 0.05) and 41.88 ± 5.40% for oxytocin-induced contractions (n=6; P<0.05). No significant difference was found between control 1 and control 2 for both spontaneous and oxytocin induced contractions.

Conclusion: The calcium sensitizer levosimendan exerted a potent relaxant effect on uterine contractility *in vitro*. This action was seen in both spontaneous and agonist induced contractions. The results from this study raise the possibility of calcium sensitizers holding potential tocolytic properties in vivo and further studies are required to investigate the potential benefits of this novel class of drugs.



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Comparing the Effect of Patient Movement upon Transabdominal Uterine EMG and Tocodynamometer Traces. Sangeeta Jain, William L Maner, Lynette B MacKay, Robert E Garfield. *Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, TX, USA.*

Introduction: Transabdominal uterine electromyography (EMG) and tocodynamometery (TOCO) data are best acquired when the patient is completely still. However, it is not always possible to have such ideal conditions in the clinic.

Objective: To determine to what degree extensive patient movement affects uterine EMG signals and TOCO signals.

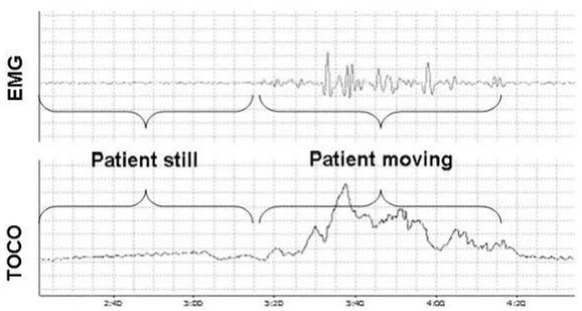
Study design: 11 pregnant term labor patients were recorded using both uterine EMG and TOCO simultaneously. Baseline recordings were obtained when patients were still, and these were used as control records. Test recordings for both devices were obtained from all patients by asking the patients to perform movements. Area under the rectified-voltage amplitude curve of the uterine EMG signals and area under the curve for the TOCO signals were found. Mean %-increase was calculated for the uterine EMG and TOCO devices (each device %-increase values were averaged over all 11 patients). Mann-Whitney rank-sum test was used to look for any statistical differences in %-increase in area for uterine EMG vs. TOCO methods ($P < 0.05$ considered significant).

Results: There was a large increase in activity (artifact) on both devices' signals during patient movement (**Figure 1**). Both devices' traces eventually returned to baseline after the patient movement stopped. TOCO movement artifact was significantly higher than EMG movement artifact (**Table 1).**

Conclusions: Both uterine EMG and TOCO signals experience artifact when patients move the uterine EMG electrodes and TOCO pressure transducer, respectively. TOCO seems to be more adversely affected by such movements. Uterine EMG may be a preferred method for monitoring contractions of laboring patients in the clinic. Supported by grant NIH R01- HD037480.

Table 1

Patient Number	TOCO % Increase	EMG % Increase
1	235	50
2	125	200
3	630	160
4	630	400
5	90	100
6	510	100
7	665	175
8	135	55
9	305	300
10	860	300
11	1425	300
Mean ± SD	510 ± 400 (* P < 0.05)	195 ± 115



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Consultant Attitudes to Delivery Following a Previous Lower Segment Caesarean Section: Personal Preferences Compared to Patient Recommendations. Shyamaly D Sur,[1] IZ Mackenzie,[2] K Murphy.[3] *[1]Dept of Obstetrics and Gynaecology, Stoke Mandeville Hospital, Aylesbury, Bucks, United Kingdom; [2]Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, Oxford, Oxon, United Kingdom; [3]Dept of Obstetrics and Gynaecology, St Mary's Hospital, London, United Kingdom.*

Objective: To investigate Consultant obstetricians' personal preferences and their professional advice to women in the management of pregnancies after a lower segment caesarean section.

Design: A structured questionnaire.

Setting: Two large medical deaneries in the United Kingdom.

Population: 219 consultant obstetricians.

Methods: Analysis by age and gender of the responses given to the structured questionnaire comparing the obstetrician's preferences for delivery for themselves or their spouse and how they would advise their patients according to the reason for the previous caesarean section.

Main outcome measures: Comparisons between patient recommendations and personal preferences for subsequent labour, spontaneous, augmented and induced and the impact of the reason for the initial caesarean section on preferred mode of delivery.

Statistics: Analyses were performed using binomial Analyses, Fisher Exact test and Relative Risk with 95% Confidence Intervals.

Results: 68% of obstetricians completed the questionnaire. None would counsel patients against labour unless there were contraindications. The majority would recommend labour for all indications for previous caesarean section investigated although in all instances, personal preferences were lower ($P<0.04$); after a failed instrumental delivery, 74% obstetricians would recommend labour but only 44% would choose that option for themselves ($p<0.01$). Overall, female obstetricians would contemplate and recommend labour more readily than male obstetricians. Labour augmentation and induction were recommended to patients more frequently (66% and 57% respectively) than chosen for personal care (57% and 52%). Reluctance for labour augmentation and induction was greatest among younger consultants.

Conclusion: Consultants have responded to consumerism and aim to meet the requests of their patients. They more readily recommend labour than they would choose for personal care, and a majority would recommend labour induction when necessary. Informed patient choice is paramount rather than attaining a target figure for women attempting to labour, and the views of those requesting delivery by caesarean section should be respected.

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How Can We Keep under Control Cesarean Section Rate? Clinical Audit of an Italian University Hospital. Elena Ciriello, Maddalena Incerti, Anna Laura Regalia, Simona Fumagalli, Elisabetta Nelli, Anna Locatelli. *Ob/Gyn, University of Milano-Bicocca, Monza, Italy.*

Objective: Cesarean delivery (CD) rate reached 37% in Italy during 2004, the higher rate observed in the North of the world. The objective of study is to assess the reasons of increase of CD rate in an Italian University Hospital with a low incidence of CD, standardized unchanged protocol of care and clinical audit implemented.

Methods: Retrospective clinical audit of all deliveries comparing demographic and obstetrics characteristics and the incidence and indications to CD in two periods (94-96 vs 04-06). We used the Robson classification of CD (AJOG 1996) to establish the groups of women contributing most to the overall CD rate.

Results:16,657 deliveries were studied. The populations in the two periods significantly differed for maternal age (16% vs 27% 35 >years , $p<0.001$), obesity (0.6% vs 1.2 % BMI >35, $p<0.001$), and gestational age (6% vs 5% ≥41.5 weeks, $p=0.001$). CD rate increased from 12.6% to 17.8%, $p<0.001$ (Table). CD increased in nullipara singleton, cephalic, term pregnancy in spontaneous labor, mostly due to dystocia; nullipara singleton, cephalic term pregnancy with induced labor, mostly due to non-reassuring fetal status and dystocia; singleton, cephalic term pregnancy with previous CD, mostly due to elective CD; singleton cephalic preterm. No changes in neonatal outcome were observed.

Conclusion: Clinical audit was useful to keep under control CD rate in our Institution in comparison with Italian reality, but it was not sufficient to maintain stable CD rate suggesting the need of other, multifaceted strategies.

	Years 94-96 % of group	Years 04-06 % of group	p	Years 94-96 % of CD	Years 04-06 % of CD	p
Nullipara single term cephalic, spontaneus labor	35	29	<0.001	5	8	<0.001
Nullipara single term cephalic, induced labor and elective CD	14	13	0.4	18	30	<0.001
Multipara single term cephalic, NO previous CD, spontaneus labor	30	31	0.01	1	1	0.2
Multipara single term cephalic NO previous CD, induced labor and elective CD	6	7	<0.001	8	9	0.5
Previous CD, single cephalic term	5	7	<0.001	34	46	<0.001
Breech	3	3	0.9	91	95	0.09
Abnormal lies	0.1	0.1	0.9	100	100	1
Multiple pregnancy	1	2	0.002	57	57	0.9

Single cephalic preterm	6	6	0.8	34	41	0.03

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Iatrogenic Versus Spontaneous Preterm Birth: Do Neonatal Outcomes Differ? Jamie Bastek, Sindhu K Srinivas, Michal A Elovitz. *OBGYN; CRRWH, University of Pennsyvlanai, Philadelphia, PA, USA.*

Objective: Preterm birth (PTB) is a leading cause of perinatal morbidity and mortality. Whether outcomes for neonates born due to spontaneous preterm labor (PTL) differ from those of neonates for whom PTB is indicated secondary to preeclampsia (PEC) is unknown. This study sought to compare differences in short-term neonatal outcomes in premature infants from spontaneous versus iatrogenic PTB.

Methods: Data was collected from two cohorts of women: (1) those admitted with spontaneous PTL (2002-2005) and (2) those admitted with PEC (2005-2007). Patients delivering 24-36.6 weeks were included (PTL: N = 240; PEC: N = 195). The incidence of NICU stay ≥8 days, antibiotic treatment ≥3 days (ABX), and composite outcome (COMP) (respiratory support, IVH, NEC, or seizure) in infants from spontaneous PTB (SPTB) were compared to infants from iatrogenic PTB (IPTB). Chi-square analysis was used to compare outcomes between women in each cohort at each gestational age category.

Results: SPTB results in greater adverse COMP neonatal outcome across gestational ages assessed (TABLE). In the very preterm infants, this increase in adverse neonatal outcome is secondary to increased need for respiratory support. Among later gestational age infants, SPTB results in a significantly longer stay in the NICU. There was no difference in length of antibiotic duration between the two cohorts regardless of gestational age.

Conclusions: The likelihood of adverse neonatal outcome differs by cause of PTB. Whether the observed increase in adverse outcomes in infants from SPTB is due to the presence of inflammation is not clear. Further investigation is required to correlate differences in short-term neonatal morbidity between these two groups with long-term adverse neonatal outcomes.

Outcome	24.27.6 wks			28.32.6 wks			33.36.6 wks		
	SPTB (%)	IPTB (%)	P value	SPTB (%)	IPTB (%)	P value	SPTB (%)	IPTB (%)	P value
COMP	97	70	0.01	74	55	0.02	24	12	0.02
RESP	97	71	0.01	71	82	0.31	20	22	0.91
IVH	51.2	20	0.10	19.5	21.7	0.31	2.6	2.4	0.76
NEC	15	0	0.37	11.5	6.7	0.42	2.6	3.2	0.83
SZ	5.1	0	0.94	1.7	0	0.87	0.9	0	0.94
NICU	84.6	90	0.52	90.8	93.3	0.42	68.6	41.6	<0.001
ABX	76.9	50	0.71	65.5	48.3	0.21	18.4	8.8	0.16

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Estimation of Maternal Mortality Rates Associated with Elective Delivery at 39 Weeks Gestation. Michael Y Divon,[1] Noa F Feldman-Leidner,[1] Nachum Sicherman,[2] Victoria Minior,[1] Asaf Ferber.[1] *[1]Obstetrics and Gynecology, Lenox Hill Hospital, New York, NY, USA; [2]Graduate School of Business, Columbia University, New York, NY, USA.*

Elective delivery at 39 weeks' gestation is a common obstetric intervention. The purpose of this study is to estimate the maternal mortality rates (MMR) associated with this ACOG – approved intervention.

There are no reliable US data describing MMR by mode of delivery. Therefore, we base our estimates on British data indicating a procedure related MMR of 2.06/100,000, 5.8/100,000 and 18.2/100,000 for vaginal, elective cesarean section (C/S) and emergency-unplanned C/S deliveries, respectively.

A decision tree model was constructed assuming that all eligible patients (approx. 2,500,000/annually in the U.S.) would be delivered at 39 weeks by either an elective C/S or an induction of labor. We further assumed that 50% of inductions would result in a vaginal delivery.

Our estimates show that the annual, delivery-related maternal mortality associated with an elective delivery of all patients at 39 weeks would be 145 and 253 for elective C/S and induction of labor, respectively.

Because vaginal delivery results in the lowest MMR, we performed a one-way sensitivity analysis to identify the impact of changing the success rate of induction on the estimated MMR. The results indicate that once the success rate exceeds 77%, this intervention would be associated with a lower MMR as compared to elective C/S. Our estimates indicate that although the overall MMR associated with elective delivery at 39 weeks is relatively low (approximately 12.5/100,000 deliveries), it is certainly not negligible. In addition, the MMR is highly dependent on the likelihood of a successful vaginal delivery following induction of labor. When the success rate for induction of labor falls below 77%, an elective C/S appears to be the safer delivery method.

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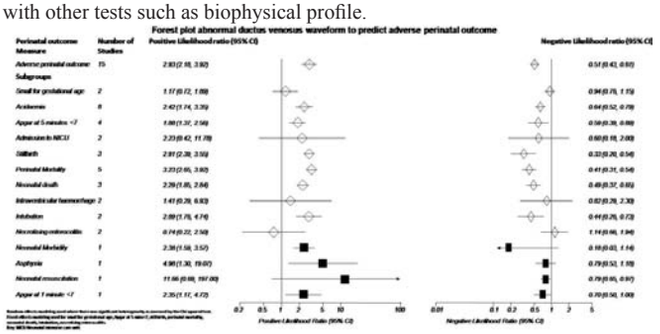
Systematic Review and Meta-Analysis of the Accuracy of Ductus Venosus Doppler To Predict Fetal Growth Restriction and Compromise of Fetal and Neonatal Wellbeing. Rachel K Morris,[1] Tara J Selman,[1] Meenakshi Verma,[1] Stephen C Robson,[2] Jos Kleijnen,[3] Khalid S Khan.[1] *[1]Academic Department of Obstetrics and Gynaecology, University of Birmingham, Birmingham Women's Hospital, Birmingham, United Kingdom; [2]School of Surgical and Reproductive Sciences, University of Newcastle, Newcastle, United Kingdom; [3]Kleijnen Systematic Reviews Ltd, York, United Kingdom.*

Objective To determine the accuracy of ductus venosus Doppler to predict fetal growth restriction and compromise of fetal and neonatal wellbeing.

Review methods Systematic searches in Medline, Embase, Cochrane Library, MEDION, reference lists, and contact with experts. All studies reporting on ductus venosus Doppler with data for a 2x2 table were selected. No language restrictions. Independent selection of studies, data extraction, and quality assessment. Meta-analysis of sensitivity and specificity and likelihood ratios was conducted when appropriate.

Results There were 15 included studies, 1463 pregnancies, all were performed in high risk pregnancies with the Doppler results used for analysis performed within 2 weeks of delivery. An abnormal ductus venosus waveform predicted adverse perinatal outcome with a pooled positive likelihood ratio (LR+) of 2.93 (95% CI 2.18-3.92) and negative likelihood ratio (LR-) of 0.51 (95% CI 0.43-0.61) (figure 1). Subgroup analysis showed the most accurate waveform index to be pulsatility index for veins >95th centile; LR+ 3.74 (95% CI 1.49-9.39), LR- 0.57 (95% CI 0.4-0.82). Subgroup analysis showed that of the measures of adverse perinatal outcome, abnormal ductus venosus best predicted birth asphyxia (cord umbilical artery pH<7.10/BE<-13) with a LR+ 4.98 (95% CI 1.30-19.07), LR- 0.79 (95% CI 0.33-1.18).

Conclusion Doppler assessment of the ductus venosus in high risk pregnancies has limited accuracy to predict adverse perinatal outcome. To assess its true value in a clinical setting we must look at its predictive accuracy when combined with other tests such as biophysical profile.



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Discussions of Substance Use during the First Obstetric Visit. Judy C Chang,[1] Diane Dado,[2] Richard Frankel,[3] Robert Arnold.[4] *[1]Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA; [2]Magee-Womens Outpatient Clinic, Magee-Womens Hospital, Pittsburgh, PA, USA; [3]Medicine and Regenstrief Institute, Indiana University School of Medicine, Indianapoplis, IN, USA; [4]Medicine, University of Pittsburgh, Pittsburgh, PA, USA.*

Background: The first obstetric visit is an opportunity to provide the pregnant patient information regarding substances that can cause potential harm to the pregnancy. Little is known about how obstetric care providers handle these topics. **Objective:** To examine patient-provider communication about substance use during the first prenatal visit. **Methods:** We audiotaped first prenatal visits and qualitatively analyzed those tapes in which patients disclosed substance use. We invited patient participants to return for a semi structured interview during which they reviewed their audiotaped conversations and described their reactions to the providers' communication styles. **Results:** 29 providers (21 residents, 5 midwives, 3 nurse practitioners) and 51 patients participated. Providers asked about smoking, alcohol and drug use in all 51 (100%) visits. 25 patients reported being smokers, 4 reported alcohol use, and 11 reported drug use. Provider responses to smoking disclosures included brief discussions of smoking effects on pregnancy, encouragement to quit/cut down, and referral to smoking cessation programs. Provider responses to alcohol or drug disclosures included only general statements regarding effects on pregnancy (e.g., "We find that this is bad for babies.") and referral to ultrasound/genetics for reassurance. Few alcohol or drug discussions assessed whether the patient had intentions or concerns regarding continued use during the pregnancy. Few discussions

addressed strategies for behavioral change. None included assessment for motivations, readiness, or barriers to change. In follow up interviews, patient participants said they expected to be asked about substance use but advised providers to ask non-judgmentally. Those who used alcohol/drugs wanted more information on potential effects of these substances on the pregnancy/fetus and appreciated the reassurance from referrals to ultrasound/genetics. **Discussion:** Counseling for risky behaviors in the first obstetric visits contained only limited discussion of the effects of the risky behaviors and primarily focused on referral—which may be a proxy for avoiding a difficult and time consuming conversations.

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Impact of Maternal Psychological Distress on Fetal Weight, Prematurity and Intrauterine Growth Retardation. Tullia Todros,[1] Giuseppe Maina,[2] Paola Saracco,[2] Maria Rosa Giolito,[3] Daniele Danelon,[1] Filippo Bogetto.[2] *[1]Department of Obstetrics and Gynaecology, University of Turin, Turin, Italy; [2]Department of Neuroscienze, University of Turin, Turin, Italy; [3]Family Planning Clinics, Turin, Italy.*

Background. There are conflicting results regarding the association of maternal psychiatric disorders or psychological distress due to stressful life events with pre-term birth and low birth weight. Aims. To investigate the association between maternal psychiatric disorders and/or stressful life events and intrauterine growth abnormality, low birth weight or preterm birth. **Method.** Three mutually exclusive and homogeneous groups of pregnant women (20 with actual psychiatric disorder, 20 with stressful life events, and 40 healthy comparisons) underwent serial fetal ultrasound examinations and uterine and umbilical artery Doppler velocimetry at 20 (±1), 28 (±1) and 34 (±1) weeks of gestational age. Subjects were recruited from all consecutive women attending two antenatal clinics. The presence of any maternal medical illness, drug treatments, fetal chromosomal and/or structural malformations, were exclusion criteria. All women recruited underwent a structured interview at 18-20 weeks for the psychiatric diagnosis (Mini International Neuropsychiatric Interview - MINI) (Sheehan *et al*, 1998); moreover, the 17-item Hamilton Rating Scale for Depression and the Hamilton Rating Scale for Anxiety were included in the assessment. The person who obtained obstetrical clinical data was blind to the results of psychological evaluations. **Results.** The three groups were comparable for: age, parity, socioeconomic status, smoking, alcohol consumption, body mass index. Gestational age at birth was not different in the three groups. Infants of women with psychiatric disorders had significantly lower birthweight (3084 ± 414 g) and higher percentage of birth weight below the 10th centile for gestational age (30%) than infants of healthy mothers (3408 ± 453 g and 5%, respectively). There was also a trend towards lower mean birth weight (3242 ± 400 g) and higher incidence of birth weight below the 10th centile (10%) in the stressful life event group. There was no significant difference among groups in the percentage of abnormal uterine or umbilical Doppler results. **Conclusions.** Maternal psychiatric disorders are associated with a lower birth-weight, but the effect is unlikely to be due to abnormal utero-placental or feto-placental vascularisation.

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Sonographic Screening for the Detection of Intrauterine Growth Restriction and Small for Gestational Age Infants in Pregnancies with Elevated Human Chorionic Gonadotropin Levels: What Is the Optimal Regimen? Tamera J Hatfield,[1] Aaron B Caughey,[2] David C Lagrew,[3] Ryan G Heintz,[1] Judith H Chung.[1] *[1]University of California, Irvine, Orange, CA, USA; [2]University of California, San Francisco, San Francisco, CA, USA; [3]Saddleback Memorial Medical Center, Laguna Hills, CA, USA.*

Objective: The purpose of this study was to evaluate antenatal ultrasound as a tool for the detection of intrauterine growth restriction (IUGR) and small for gestational age (SGA) infants among subjects with elevated human chorionic gonadotropin (hCG) levels on second trimester serum screening. Although IUGR has been linked to elevated hCG levels, the optimal screening regimen for antenatal sonographic surveillance has not been previously established.

Methods: A retrospective cohort study was performed at Saddleback Memorial Medical Center where serial ultrasounds from 26 weeks-delivery are generally recommended for patients with hCG levels > 2.0 MOM. All pregnancies were dated by second trimester ultrasound ± last menstrual period. Subjects with an hCG > 2.0 MOM, who had at least one antenatal ultrasound evaluation for IUGR, were identified from an electronic ultrasound database used for clinical report generation. Ultrasound data were then linked to an obstetrical birth outcomes database for relevant demographic/delivery information using unique identifiers. IUGR was defined as a sonographic estimated fetal weight (EFW) <10%ile for the estimated gestational age (EGA). SGA was defined as an actual birthweight <10%ile for the EGA at the time of delivery.

Results: From 1999-2007, there were 659 subjects with elevated hCG levels who underwent antenatal ultrasound surveillance for IUGR and who had known delivery information. A total of 1708 ultrasound examinations were performed. The median number of examinations per subject was 3 with a range from 1-5 examinations per subject. The incidence of IUGR and of SGA were 5.0% (n=33/659) and 7.3% (N=48/659), respectively. No fetus with IUGR demonstrated absent or reverse end diastolic umbilical artery doppler flow. Antenatal ultrasound examinations only identified 31.3% (N=15/48) of SGA infants. However, the sensitivity for the detection of SGA was 100% when an EFW cut-off of 75% was used.

Conclusions: Although the majority of SGA infants did not demonstrate growth restriction on antenatal ultrasound, a sonographic EFW > 75%ile appears to be a safe cut-off to rule out fetuses at risk for SGA.

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Neonatal Dry Lung Syndrome after PPROM: Reason To Change Intrauterine Referral Practice in the Netherlands? Ellen S Hoogakker,[1] Christiaan V Hulzebos,[2] Gerda G Zeeman.[1] *[1]Obstetrics and Gynecology, University Medical Center Groningen, Groningen, Netherlands; [2]Pediatrics, Division of Neonatology, University Medical Center Groningen, Groningen, Netherlands.*

BACKGROUND: Neonatal Dry Lung Syndrome (DLS) is a distinct clinical entity following PPROM, mimicking pulmonary hypoplasia but with dramatic respiratory improvement during the first 24-48^{h}. Its pathogenesis implies complete collapse of small airways to a degree that capillary forces impede distension by ordinary ventilatory pressures. In the Netherlands, women with PPROM remain admitted at a level III NICU perinatal center until they reach 32 wks. When they are referred back to their community hospital, unless they live in the neighborhood of the tertiary center. We question this referral pattern because we still see severe respiratory problems occur ≥ 32 wks. We sought to determine the prevalence of such morbidity, in particular DLS, in women with PPROM who deliver ≥ 32 wks.

METHODS: Retrospective descriptive study of neonatal outcome data of singleton pregnancies complicated by PPROM between 24-32 wks, who deliver ≥ 32 wks (latency > 24^{h}), during the 4-yr period of 2002-2006 at a single academic center. Data were extracted from medical records and electronic department databases.

RESULTS: 108 PPROM pregnancies were identified. All but 2 received at least 1 full course of steroids. 27 (25 %) delivered ≥ 32 wks. 3 newborns born at the community hospital needed emergency transportation to a level III NICU for respiratory morbidity. Neonatal outcome data (means ± SD) are listed in the table. There were no cases of late onset sepsis, NEC or perinatal mortality.

CONCLUSIONS: Respiratory morbidity still occurs after PPROM with delivery ≥ 32 wks. Further investigation of pregnancy-related characteristics, such as the presence of anhydramnios and the latency period, with regards to DLS is needed. Modification of current referral practice depends upon complete data derived from all Dutch level III perinatal care centers (n =10).

	Tertiary care center (n = 8)	Community hospital (n = 19)
mean birthweight (grams)	2037 (± 363)	2214 (± 542)
EGA @ PPROM (weeks)	28.3 (± 3.4)	30.6 (± 0.8)
EGA @ birth (weeks)	33.4 (± 0.8)	34.4 (± 2.2)
ventilator requirement (n)	2	3
Days on ventilator	1.0 (± 2.4)	0.63 (± 1.6)
Days on CPAP	3.3 (± 7.6)	0.42 (± 1.2)
RDS (n)	1	2
Pneumonia (n)	0	1
Pneumothorax (n)	1	1
Dry Lung Syndrome (n)	0	1
IVH (n)	0	2
Early onset sepsis (n)	1	1

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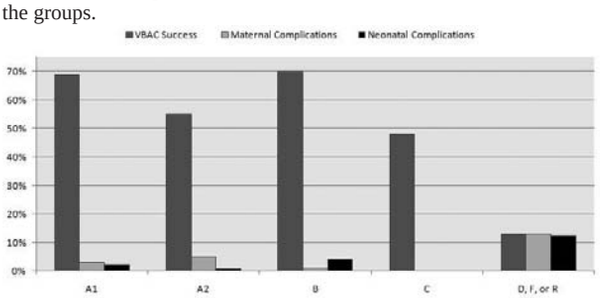
Relationship between Severity of Maternal Diabetes and VBAC Success in Women Undergoing Trial of Labor. Clint M Cormier.[1,2] *[1]Obstetrics, Gynecology, and Reproductive Sciences, University of Texas Health Sciences Center at Houston, Houston, TX, USA; [2]NICHD MFMU Network, Bethesda, MD, USA.*

Objective: To compare maternal and neonatal outcomes of diabetic women attempting VBAC (vaginal birth after cesarean delivery) based on White's Classification.

Study Design: This is a secondary analysis of an observational study conducted

at 19 medical centers of women attempting term VBAC. Diabetic women with singleton gestations, one prior cesarean delivery, and cephalic presentation who underwent a trial of labor (TOL) were included. VBAC success rates, maternal and neonatal complications were compared based on White's Classification.

Results: Of 17,898 women who underwent trial of labor, 624 met all study criteria (Class A_1=356, A_2=169, B=70, C=21, D, R, or F=8). VBAC success rates, combined maternal complication rates (includes uterine rupture, uterine dehiscence, need for hysterectomy, deep vein thrombosis, need for transfusion, and maternal death), and combined neonatal complication rates (includes 5 minute Apgar <4, cord arterial ph <7.0, seizures, hypoxic-ischemic encephalopathy, and neonatal death) for each category of White's classification are shown in the figure. Cochrane-Armitage test for trend showed decreased VBAC success with increasing White's Class (P=0.003). However, logistic regression analysis of VBAC success, when adjusted for inductions and gestational age, showed D,F and R diabetics to be significantly lower than A_1 (OR 11.8, 1.9-101.2) and B,(15.4 1.7 -138.0) but not A_2 or C. Maternal and neonatal complications were rare, and not found to be different between the groups.



Conclusion: VBAC success rates are lowest in patients with most severe forms of diabetes (D, F, or R). Maternal and neonatal complication rates are low and not related to White's classification.

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The Association of Macrosomia and Perinatal Outcomes in Patients with and without Gestational Diabetes Mellitus. Tania F Esakoff, Yvonne W Cheng, Teresa Jones, Aaron B Caughey. *Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA.*

Objective: To determine if an association exists between macrosomia (birthweight > 4000g) and perinatal outcomes in women with and without gestational diabetes mellitus (GDM).

Study Design: This is a retrospective cohort study of 36,241 singleton pregnancies. The study cohort was stratified by the diagnosis of GDM, with the presence or absence of macrosomia as the dependent variable. Perinatal outcomes examined included neonatal hyperbilirubinemia, hypoglycemia, respiratory distress syndrome (RDS), shoulder dystocia and Erb's palsy. Chi-square tests were performed as well as multivariable analyses controlling for confounders, using p<0.05 to indicate statistical significance.

Results: In women diagnosed with GDM, macrosomia is associated with a higher frequency of hypoglycemia, respiratory distress syndrome, shoulder dystocia and Erb's palsy. Though the prevalence of these outcomes is relatively decreased in patients without GDM, they are still more prevalent in macrosomic patients.

Conclusions: Macrosomia is associated with a higher prevalence of adverse perinatal outcomes in women with and without GDM. Therefore, it is important to evaluate neonates with birthweights greater than 4000 grams for hypoglycemia and unrecognized Erb's palsy.

Table 1: Prevalence of perinatal outcomes in the presence and absence of macrosomia

	+GDM/ -Macro-somia	+GDM/ +Macro-somia	OR p-value	-GDM/ -Macro-somia	-GDM/ +Macro-somia	OR p-value
Hyperbili-rubinemia	10.4%	13.2%	1.6 p=0.90	9.1%	7.6%	0.9 p=0.23
Hypogly-cemia	**2.6%**	**5.3%**	2.6 p=0.04	**1.2%**	**2.4%**	2.1 p=0.00
RDS	**1.5%**	**4.0%**	3.1 p=0.03	**1.2%**	**1.7%**	1.5 p=0.02
Shoulder dystocia	**1.6%**	**10.5%**	16.4 p=0.00	**0.9%**	**6.0%**	9.2 p=0.00
Erb's Palsy	**0.2%**	**2.6%**	41.9 p=0.00	**0.1%**	**0.7%**	7.6 p=0.00

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Obesity in Pregnancy – What Do Patients Think? Julie K Phillips, Cynthia Argani, Joe Richardson, Janice Henderson. *Gynecology and Obstetrics, Johns Hopkins Hospital, Baltimore, MD, USA.*

Objective

Obesity in pregnancy is a modern epidemic with the potential for adverse perinatal outcomes. Our objective was to evaluate and compare patient attitudes and perceptions about diet, weight gain, and nutrition in pregnancy as well as to evaluate the influence of education on their BMI status.

Study Design

With IRB approval, all obstetrical patients presenting for prenatal care at one prenatal site between September 2006 and July 2007 at Johns Hopkins Hospital were asked to complete a questionnaire which included demographic data, dietary practices, knowledge of risks of obesity in pregnancy and understanding of normal weight gain during pregnancy. Chi-square and Fisher's exact tests were used to compare perceptions about weight gain during pregnancy and level of education by BMI stratification. BMI was calculated using pre-pregnancy weight and was categorized as overweight (BMI 26-30), obese (BMI 30-35), and morbidly obese (BMI >35).

Results

170 patients were surveyed, of which 114 were able to be analyzed and BMI calculated. Of these, 43% were of normal weight, 17% were overweight, and 24% were obese, of which 10% were morbidly obese. There was a statistically significant difference between level of education between those of normal BMI and those patients who were morbidly obese (p=0.005), with 65% of patients with a normal BMI having a college level of education or higher versus 10% of morbidly obese patients completing college. A similar percentage of patients in the normal BMI and obese groups (17% and 19%) thought that gaining less than 15 pounds during pregnancy was appropriate. On the other hand, 6% of patients in the normal BMI group versus 21% of those in the obese BMI groups felt that it was appropriate to gain greater than 35 pounds during pregnancy. Surprisingly, 30% of obese patients felt that they were of appropriate weight (p=0.004).

Conclusion

A significant proportion of our obstetrical patients are obese and many do not perceive themselves to be obese. While our finding of an inverse correlation between level of education and BMI may be confounded by socioeconomic status, our results suggest that in order to address the problems of obesity in our population an important first step will be improving the education of our reproductive age women regarding normal weight gain and nutrition in pregnancy. This may have a significant impact on improving pregnancy outcomes of today's obstetrical population.

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Flat (<10%) Response to the Oral Glucose Challenge Test (GCT) and Pregnancy Outcomes. Arianna Prada, Camilla Bulfoni, Anna Maria Marconi. *Obstetrics and Gynecology, University of Milano, DMSD San Paolo, Milano, Italy.*

Objective: The aim of the study was to evaluate the impact of a "flat" oral GCT upon the outcome of pregnancy. The GCT was considered flat when the difference between the basal value and the after load value was ≤ 10%.

Methods: We prospectively analyzed the outcomes of 410 pregnancies who delivered at our Department. Inclusion criteria were singleton pregnancy; BMI <30 kg/m^2, absence of major risk factors for diabetes and of pregestational diabetes. 50 g 1-hour oral GCT was performed at 24 - 28 weeks of gestation. Women were subdivided into 3 groups according to the result of the GCT: Group 1= negative (load glucose >10% and <140 mg/dl) 327 women (79.8%); Group 2=flat (load glucose ≤10% than basal and <140 mg/dl) 51 women (12.4%); Group 3=positive GCT/negative OGTT, 32 women (7.8%). Data are mean ± SD. Differences were calculated with the Student *t* test for unpaired samples and χ^2 test. Regression analysis were performed by the least squared method. *P*-values were considered significant at $p<0.05$.

Results: The characteristics and obstetric outcomes in the three groups are presented in the Table 1. In all patients there was a significant linear relationship between the load and basal glucose values (load value=66.8+0.6 basal value; r=0.2; p<0.001) and between birthweight and load values (birthweight=2978.3+2.8 load value; r=0.15; p<0.02). The relationships were significant also in Group 1 and 2 separately but not in Group 3.

Conclusion: In this preliminary study we found no major outcome differences in women with flat GCT compared to women with normal and GCT positive/ OGTT negative results. It seems important, however, to futher investigate the meaning of a flat curve in a bigger population and/or by means of metabolic studies with the use of stable isotopes.

TABLE 1	Group 1	Group 2	Group 3	p 1 vs 2	p 1 vs 3	p 2 vs 3
Age years	32.1±5.1	30.3±5.9	34.4±4.3	0.02	0.01	0.001
Caucasian %	88.4	82.3	87.5	ns	ns	ns
BMI kg/m2	21.9±3	21.9±2.9	22.4±2.8	ns	ns	ns
Basal glucose mg/dl	76.4±7.3	81.1±7.9	79.4±2.8	0.001	0.04	ns
Load glucose mg/dl	112± 15.7	81.6± 10.5	154.6± 11.5	0.001	0.001	0.001
Weeks at delivery	39.3±1.3	39.3±1.3	39±1.4	ns	ns	ns
Birthweight grams	3287± 419	3241± 394	3393± 470	ns	ns	ns
Nulliparous %	57.8	47.1	71.9	ns	ns	ns
Pregnancy complications %	10.1	7.8	15.6	ns	ns	ns

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Pre Pregnancy Body Mass Index (BMI) and Pregnancy Outcome. Monica Moneta, Arianna Prada, Anna Maria Marconi. *Obstetrics and Gynecology, University of Milano, DMSD San Paolo, Milano, Italy.*

Objective The aim of our study was to determine the impact of pre pregnancy BMI upon pregnancy outcome

Materials and Methods We retrospectively analyzed pregnancy outcome in 5854 singleton pregnancies. Women were grouped by BMI (weight kg/height m^2) into 4 groups based on the WHO criteria (see table 1). Data are presented as mean±SD. Statistical methods included Student *t* test for unpaired samples, χ^2 test and univariate and multivariate logistic regression models. Values were considered significant at $p<0.05$.

Results Characteristic of the study population and obstetric outcomes are presented in table 1. The probability to be primiparous and to deliver babies <10° centile decreased significantly with BMI whereas the risk of cesarean section, of post partum hemorrhage at vaginal delivery and to deliver babies >90° centile increased significantly. Also the risk to develop preeclampsia and gestational diabetes was increased, although not significantly, with BMI. Compared to lean and normal, obese were more likely to be hypertensive and diabetic before pregnancy (p<0.001) and to start pregnancy without any medical and obstetrical risk but obesity (85.6% vs 90.5 and 90.2%; p<0.01). African women exhibited the highest BMI and the highest rate of obesity (Table 2).

Conclusions We confirm that obese women have an increased risk of pre-pregnancy and pregnancy complications: less than 50% have an uncomplicated pregnancy. At delivery there is an increased risk of cesarean section and post partum haemorrhage.

Table 1

	Lean <18.5; n=529 (9%)	Normal 18.5-24.9; n=4027 (68.8%)	Overweight 25-29.9; n=972 (16.6%)	Obese ≥30; n=326 (5.6%)	
Age (years)	30.4±6	31.4±5.4	31.5±5.3	31.3±5.7	
BMI	17.5±0.9	21.5±1.7	26.9±1.4	33.4±3.7	
Weeks at delivery	39.1±1.7	39.2±2.2	39.2±2.6	39.3±3.7	
Birtweight <10° %	13.4	9.4	7.4	5.5	0.02
Birthweight >90° %	3.6	7	10.2	17.2	0.02
Para 0 %	49.5	42.9	35.9	28.5	0.001
Cesarean section %	20.8	23.9	30.3	33.1	0.01
Gestational diabetes %	2.1	2.9	4.6	9.2	
Preeclampsia %	0.5	1.1	1.1	4	
% Post partum hemorrhage ≥500 ml*	19.6	24.1	25.4	32.1	0.03

* at vaginal delivery

Table 2

	West Europe n=4963	East Europe n=128	Africa n=319	Asia n=213	South America n=231
BMI	22.5 ± 3.9	22.4 ± 3.7	25.5 ± 5.1	22.1 ± 3.5	23.2 ± 3.5
Lean %	9.2	13.3	5.3	12.2	4.8
Normal %	70.3	68	46.4	72.3	64.9
Overweight %	15.4	13.3	31.7	12.7	26.8
Obese %	5.1	1.4	16.6	2.8	3.5

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Maternal Body Mass Index as a Predictor of Abnormal Uterine Artery Doppler Indices. Kimberly W Hickey,[1] Nori Gilo,[1] Renee Evans,[2] Alessandro Ghidini,[2] Sarah H Poggi.[2] *[1]Maternal Fetal Medicine, Georgetown University Hospital, Washington, DC, USA; [2]Perinatal Diagnostic Center, Inova Alexandria Hospital, Alexandria, VA, USA.*

Background: Maternal uterine artery (UtA) Doppler indices can be used to predict fetal growth restriction, preeclampsia, and perinatal death. However, the effect of maternal obesity (a condition associated with high risk of adverse obstetric outcomes) on these indices has not been adequately evaluated.

Methods: In a cohort of high risk, singleton pregnancies (n=268), utA Doppler indices, evidence of notching, and body mass index (BMI) were obtained. The effect of BMI on average resistance index (RI) of right and left UtA was evaluated, with and without adjustment for fourteen demographic, clinical, and sonographic risk factors, using univariate and multiple regression analyses, with $p<0.05$ considered significant.

Results: Univariate analysis revealed a trend for positive correlation between average RI and BMI (RI=0.138, p<0.057). Multiple regression analysis controlling for maternal age, parity, past medical history, chronic hypertension, maternal pulse, estimated gestational age at service, and obstetrical outcomes showed that BMI was independently and significantly associated with UtA RI (p=0.002), with increases of 0.002 unit of RI for every one kg/m^2 increase in BMI. Presence of bilateral and unilateral notching was not significantly affected by BMI at multiple regression analyses (p=0.31 and p=0.14, respectively.

Conclusions: Maternal BMI is significantly associated with UtA RI and extremes of BMI may lead to mis-estimation of perinatal risk in women using an uncorrected RI. Consideration should be given for development of mathematical models with integration of BMI to correct estimated UtA RI.

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Baboon Placental System N Amino Acid (AA) Transporter Protein Is Down-Regulated by Moderate Global Maternal Nutrient Restriction (MNR). Natalia E Schlabritz-Loutsevitch,[1,2] Cun Li,[1,2] Laura Cox,[2] Peter W Nathanielsz.[1,2] *[1]Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; [2]Department of Genetics, Southwest National Primate Research Center, Southwest Foundation for Biomedical Research, San Antonio, TX, USA.*

Introduction: AA delivery to the fetus involves several placental transport systems (e.g. A, N, t, l). SNAT3 (SN1, SLC38A3) is the system N AA transporter for substrates glutamine (GL) , histidine, and asparagine (ASP). **Goal:** we explored the role of precisely controlled MNR on placental SNAT 3 protein abundance at 0.9 gestation (G) in a well documented animal model, the pregnant baboon.**Methods:** Baboons fed *ad lib* (CTR; n= 7) and nutrient restricted (MNR; n=6) were randomly assigned to receive 70% of *ad lib* fed CTR intake from 0.16G and delivered by Csection at 0.9 G. Western blot (WB) and immunohistochemistry (IHC) were performed for SNAT 3 (Antibody sc-33445, Santa Cruz, USA). Data M ± SEM. Significance set by two tail t-test p<0.05. **Results:** SNAT3 localized in syncytiotrophoblast (ST) (Fig.1). SNAT3 abundance (WB) were lower in MNR vs. CTR placentas (13.8 ± 1.08 vs 21.8 ±2.55 respectively; p<0.05)). **Discussion:** Previously we showed in this MNR model at 0.5G that MNR influences imaternal to fetal GL and ASP ratio (1) and MNR does not change SNAT3 placental mRNA (2) suggesting translational but not transcriptional regulation in MNR. **Conclusion:** We demonstrate for the first time SNAT3 expression in the baboon placenta and its regulation by maternal nutritional status.

1.Wu, G., Jenkins, S.L., Schlabritz-Loutsevitch, N., Nathanielsz, P.W. (2005) JSGI 12(2-Suppl): 122A, Abstract 117. 2. N. Schlabritz-Loutsevitch, L. Cox, G. Hubbard and P. W. Nathanielsz (2007). SSR Abstract # 242, P.35.

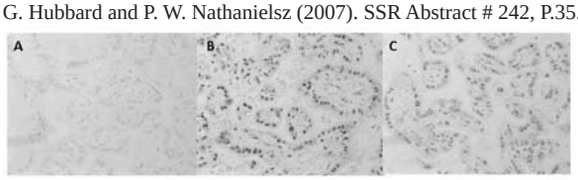

Figure1: SNAT3 IHC staining in baboon placenta at 0.9G: pre-absorbed -ve control (A) ; CTR (B) and MNR (C).

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Development of a Mouse Model of Obesity in Pregnancy. Helen N Jones,[1] Laura A Woollett,[2] Theresa L Powell,[1] Thomas Jansson.[1] *[1]Obstetrics and Gynecology; [2]Pathology and Laboratory Medicine, University of Cincinnati, Cincinnati, OH.*

Objective: Overweight and obese women often give birth to larger babies, which is associated with traumatic birth injuries and an increased risk to develop obesity, diabetes and hypertension in childhood and later in life. The mechanisms underlying fetal overgrowth in obese pregnant women are largely unknown. The aim of this study was to establish a mouse model of obesity/high fat diet in pregnancy. We hypothesized that a moderately high fat diet prior to and during pregnancy would result in increased maternal adiposity, fetal overgrowth and a metabolic profile similar to that of obese

pregnant women. **Method:** C57BL/6J female mice were fed control (C, 11% of energy from fat) or isocaloric high fat (HF, 32% of energy from fat) diets ad libitum for 8 weeks prior to mating and during gestation. At gestational day 18.5 maternal blood samples were obtained to measure adiponectin, leptin and cytokine levels and maternal fat pads were isolated and weighed. In a sub set of animals measurements of transplacental transport of neutral amino acids and glucose were performed *in vivo* under ketamine anesthesia using ^{3}H-MeAIB, and ^{14}C-glucose. **Results:** No significant differences were observed in maternal pre-pregnancy bodyweight, total caloric intake, weight of the dam at E18.5 or litter size between treatment groups. Fetal weight was increased in the HF group by 45% ($p<0.05$). Maternal adiponectin levels were significantly ($P<0.01$, n=18) decreased (HF 45 ± 8, C 69±15 μg/ml) and leptin levels increased by 60% in animals fed a high fat diet, but this difference did not reach statistical significance (n=6). Adiposity (fat pad weight) was increased by 7% ($P<0.05$, n=10 in the dams fed high fat diet, however no difference was observed in maternal IL-6 levels and neither group had measurable levels of TNF-α. Maternal red blood cell lipid profiles were altered in high fat animals with an increase in stearic and linoleic acids but decreased oleic acid levels. Preliminary data showed that placental uptake and transfer to the fetus of glucose and MeAIB were increased by at least 50% in dams fed high fat diet. **Conclusion:** This murine high fat diet model has several features consistent with human obesity in pregnancy and the maternal metabolic environment is similar to that seen in the human. The increase in placental uptake and transfer of nutrients constitute a key mechanism underlying fetal overgrowth in overweight/obesity in pregnancy.

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Maternal Overnutrition Does Not Upregulate Hepatic 11βHSD1 mRNA Expression of the Fetal and Postnatal Lamb. Leewen Rattanatray,[1,2] Beverly S Muhlhausler,[2] I Caroline McMillen.[2] *[1]Department of Physiology, Adelaide University, Adelaide, South Australia, Australia; [2]Sansom Institute, University of South Australia, Adelaide, South Australia, Australia.*

Objective: Epidemiological studies have demonstrated a positive relationship between maternal overnutrition and the development of the metabolic syndrome in the offspring. We previously reported that lambs of Well Fed ewes have increased plasma glucose levels in early life, this may be a consequence of altered hepatic glucose production. Increased 11βHSD1 expression is associated with an increase in intracellular cortisol, promotion of hepatic insulin resistance and a consequential increase in gluconeogenic activity.

Hypothesis: We hypothesised that maternal overnutrition in late gestation in the sheep results in increased hepatic expression of 11βHSD1 in the lamb before and after birth.

Methods: Ewes were provided with either 100% (Control,C) or 160% (Well Fed, WF) of maintenance energy requirements from 115d gestation until delivery. Post-mortem was performed on either 139±141d gestation (C=6,WF=8) or and postnatal day 30 (C=12,WF=9). Plasma glucose and leptin concentrations in the fetuses and lambs were determined. The relative hepatic mRNA expression of 11βHSD1 and reference gene RpP0 were determined by qRT-PCR.

Results: Relative liver weight was significantly higher in lambs of WF ewes compared to C at 30d ($P<0.05$). The expression of 11βHSD1 mRNA was significantly higher in the postnatal compared to the fetal lamb ($P<0.001$) independent of maternal nutritional treatment. However, there was no effect of maternal overnutrition on the hepatic expression of 11βHSD1 mRNA before or after birth. There was no effect of prenatal nutrition on fetal or postnatal plasma cortisol concentrations. The expression of 11βHSD1 in the liver of lambs of WF, but not C ewes, was inversely related to plasma glucose concentrations in the first 24hrs after birth (r^2=-0.78, P=0.002).

Conclusions: We have therefore demonstrated that exposure to prenatal overnutrition results in an inverse relationship between 11βHSD1 mRNA expression in the liver at 30d and plasma glucose concentrations on the first day of life. This suggests that exposure to high glucose levels before and immediately after birth results in a reduced expression of hepatic 11βHSD1. We would expect a reduction, rather than promotion of intra-hepatic cortisol production, and therefore it is unlikely to explain the hyperglycemia present in lambs of WF ewes in early life.

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Maternal and Neonatal Outcomes Associated with Obesity in Women with Type II and Gestational Diabetes Mellitus. Gladys A Ramos, Alethea A Hanley, Jennifer Aguayo, Carri R Warshak, Thomas R Moore. *Reproductive Medicine, University of California, San Diego, Sand Diego, CA, USA.*

Objective: To evaluate the relationship between maternal obesity on maternal and neonatal outcomes of women with Type II and gestational diabetes mellitus (GDM).

Study Design: A retrospective study of obese and non-obese pregnant women with Type II or GDM treated with diet, glyburide and insulin. Neonatal and maternal outcomes were assessed. Statistical methods included bivariate analyses.

Results: Between 2001-2004, 255 mother and neonate pairs were identified. Ninety-seven (38%) of mothers were classified as obese (BMI≥30) based on their first pregnancy weight. Glycemic control at 36 weeks was superior in the non-obese group (Table 1). There were no differences in glycemic control during the last week of pregnancy. Obesity was significantly associated with increased maternal weight gain during pregnancy. Mean birth weights, ponderal indices and rates of macrosomia were significantly higher in infants born to obese women when compared to non-obese (Table 1). Primary cesarean deliveries rates were comparable. The rate of neonatal hypoglycemia, hyperbilirubinemia, phototherapy and neonatal ICU admissions did not differ between obese and non-obese diabetic women (Table 1). Although not statistically significant, there was a trend towards an increased rate of birth injuries in the obese group. A similar comparison between obese and non-obese women treated with medication (glyburide or insulin) demonstrated a higher mean birth weight (3675g±593 vs. 3434g±520, p=.005) and higher rate of macrosomia (11 vs. 9%, p=0.04). There were no differences in glycemic control, cesarean delivery rates and other neonatal outcomes between the obese and non-obese treated with medication.

Conclusion: Obese women with Type II and GDM give birth to larger infants than their non-obese counterparts and have a higher incidence of fetal macrosomia. There was a trend towards increased rate of birth injuries in the obese group. Despite these differences other maternal and neonatal outcomes were similar which may be a reflection of glycemic control.

Table 1. Maternal and Neonatal Outcomes Associated with Obesity in Type II and Gestational Diabetes Mellitus.

Outcome	Obese (n=97)	Non-Obese (n=158)	p value
Diet Controlled (%)	19 (20)	47 (30)	0.79
Pharmacotherapy (%)	78 (80)	111(70)	
Weight Gain (lb)	26±13	21±17	0.01
Glycemic Control			
36 Weeks GA:			
Fasting (mg/dL)*	92.7±16	87±13	0.008
Postprandial (mg/dL)*	132.6±28	124.6±19	0.03
Last Week of Pregnancy:			
Fasting (mg/dL)*	89.6±14	86.4±19	0.26
Postprandial (mg/dL)*	123.4±23	123±16	0.91
Primary Cesarean Delivery (%)	20 (20)	26 (17)	0.41
Neonatal Outcomes:			
Birth Weights (grams)*	3691±619	3434±520	<0.001
Macrosomia (%)	28 (29)	19 (12)	<0.001
Ponderal Index	2.77±0.37	2.66±0.36	0.02
Hypoglycemia (%)	23 (24)	27 (17)	0.19
Hyperbilirubinemia (%)	15 (16)	23 (15)	0.84
NICU Admission (%)	26 (27)	38 (24)	0.66
Birth Injury (%)	6 (2.4)	2 (0.8)	0.057

* Values are mean ± standard deviation
GA, Gestational Age

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Maternal Obesity Accelerates Pancreatic β-Cell Mitotic Rate and Insulin Secretion in Fetal Sheep. Stephen P Ford,[1] Liren Zhang,[1] Mei J Zhu,[1] Bret W Hess,[1] Gary E Moss,[1] Peter W Nathanielsz.[1,2] *[1]Center for the Study of Fetal Programming, Department of Animal Science, University of Wyoming, Laramie, WY, USA; [2]Department of Obstetrics and Gynecology, University of Texas Health Sciences Center, San Antonio, TX, USA.*

Background: Maternal obesity is accompanied by short and long term unwanted effects on fetal development. Last year we reported that fetuses of obese (OB) ewes fed 150% of NRC recommendations from -60 to 75 days gestation (dG) were heavier ($P<0.05$) than fetuses of control (C) ewes maintained on 100% NRC. Absolute and relative pancreatic weight was heavier in fetuses of OB

ewes (OBF) than fetuses from C ewes (CF), and β-cell numbers, and insulin concentrations increased in pancreatic tissue. IGF-1 and cortisol (hormones known to stimulate islet development) were elevated in OBF blood.
Hypothesis: We hypothesized that increased pancreatic β-cell numbers in OBF result from increased mitosis and/or decreased apoptosis.
Methods: Using the dietary approach above, pancreatic tissue from (OBF) (n=5) and CF (n=5) were paraffin embedded, and evaluated for proliferating cell nuclear antigen (PCNA; DNA replication marker) using anti-PCNA (Santa Cruz Biotechnology, Santa Cruz, CA) or phosphor-histone H3 (pHH3; cell mitotic marker) using anti-pHH3 (Upstate, Lake Placid, NY) within insulin positive cells (anti-porcine insulin, Dako, Carpinteria, CA) via double-label immunofluorescence. β-cell apoptosis was determined by TUNEL assay (Roche Molecular Biochemicals, Mannhiem, Germany) and insulin immunostaining. Frozen (-80^{0}C) pancreatic tissue was used for PCNA and pHH3 western blotting.
Results: Percentage of β-cells staining positively for PCNA and pHH3 were greater in OBF pancreatic tissue than CF (12.2 ± 0.6 vs. 10.3 ± 0.3%; 1.2 ± 0.1 vs. 0.6 ± 0.1%, respectively; P<0.05). In contrast, no differences in β-cell apoptosis were observed between groups (1.0 ± 0.1 vs 1.4±0.2%). Western blot analysis confirmed the increases (P<0.05) in both PCNA (1.3 ± 0.1 vs. 0.9 ± 0.1 Arbitrary Units, AU) and pHH3 content (1.5 ± 0.1 vs. 1.2 ± 0.1 AU) in OBF pancreatic tissue versus CF.
Conclusions: We conclude that the increased β-cell numbers and insulin content in midgestation OBF results from increased mitotic rate rather than decreased programmed cell death. Failure of the pancreas to return to a normal cellular composition and function postnatally could lead to obesity, altered secretion and diabetes in offspring. NIH INBRE 1P20RR16474.

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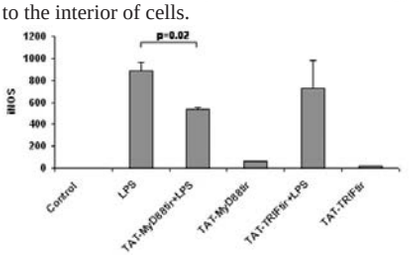
Modulation of Toll-Like Receptor Signaling In Vitro Using a 'Poison Subunit' Fused to the TAT Protein Transduction Domain. Yana Filipovich,[1] Emmet Hirsch.[1,2] [1]*Department of Ob/Gyn, Evanston Northwestern Healthcare, Evanston, IL;* [2]*Department of Ob/Gyn, Feinberg School of Medicine, Northwestern University, Evanston, IL.*
Objective: Toll-like receptors (TLR) are membrane proteins that initiate innate immune responses to pathogens by recognizing their specific molecular constituents. TLR-4 is the receptor for lipopolysaccharide (LPS), and after ligand binding it transduces signals intracellularly via two pathways, known as the MyD88 (myeloid differentiation primary response gene 88)-dependent and the MyD88-independent pathways. The MyD88-independent pathway depends upon the function of an adaptor protein known as TRIF (Toll/IL-1 receptor (TIR)-domain-containing adaptor inducing IFN-β). Both MyD88 and TRIF contain TIR domains that may antagonize the functions of the respective full-length proteins. The objective of this project was to test whether such antagonism of LPS-induced signaling could be accomplished in vitro by fusing either of these TIR domains to the TAT protein transduction peptide (which efficiently delivers exogenous proteins to the intracellular compartment).
Methods: Recombinant TAT-MyD88tir and TAT-TRIFtir fusion proteins were generated by standard cloning methods followed by expression and protein extraction under denaturing conditions from bacterial cultures. Two cell lines (the IEC-6 rat intestinal epithelial line and the RAW264.7 mouse macrophage line) were pre-treated with either one of the above fusion proteins or medium alone 30 minutes prior to 4 hours of incubation with LPS. The expression of inducible nitric oxide synthase (iNOS), used as a marker of TLR-4 activation, was determined by real-time multiplex RT-PCR and normalized to the expression of a housekeeping gene (GAPDH).
Results: Treatment with TAT-MyD88tir, but not TAT-TRIFtir, significantly diminished iNOS expression in both cell lines (see Figure for RAW264.7 cells).
Conclusion: LPS-induced responses can be modulated by a 'poison subunit' of the MyD88 adaptor protein. Fusion of this subunit to that TAT-protein transduction domain may be an effective strategy for delivery of such proteins to the interior of cells.



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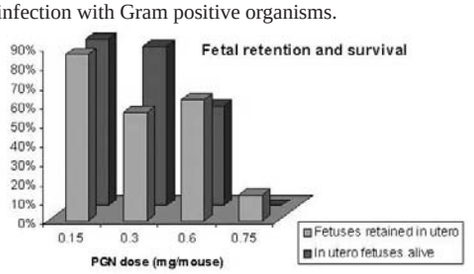
Preterm Delivery, Auto-Induction of TLR-2 and Fetal Retention and Survival after TLR-2 Activation in a Mouse Model. Vladimir Ilievski,[1] Emmet Hirsch.[1,2] [1]*Department of Ob/Gyn, Evanston Northwestern Healthcare, Evanston, IL;* [2]*Department of Ob/Gyn, Feinberg School of Medicine, Northwestern University, Chicago, IL.*
Background: Recent data support an important role for toll-like receptors (TLR) in bacterially induced labor. TLR engagement is the initial step in the innate immune response. TLR-2 mediates cellular responses to gram-positive organisms via their membrane lipoproteins, glycolipids, and peptidoglycans (PGN). We have demonstrated previously that preterm delivery can be induced in the mouse by intrauterine administration of PGN in a dose-responsive fashion.
Objective: The objective of this study was to investigate the impact of activation of TLR-2 on fetal survival and expression of TLR-2 in the uterus.
Methods: One uterine horn of preterm pregnant CD-1 mice at approximately 75% of gestation was injected with PBS or PGN (0.15, 0.3, 0.6 or 0.75 mg per mouse) in a volume of 100 μL. Necropsy was performed 48 hours later and retention and survival of fetuses in utero was recorded. For gene expression studies, the untreated left uterine horn of each animal (excised prior to injection of the right horn) was used as a negative control for the injected right horn, harvested 5 hours after treatment with 0.5mg PGN per mouse. Total uterine RNA was extracted and mRNAs were quantified by real-time RT-PCR in comparison to the housekeeping gene GAPDH.
Results: Increasing doses of PGN were associated with more complete evacuation of the uterus (see Figure). Among fetuses retained in utero, survival diminished proportionally with increasing PGN dose. Treatment with PGN significantly increased TLR-2 mRNA by a factor of 12.
Conclusion: TLR-2 activation has a dose-responsive effect on preterm delivery and on fetal retention and survival in utero. PGN induces uterine expression of TLR-2 in a positive feedback loop. This has implications for intrauterine infection with Gram positive organisms.



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Activation of TLR-3 in the Trophoblast Is Associated with Preterm Delivery. Kaori Koga, Paulomi B Aldo, Bing Peng, Sara Fill, Ingrid Cardenas, Gil Mor. *Obstetricsw and Gynecology, Yale University School of Medicine, New Haven, CT, USA.*
Introduction: TLRs are key components of the innate immune system which recognize conserved sequences on the surface of pathogens and trigger effector cell functions. The placenta, and more specifically the trophoblast, may play an important role in the response to infection. Previously, we described the expression of TLR-3 by human trophoblast and their ability to respond to polyinosinic-polycytidylic acid (polyI:C), a synthetic double strand RNA which mimics viral RNA. In the present study we evaluate the effect of polyI:C in mouse pregnancy and characterize the local and systemic response.
Material and Methods: Human first trimester trophoblast cell line, HTR8, was treated with polyI:C. C57B/6 wild type and TLR-3 knock out mice were injected intraperitoneally with PolyI:C at 16.5 gestation day. Cytokine and chemokine level were determined in supernatant and lysates using the Bio-Rad multiplex assay and analysis was done using the Bio-Plex100 IS.
Results: PolyI:C induced cytokine (IL6, IL1β and IL1α) and chemokine (IL8, RANTES, GROα, MCP1 and MIP1β) secretion and production by human cultured trophoblast in a time (24-48 h) and a dose (2-25 μg/mL) dependent manner. Injection of polyI:C to C57B/6 wild type mice induced preterm delivery within 24 h at a dose of 9 mg/kg body weight. No effect was observed in TLR-3 knock out mice. A robust systemic (spleen and serum) and local (placenta and amniotic fluid) inflammatory response was observed 2 and 4 h following polyI:C treatment. Trophoblast cell cultures from TLR-3 KO mice confirmed that the response to PolyI:C is TLR-3 dependent.
Conclusion: We demonstrate that viral infection may trigger an immune response leading to preterm labor. Furthermore we show that the trophoblast

is able to recognize and respond to viruses through the expression of TLR-3. Our findings provide a novel mechanism of pathogenesis of preterm labor associated with TLR-3 mediated inflammatory response.

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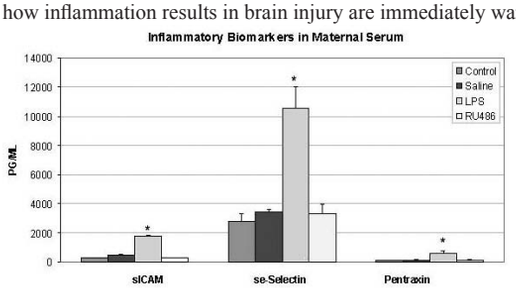
Preterm Labor Is Insufficient To Evoke Fetal Brain Injury: Activation of Inflammatory Pathway Is an Essential Mechanism. Michal A Elovitz, Juan Gonzalez, Jinghua Chai, Amy Bentz, Irina Burd. *OBGYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

Introduction: Adverse neurological outcome is a major cause of neonatal morbidity after a preterm birth (PTB). A growing body of evidence demonstrates the involvement of inflammatory pathways in PTB and implicates these pathways as a causative factor in fetal brain injury. However, activations of cytokine pathways in normal labor (whether iatrogenic preterm or at term) has been observed. What remains understudied is the effect of labor on the preterm fetal brain and whether an inflammatory stimulus is essential for fetal brain injury.

Methods: 2 mouse models were utilized: (1) a model of intrauterine inflammation (LPS into uterine horn) (n=9); controls received intrauterine saline (n=9) and (2) non-infectious model of PTB using RU486 SQ(150ug/dam)(N=5); controls received no intervention (N=6). 6 hours later amniotic fluid (AF), fetal brains and maternal serum (MS) was collected. ELISAs were performed to assess CxCL10 and sL-Selectin in AF and pentraxin, sICAM and se-Selectin in MS. IL-1b, IL-10, and IL-6 mRNA expression were assessed in fetal brains by QPCR. Confocal microscopy (NF200, MAP staining) of neuronal cultures from all 4 groups were performed and number of dendritic processes recorded.

Results: In both models, dams had vaginal bleeding by 6-8 hours with 100% PTB by 24 hrs. See figure. AF CxCL10 was 4.3-fold increased in LPS (0.002) and sL-selectin was 65-fold increased (<0.001). No change in AF from RU486 was observed. IL-10 mRNA was 2.5 fold increased (P=0.005), IL-1B 52.4 fold (P=0.002), and IL-6 6.2-fold (p=0.03) in LPS-exposed fetal brains compared to saline. Fetal brains from RU486 dams had no increase in cytokine levels. LPS, but not RU486, resulted in significantly alterations in neuronal morphology and decrease dendritic processes (P<0.05).

Conclusions: Preterm labor, in of itself, does not result in fetal brain injury. Novel biomarkers of inflammation in AF and MS correlate with fetal brain injury as assessed by elevated cytokines and neuronal injury. Strategies to indentify how inflammation results in brain injury are immediately warranted.



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Increased Cerebellar Granule Cell Number Following Prenatal Injection of Lipopolysaccharide – A Possible New Animal Model for Autism. Markus Gantert,[1] Imke AJ van Kooten,[2,3] Hanna Hurter,[1] Harry WM Steinbusch,[3] Herman van Engeland,[2] Yves Garnier,[1] Patrick R Hof,[4] Boris W Kramer,[5] Peter Mallmann,[1] Christoph Schmitz.[3] *[1]Department of Obstetrics and Gynecology, University Hospital of Cologne, Cologne, NRW, Germany; [2]Department of Child and Adolescent Psychiatry, Rudolph Magnus Institue of Neuroscience, University Medical Center Utrecht, Utrecht, Netherlands; [3]Department of Neuroscience and European Graduate School of Neuroscience (EURON), Maastricht University, Maastricht, Netherlands; [4]Department of Neuroscience, Mount Sinai School of Medicine, New York, USA; [5]Department of Paediatrics and Neonatology, University Hospital Maastricht, Maastricht, Netherlands.*

Autism is a neurodevelopmental disorder with a strong genetic component and several known environmental risk factors, such as infection. In addition, its onset of etiology is likely to occur during prenatal development. We propose that subjecting fetal sheep via amniocentesis to the bacterial endotoxin lipopolysaccharide (LPS) injected to the amniotic fluid at gestational day (GD) 110 will result in morphological alterations in the offsprings' cerebellum resembling alterations found in the cerebellum of patients with autism. Using high precision design-based stereology, we investigated mean total- and layer-specific volume and mean total granule and Purkinje cell (PC) number in the cerebellum of 6 LPS infected animals and 3 controls. The results of the present study showed preserved volumes of the total cerebellum as well as of the molecular layer, outer and inner granular cell layers and white matter. Interestingly, compared to controls, the LPS infected brains showed a statistically significant increase (+20.4 %) in the mean total number of granule cells, whereas the PCs did not show any difference between the groups. These seemingly paradoxical results might be explained by (1) the so-called time of origin of these neurons, i.e. the PCs develop prenatally whereas the granule cells develop postnatally or (2) the direct correlation between PCs and granule cell number in the cerebellum. These results might contribute, as an animal model, to our understanding of the biological basis for interindividual differences in morphological alterations found in the brains of patients with autism.

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The Stimulating Effect of LPS on Cytokines and Prostaglandin-Endoperoxide Synthase 2 (PTGS2) in Human Placental Trophoblast Cells Is Dependent upon the Sex of the Fetus. Maryam Yeganegi,[1,3] Carole S Watson,[3] Sung Kim,[2] Gregor Reid,[2] John RG Challis,[1] Alan D Bocking.[1,3] *[1]Dept. of Physiology & Ob/Gyn, University of Toronto, Toronto, ON, Canada; [2]Dept. of Microbiology & Immunology, University of Western Ontario, London, ON, Canada; [3]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada.*

Objective: Previous studies have shown that there is a higher incidence of spontaneous preterm birth and poorer neonatal outcome in pregnancies with a male fetus. In vitro studies also report a sex dependent pattern in different placental enzymatic systems. We have shown previously that *Lactobacillus rhamnosus* GR-1 supernatant is able to antagonize the actions of LPS on cytokines and PTGS2 in placental trophoblasts. We hypothesize that fetal sex will influence the production of cytokines and prostaglandin regulating enzymes in LPS and lactobacilli treated placental trophoblast cells. **Methods:** Term placentae were collected from women undergoing elective Caesarean section. Placental trophoblasts were isolated using established primary culture protocols. Cells were pretreated with lactobacilli supernatant and subsequently treated with LPS. PGDH and PTGS2 expression levels were measured by Western Blot analysis and TNF-α, and IL-10 concentrations measured by ELISA. **Results:** LPS stimulation caused a marked increase in production of TNF-α (30.9 pg/ml to 1175.3 pg/ml, n=11, P<0.05), an effect that was greater in placentae of the male fetuses (1516.6 pg/ml, n=6) compared to female fetuses (562.5 pg/ml, n=5). Lactobacilli supernatant abolished this response in both sexes. LPS-activated trophoblasts from placentae of the male fetuses showed an increase in IL-10 production (n=5, P<0.05) and PTGS2 expression (n=4, P<0.05). However, there was no response to LPS in placentae of the female fetuses. Lactobacilli supernatant up-regulated PGDH (n=8) by 63%, and this effect was greater in placentae of the female fetuses (n=4). **Conclusion:** We conclude that human placentae from pregnancies carrying male fetuses are more responsive to LPS by producing more pro- and anti- inflammatory cytokines, as well as PTGS2. Conversely, placentae of the female fetuses upregulate PGDH with lactobacilli treatment. These findings may explain the underlying mechanism for the higher incidence of preterm birth and adverse pregnancy outcomes seen with male fetuses in the clinical setting.

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Intraamniotic Infection (IAI) Upregulates the Vascular Endothelial Growth Factor (VEGF) Receptor, Neuropilin-1, in Term Decidua: Implications for Infection-Related Preterm Birth. Victoria V Snegovskikh,[1] Frederick Schatz,[1] Umit A Kayisli,[1] Felice Arcuri,[2] Paulo Toti,[2] William Murk,[1] Lynn Buchwalder,[1] Rebecca Caze,[1] Mizaner Rahman,[1] Charles J Lockwood,[1] Errol R Norwitz.[1] *[1]Ob/Gyn, Yale University, New Haven, CT, USA; [2]Human Pathology and Oncology, University of Siena, Sienna, Italy.*

OBJECTIVE: Aberrant angiogenic factor expression and function has been implicated in pregnancy complications, including preeclampsia and preterm birth. We have previously shown that IAI is associated with increased VEGF expression in term decidua, and that IL-1B stimulates VEGF production and expression of the VEGF receptor, neuropilin-2 (but not Flt-1 or KDR) in term decidual stromal cells (DSCs) (Snegovskikh et al. SGI Abstract #264, 2007). This study investigates whether IAI is associated also with altered expression of neuropilin-1.

METHODS: (i) Immunohistochemistry (IHC) was performed on tissue sections of term decidua with or without clinical / histologic evidence of IAI (n=3 for each). Neuropilin-1 expression was scored by an investigator blinded to the identity of the samples. (ii) Cultured term DSCs were retrieved from elective cesarean (n=6), purified, and depleted of leukocytes. After treatment

with 10^{-8}M estradiol (E2), 10^{-7}M medroxyprogesterone acetate (MPA), both, or vehicle for 7 days, DSCs were stimulated with IL-1B (1-10 ng/mL), TNFa (1 ng/mL), or thrombin (2.5 IU/mL) for 24h. Since no ELISA exists for neuropilin-1, protein expression was determined by immunocytochemistry (ICC). (iii) Total RNA was extracted and the effect of IL-1B on neuropilin-1 mRNA expression measured by real-time RT-qPCR and corrected for B-actin mRNA.

RESULTS: Neuropilin-1 expression in term decidua was increased in tissues with IAI vs controls ($p<0.05$), and localized primarily to DSCs. Using ICC, an increase in neuropilin-1 was noted after stimulation with IL-1B and TNFa, but not thrombin. IL-1B increased neuropilin-1 mRNA expression in DSCs by 6.5±1.6-fold (from 28.0±17.7 to 240.9±189.4 neuropilin-1 mRNA/B-actin mRNA; $p=0.031$).

CONCLUSIONS: IAI is associated with increased expression of the VEGF receptor, neuropilin-1, in term decidual tissues. IL-1B and TNFa (but not thrombin) stimulated neuropilin-1 expression in term DSCs, and this effect appears to be mediated at the level of gene transcription. Since aberrant VEGF function alters vascular permeability, these data provide a mechanism by which IAI can promote 'decidual activation' and preterm labor.

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Mast Cells Traffic into Myometrium in Association with Labour at Term. Abdul H Khan,[1] Anne Young,[1] Jane E Norman,[1] Iain B McInnes,[2] Malcolm C Shepherd,[2] Robert JB Nibbs,[2] Scott M Nelson.[1] [1]*Reproductive & Maternal Medicine, University of Glasgow, Glasgow, United Kingdom;* [2]*Immunology, Infection & Inflammation, University of Glasgow, Glasgow, United Kingdom.*

OBJECTIVE: Asthma is associated with inappropriate activation of airway smooth muscle, chemokine expression and accumulation of mast cells which drive smooth muscle reactivity. Labour is similarly associated with smooth muscle activation and expression of CXCR1 and CXCR2 ligands. The role of mast cells in human parturition is unknown; however, mast cell products can stimulate myometrial contractions and preterm labour in animal models. We have quantified mast cells in association with human labour and determined whether they express CXCR1 and CXCR2.

METHODS: Lower segment myometrial and cervical biopsies were taken at term caesarean section from women not in labour (NIL) (myometrium n=8; cervix n=9) and in labour (IL) (myometrium n=8; cervix n=9). Mast cells were localised in myometrial and cervical sections by ICC with a primary antibody against c-kit. The number of cell transects in 10 randomly selected high-powered fields (400x) was quantified blindly by two observers for each specimen, with median density and interquartile range (IQR) calculated. Back-to-back ICC was performed to determine whether c-kit co-localised with the chemokine receptors CXCR1 and CXCR2.

RESULTS: Mast cells were in close association with myometrial smooth muscle in non-labouring lower segment myometrium. Labour was associated with a significant influx and increase in mast cells numbers (NIL median 11.9, IQR 6.7 - 19.8; IL median 26.9, IQR 16.1 - 40.2, $p=0.025$). In contrast no significant increase in mast cells was observed in cervical tissue in association with labour (NIL median 19.0, IQR 4.6 - 35.5; IL median 7.9 IQR 3.5 - 10.4, $p=0.17$). Analysis of chemokine receptor expression demonstrated co-localisation of CXCR1 to c-kit positive cells present within the myometrium.

CONCLUSIONS: Human labour at term is associated with an increase in mast cells within the myometrium, with close approximation to smooth muscle bundles. These mast cells express the chemokine receptor CXCR1, the ligands of which we have previously shown to be up-regulated in labouring myometrium. Mast cells are not accumulated in cervix in association with labour suggesting a less critical role in cervical ripening. Further analysis of the role of mast cells in modulating myometrial smooth muscle physiology is warranted.

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Progestins and the Glucocorticoid Receptor in Human Myometrial and Amnion-Derived WISH Cells. Alison J Tyson-Capper, Stephen C Robson. *Surgical & Reproductive Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Background and objectives: Progesterone (P) can reduce the risk of preterm birth in some high risk women. In this context there is accumulating evidence that this, at least in part, may be due to anti-inflammatory and immunoregulatory properties of P. Target tissue responsiveness to P is considered to be determined by the progesterone receptors (PR) and nuclear co-factors that directly interact with PR.

PR and glucocorticoid receptors (GR) share several structural and functional characteristics, including similarities in DNA sequence recognition by binding to the same hormone response elements. PR and GR interact with similar chaperones in the absence of ligand and with a similar group of co-activators in the presence of hormones; both can display comparable anti-inflammatory activities under specific physiological conditions. In this study we aimed to investigate whether the anti-inflammatory properties of progestins may be mediated by PR and GR signalling.

Methods: Primary cultures of non-pregnant and term pregnant human myometrial (passage 1-2) and WISH cells were serum starved for 24hrs and treated with 17-hydroxyprogesterone (17-HP), progesterone (P), dexamethasone (Dex) and immunofluorescent staining and immunoblotting analyses performed. In some experiments cells were pre-treated with RU486 (a PR/GR antagonist) or Org 31710 (a pure PR antagonist).

Results: In the absence of hormone GR appeared to be predominantly cytoplasmic, whereas, upon treatment with 17-HP and P (1 and 10μM) and Dex (10nM) GR was abundant within the nuclei of myometrial cells. Immunoblotting analyses demonstrated that levels of GR progressively increased within the nuclear fractions of both pregnant myometrial and WISH cells in response to increasing concentrations of P (100nM to 10μM), and decreased sequentially within cytoplasmic fractions. In the presence of Org31710 GR protein levels remained constant within the cytoplasm. There also appeared to be a slight increase in GR expression, though not statistically significant ($P>0.05$) within both cells types in response to P.

Conclusion: In this study we show that GR is activated by 17-HP and P and translocates to the nuclei of human myometrial and amnion-derived cells. In addition, levels of GR increase in response to P. Whether P and 17-HP act as agonists or antagonists for GR in the regulation of hormone response genes associated with the onset of term and preterm labour remains to be elucidated.

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Unraveling the Mechanisms of Fetal Injury in Inflammation-Induced Preterm Birth: Fetal Macrophage Activation and Erythropoeisis. Ernst M Linda,[2] Gonzalez Juan,[1] Ofori Ella,[1] Elovitz Michal.[1] [1]*Department of Obstetrics and Gynecology; CRRWH, University of Pennsylvania, Philadelphia, PA, USA;* [2]*Department of Pathology and Laboratory Medicine, Children's Hospital of Pennsylvania, Philadelphia, PA, USA.*

Objective: Using a mouse model of inflammation-induced preterm birth (PTB), we have demonstrated dramatic cytokine elevations in the uterus and placenta with concomitant, though less dramatic, cytokine elevations in the fetal liver and brain, associated with neuronal injury. Because precise mechanisms of fetal injury in PTB remain unclear, we sought to examine inflammatory cell trafficking, and target organ damage by histopathologic assessment of the placenta, fetus, and fetal brain.

Study Design: 6 hours after intrauterine infusion of saline or LPS into the right lower uterine horn of CD-1 mice, the left upper horn, with the gestational sacs(GS) in situ, was removed en bloc(n=6 per group) each with 2-4 GS with15 fetuses/treatment group. Specimens were fixed, bisected and processed for histology and IHC. Inflammatory and hematopoietic cells were quantified using PAS, GATA-1 (erythroid precursors), CD3, and BM8 (macrophage-MP) within the placenta, liver, extremity mesenchyme, brain and leptomeninges. The presence of hemorrhage, necrosis, and apoptosis (H2AX stain) was assessed. Erythopoietin (EPO) levels were measured in brain and liver by ELISA.

Results: More neutrophils were present in maternal decidual vessels in LPS compared to saline ($P=0.02$). In LPS-exposed, fetal MP were increased in the placenta ($P=0.007$), fetal extremity mesenchyme ($P=0.018$), fetal liver ($P=0.005$) and leptomeninges ($P=0.013$) but not in the brain or spinal cord compared to saline. No necrosis, hemorrhage or increased apoptosis was noted in the fetal brains. 69% of LPS-exposed fetuses and 0% of saline-exposed had liver hemorrhages ($p< 0.001$). Increases in nucleated erythrocytes and erythroid precursors were found in fetal vasculature of the placenta in LPS-exposed ($P=0.004$). EPO levels were not elevated in either group.

Conclusion: Intrauterine LPS infusion induces acute inflammation predominantly in the maternal circulation of the placenta. In the fetus, there is widespread MP activation, liver hemorrhage and increased erythroid precursors seen in the fetal circulation of the placenta. Although histologic evidence of CNS damage was not evident, the increased MPs present in the leptomeninges may play an important role in inflammatory-mediated CNS damage.

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Non-Toll-Like Innate Immune Proteins: Do They Change during Pregnancy? Juan M Gonzalez, Hua Xu, Ella Ofori, Michal A Elovitz. *OBGYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

Introduction: TREM-1 (trigger receptors expressed on myeloid cells) is an important regulator of innate immunity. The natural ligand for TREM has not been identified. Activation of TREM-1 in the presence of toll-like receptors results in substantial amplification of the host inflammatory response (Klesney-Tait et al Nature Immunology 2006). Since inflammatory pathways are implicated in adverse pregnancy outcomes, this novel mediator of inflammation may play a critical role in preterm birth (PTB). Therefore, we sought to determine TREM-1 expression in the uterus, cervix, and placenta across gestation and to determine if TREM-1 levels are altered by intrauterine inflammation.

Methods: In CD-1 mice, TREM-1 was investigated in non-pregnant (NP) and throughout gestation E 15, E 18 (N=3 -6 per group). Uterine, cervical, and placental tissues were harvested. Using an established mouse model of inflammation-induced PTB, uterine tissue was collected 6 hours after intrauterine infusion of saline (N=3) or lipopolysaccharide (LPS) (N=3). For a non-pregnant model, using CD-1 mice, LPS (N=3) or saline (N=3) was injected into the uterine horn following same procedures as with pregnant mice. Uteri were harvested 6 hrs later. Quantikine ® Mouse TREM-1 Immunoassay was utilized for these studies. Statistical analysis was performed using One-way Anova followed by pair-wise comparison if statistical significance was reached ($P<0.05$)

Results: TREM levels are significantly different between NP and pregnant uterine tissues ($P=0.002$). E15 and E18 TREM expression is significantly increased 2.3 and 2.5-fold compared to NP ($P=0.004$ and 0.001 respectively). TREM-1 levels in the placenta and cervix were not significantly different between E15 and E18. TREM levels increased about 4-fold in the uterus after intrauterine infusion of saline or LPS compared to E15 controls. In non-pregnant, TREM levels were significantly different ($P=0.05$) with a 17-fold increase in TREM expression in uteri exposed to LPS or saline compared to controls.

Conclusions: Non-toll-like innate immune proteins are differentially regulated during pregnancy compared to the non-pregnant state. The role of TREM-1 in inflammation-induced PTB requires further study. Research is warranted to determine if uterine up-regulation of TREM in gestation is associated with an increased likelihood of responding to pathogens or severe as a protective mechanism.

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Reduced Plasma Levels of Vitamin D in Caucasian Women at Term Are Associated with Increased Rate of Infection. Chander P Arora,[1] Adegoke Adeniji,[1] Susan E Jackman,[1] Babak Forooghi,[1] Isaac Mostadim,[1] Phillip Yadegari,[1] Calvin J Hobel.[2] *[1]Og-Gyn, Cedars-Siani Medical Center, Los Angeles, CA, USA; [2]University of California Los Angeles, Los Angeles, CA, USA.*

BACKGROUND: Vitamin D plays an important role in human pregnancy by acting as a regulator of immunity at the fetal-maternal interface. Inflammatory changes associated with pro-inflammatory cytokines were reduced by vitamin D while anti- inflammatory cytokines were increased in T lymphocytes. Vitamin D status has been defined as deficiency (<37.5nmol/l), insufficiency (37.5 to 80 nmol/l) and sufficiency (>80nmol/l) .

OBJECTIVE: To assess the involvement of Vitamin D in the occurrence of maternal infection during pregnacy in women with term deliveries.

HYPOTHESIS: Vitamin D metabolism could affect the rate of infection during pregnancy.

STUDY DESIGN Plasma levels of 25(OH)D were determined by ELISA and the rate of infection was recorded in a Behavior in Pregnancy Study. In this study, 626 ethnically diverse women were evaluated at 18-20 weeks (T1), 28-30 weeks (T2) and 34-36weeks (T3). Maternal infections were documented at each stage as well as at baseline visit with history of infection in current pregnancy. Of these subjects who delivered at term two groups (58 with no infection during pregnancy, 101 with infection or history of infection) were matched further for non-smoking status, non-diabetics, ethnicity and maternal age. Plasma from these were assayed for 25(OH)D and analyzed for the rate of maternal infection using Fisher´s exact test or Chi-square test.

RESULTS Although the women delivered at term, the levels of 25(OH)D in Caucasians were significantly lower in the subjects with infection than the ones without ($p<.001$). Women with vitamin D insufficiecy in the first trimester were more likely to develop infection during pregnancy (46.2 nmol/l ± 6.8 at T1, 28.4 nmol/l ± 4.1 at T2 and 30.2 nmol/l ± 3.6 at T3; all $p<.001$) but not subjects with sufficient vitamin D at T1(87.3 nmol/l ± 5.4 at T1, 27.9 nmol/l ± 3.8 at T2 and 32.8 nmol/l ± 4.6 at T3; all $p<.001$).

CONCLUSION The results reveal a positive association between 25(OH) D concentrations and greater risk of infection. Vitamin D deficiency or even insufficiency may, therefore be involved in the pathogenesis of maternal infection during pregnancy. It is probable that vitamin D deficiency or even insufficiency could modulate the maternal susceptibility to infection during pregnancy by a proinflammatory mechanism.

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Intraperitoneal Heat Killed Group B Streptococcus Induces Caspase Independent Apoptosis in the Pregnant Mouse Placenta. Ozlem Equils,[1] Chantelle Moffatt-Blue,[1] Vladimir Ilievski,[2] Emmet Hirsch.[2] *[1]Pediatrics, Cedars-Sinai Medical Center/UCLA, Los Angeles, CA, USA; [2]Obstetrics and Gynecology, Evanston Northwestern Healthcare and Northwestern University, Evanston, IL, USA.*

Objective: Preterm delivery is one of the most important causes of infant mortality and morbidity in the US. Multiple factors lead to preterm delivery; however infection is considered the cause of a large proportion of such cases. *In vitro* and *in vivo* observational data suggest that infection leads to caspase activation and apoptosis in the placenta and membranes, however currently there are no data on the role of apoptosis in the pathogenesis of infection associated preterm delivery. Here we used group B streptococcus (GBS) as a model pathogen and examined the role of caspase dependent and independent apoptosis in preterm delivery.

Methods: We injected ($7.5x10^8$) heat killed group B streptococcus organisms (HK-GBS) intraperitoneally (i.p.) in 14.5 day pregnant C57B/L6 mice. The mice were euthanized at 5 hr (n=4) and 14 hr (n=6), the placentas and membranes were removed and assessed for apoptosis by TUNEL staining. Caspase 3 activation and expression were determined by immuno-histochemistry (IH) and Western blotting. The effect of apoptosis on preterm delivery was assessed by i.p. treating the pregnant mice with PBS (n=3), DMSO (n=4) or pancaspase inhibitor Z-VAD-FMK (n=6) prior to HK-GBS and observing the animal for delivery for 48 hrs.

Results: There was a time dependent, GBS-induced increase in apoptosis by TUNEL assay and caspase 3 activation in the placenta and membranes. In addition HK-GBS-induced the expression of caspase 3 and caspase independent m-calpain in the placenta. Z-VAD-FMK (10 mg/kg), at the maximum concentration that did not induce maternal illness, did not prevent HK-GBS-induced preterm delivery.

Conclusions: Caspase dependent and independent mechanisms are activated in the placenta upon exposure to GBS. Systemic adminstration of a pan-caspase inhibitor did not impact upon the occurance or timing of bacterially induced preterm delivery. Further studies are needed to assess the role of apoptosis in the pathogenesis of infection associated preterm delivery.

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Complement Activation and Spontaneous Preterm Birth. Anne M Lynch,[1] Ron S Gibbs,[1] James R Murphy,[2] Margaret C Neville,[1] Tim Byers,[3] Patricia C Giclas,[4] Jane E Salmon,[5] Trisha M Van Hecke,[1] Holers V Michael.[6] *[1]Obstetrics and Gynecology, University of Colorado at Denver and Health Sciences Center (UCDHSC), Aurora, CO, USA; [2]Division of Biostatistics and Bioinformatics, National Jewish Medical and Research Center (NJMRC), Denver, CO, USA; [3]Department of Preventive Medicine and Biometrics, UCDHSC, Aurora, CO, USA; [4]Department of Pediatrics, Division of Allergy and Immunology, NJMRC, Denver, CO, USA; [5]Hospital for Special Surgery, Weill Medical College, Cornell University, New York, NY, USA; [6]Dept. Medicine and Immunology, UCDHSC, Aurora, CO, USA.*

Objective: The complement system protects the fetus and placenta against infection and disposes of immune complexes, ischemic, necrotic and apoptotic cells. The objective of this study was to determine if the complement activation fragment Bb in early pregnancy was associated with early (<34 weeks) or late (34-37 weeks) spontaneous preterm birth (SPTB).

Methods: Plasma samples were drawn on 784 women at less than 20 weeks gestation. The cohort was followed throughout pregnancy. Exclusion criteria included: multiple births, deliveries <20 weeks gestation, medically indicated PTB, maternal uterine anomalies/abnormalities, congenital or chromosomal anomalies in the baby and maternal medical disease. Analysis included univariable and multivariate logistic regression.

Results: The incidence of SPTB was 4.9%. Thirteen women (1.7%) had an

early and 25 (3.2%) had a late SPTB. The mean ± SD gestational age at blood draw was 12 ± 2 weeks. The median level of Bb was higher in women with early as compared with late SPTB or term births (P= 0.047 for trend). Women with Bb in the top quartile were 4.7 times more likely to have an early SPTB as compared with women who had lower levels of Bb (95% CI 1.5 to 14, P = 0.003). There was no association between Bb and late SPTB (RR= 0.8, 95% CI = 0.3 to 2). The adjusted OR of an elevated Bb for early SPTB was 4.3 (95% CI = 1.3 to 14, P= 0.02). When the analysis was restricted to the 38 women with SPTB the RR of an elevated Bb for early SPTB was 3.1 (95% CI 1.3 to 7.5, P = 0.03).

Conclusions: A significant relationship was found between an elevated Bb in early pregnancy and early SPTB suggesting inflammatory events in early pregnancy, perhaps infection-related, are part of the pathogenic mechanisms.

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Uterine Toll-Like Receptor (TLR) Expression and Response to LPS-Induced Preterm Delivery. Mark Phillippe, Karen H Oppenheimer, Leigh M Sweet. *Dept. of Obstetrics & Gynecology, University of Vermont College of Medicine, Burlington, VT, USA.*

Objective: Genital tract infection and/or inflammation appears to contribute to the majority of PTDs preceding 30 weeks of gestation. PTD in humans has been associated with colonization and/or infection with a variety of different organisms including gram positive and negative bacteria, mycoplasma, ureaplasma, trichomonads, malaria parasites and viruses. The innate immune response to these pathogens is produced by a family of pattern-recognition cell membrane receptors known as the Toll-like receptors (TLRs). These studies sought to characterize the TLR isoforms expressed in the preterm mouse uterus, and their modulation during lipopolysaccharide (LPS)-induced PTD.

Methods: Using sterile surgical technique, day-15 pregnant CD-1 mice underwent intrauterine injection of 250 µg LPS. Subsequently, the mice were euthanized at 0, 2, 6, 12, 18 and 24 hours after LPS injection. Uterine tissue was harvested and placed in RNA*later*; subsequently total RNA was isolated using the Trizol reagent and genomic DNA was removed using TURBO DNA-free. cDNA was made using iScript cDNA Synthesis Kit. PCR primers were designed using published mouse Tlr gene sequences. Real-time quantitative RT-PCR was performed using the Power SYBR Green master mix and an ABI Prism 7000 multicycler. To confirm TLR amplicon sizes, the RT-PCR products were visualized on a 2% agarose/TBE gel stained with GelRed.

Results: These studies have confirmed mRNA expression of all 12 of the reported mouse TLR isoforms. These TLR amplicons range in size from 83 to 200 bp as expected; amplicon sequences are pending. Quantitative RT-PCR studies performed using uterine tissues from five mice at each time point demonstrated that at 6 hours after LPS injection, TLR3 increased 3-fold and TLR6 increased 2-fold (both $p<0.05$). In contrast, the expression of TLR4 (the ligand for LPS) remained stable during the 24 hours after LPS; the expression of TLR1, 5, 7, 8, 9, and 13 also remained stable. TLR2 expression trended upward and TLR11 trended downward, although neither was statistically significant.

Conclusions: These studies have confirmed expression of all 12 TLRs within the preterm pregnant mouse uterus, along with characterization of their modulation during LPS-induced PTD. These observations are important because they contribute to our understanding of the immunologic signaling events leading to PTD. (Funded by NIH HD044747).

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UDP-Glucose and Its Receptor P2Y14 as a New Innate Immune System in the Female Reproductive Tract. Toru Arase, Tetsuo Maruyama, Hiroshi Uchida, Takashi Kajitani, Masanori Ono, Maki Kagami, Hironori Asada, Yasunori Yoshimura. *Obstetrics and Gynecology, Keio University, Shinjuku-ku, Tokyo, Japan.*

OBJECTIVE: Innate immune system involving Toll-like receptors has recently emerged in the female reproductive tract (FRT). We hypothesize that there may exist new other mucosal immunity in FRT. Recently, it has been reported that P2Y14, a G protein-coupled P2Y receptor for UDP-glucose (UDP-G), is involved in the lung epithelial immune system. The aim of this study is to investigate whether UDP-G and P2Y14 have a potential as the defense immune system in FRT, in particular endometrium.

MATERIALS AND METHODS: We obtained human endometrial tissues from consenting reproductive-aged patients. The spatiotemporal expression of P2Y14 in human and mouse endometrial tissues was analyzed using RT-PCR and IHC. Isolated human endometrial cells and a human endometrial epithelial cell line, Ishikawa, were cultured, treated with UDP-G, and subjected to RT-PCR analysis for IL-8 mRNA expression. We also measured the IL-8 secretion using ELISA. Small interfering RNA was used to knock down P2Y14 expression. The chemotactic activity of UDP-G on neutrophils was tested using transwell assay with Ishikawa cells. Lastly, mouse uterine tissues were incubated with UDP-G and subjected to RT-PCR analysis for mRNA expressions of KC and MIP-2, the IL-8 homologues in mice.

RESULTS: P2Y14 was exclusively expressed in the glandular and luminal epithelium both in human and mouse uteri. Treatment with UDP-G induced the secretion of IL-8 in Ishikawa and human endometrial glandular cells, but not stromal cells, in a dose- and a time-dependent manner. P2Y14 knockdown abrogated UDP-G-induced IL-8 production. Treatment with UDP-G also significantly increased the chemotaxis of neutrophils, which was attenuated by co-addition of anti-human IL-8 neutralizing antibody. In the mouse uterus stimulation of UDP-G significantly up-regulated the expressions of KC and MIP-2 mRNA.

CONCLUSIONS: UDP-G is an endogenous molecule and released into the extracellular environment in a lytic manner after cell damage. Taken together, our results collectively substantiate a model in which UDP-G released from endometrial cells damaged by infection stimulates IL-8 production via P2Y14 in endometrial glandular epithelium, which, in turn, recruits neutrophils thereby preventing the progression of infection. Thus, UDP-G and its receptor P2Y14 may be involved in the trans-species mucosal immune system in FRT.

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Maternal Azithromycin (AZI) Therapy for Ureaplasma Intraamniotic Infection (IAI) Prevents Advanced Fetal Lung Lesions in Rhesus Monkeys. Miles J Novy,[1] Drew W Sadowsky,[1] Peta L Grigsby,[1] Lynn B Duffy,[2] Kenneth B Waites.[2] *[1]Repro Sci, Oregon National Primate Research Ctr, Beaverton, OR, USA; [2]Pathology, Univ of Alabama at Birmingham, Birmingham, AL, USA.*

Objectives. *U. parvum* IAI results in chorioamnionitis, preterm labor and fetal pneumonia. Maternal AZI therapy clears amniotic fluid (AF) and fetal infection (JSGI 2007; 14, 2(*suppl*), abst #31) but *in utero* effects on fetal lung remain to be established. We have examined the relationship between duration of IAI and subsequent AZI treatment on the severity of fetal lung histopathology. We hypothesized that early treatment would prevent the development of advanced lesions, while late treatment may reduce the severity of lung damage.

Study Design. Thirteen chronically instrumented rhesus monkeys received intraamniotic inoculation of *U. parvum* (serovar 1; 7-14 x 10^7 cfu) at 130 ± 1.2 days gest. age (dGA, mean ± SEM, term=167 d). Six of these animals received maternal i.v. AZI (12.5mg/kg q12h or q6h for 10 d) either alone (n=3) or in combination (n=3) with dexamethasone (DEX; 4mg/kg/d i.v. for 4d) and indomethacin (INDO; 100mg/d p.o for 5d). Tissues were obtained at cesarean section for histopathologic assessment. Leukocytic infiltration of aveolar spaces and septal walls, type II pneumocyte hyperplasia and peribronchiolar lymphocytic aggregates were scored as absent (0), minimal (1), moderate (2) and severe (3).

Results. Inoculation-to-delivery interval was 18-25d for combined treatment groups and was similar to long duration infection without treatment (15-21d). Treatment effects were tabulated as mean scores and compared as follows: Control (n=3), score 1; Short duration infection (3-8d; n=4), score 8; Long duration infection (15-21d; n=3), score 12; Short duration infection + treatment (n=5), score 4; Long duration infection + treatment (n=1), score 5.

Conclusions. Histopathologic findings of fetal pneumonia progressively worsen with duration of *U. parvum* IAI. Early maternal AZI treatment prevents development of advanced lung lesions, while later treatment may reduce the severity of fetal lung damage. Our results suggest that *in utero* treatment of IAI may lower the risk of neonatal bronchopulmonary dysplasia. Support: NIH HD06159, RR00163.

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Inhibition of c-Jun-N-Terminal Kinase Signalling Delays LPS-Induced Preterm Delivery and Reduces Mortality in the Mouse. Grisha Pirianov,[1] Vasso Terzidou,[1] Simon Waddington,[2] Huseyin Mehmet,[1] Phillip R Bennett.[1] *[1]Department of Reproductive Biology, Institute of Reproductive and Developmental Biology, Imperial College London, London, United Kingdom; [2]Department of Haematology, Haemophilia Centre and Haemostasis Unit, Royal Free and University College Medical School, London, United Kingdom.*

BACKGROUND & OBJECTIVES

Clinical evidences show that intrauterine infection is a common trigger for preterm birth, and a risk factor for the subsequent development of neurodevelopmental abnormalities such as cerebral palsy in both term and preterm neonate. In animal studies injection of bacterial lipopolysaccharide (LPS) causes a preterm birth and range of lesions in the developing neonatal

brain associated with adverse neurodevelopmental outcome. LPS triggers proinflammatory responses through toll-like receptor-4 (TLR4). Mitogen activated protein kinases including c-Jun-N-terminal kinase (JNK) have been reported to be implicated in TLR4 signalling pathways and play important role in both onset of labor and brain injury.

In the present study, we used a mouse model of intrauterine infection-associated preterm labor to determine whether the administration of specific inhibitor of JNK signaling, D-JNK inhibitory peptide (D-JNKI) can (i) inhibit JNK activity *in vivo*, (ii) delay LPS-induced preterm delivery, and (iii) improve neonatal outcome in the presence of intrauterine inflammation.

METHODS & RESULTS

Intrauterine administration of TLR-4 specific LPS to CD1 pregnant mice at 16 day of pregnancy caused preterm delivery after 18 to 24 h with 70% pup mortality. *In vitro* kinase assay demonstrated the activation of JNK in response to LPS in the maternal uterus and fetal brain. Furthermore, the brain specific JNK3 was found to be the major JNK isoform activated by LPS in the fetal brain. Co-administration of D-JNKI with LPS to pregnant mice delayed significantly ($p<0.007$) LPS-induced preterm delivery and reduced pup mortality up to 15%. This was associated with inhibition of JNK activity in both maternal uterus and fetal brain. In addition, we have found that treatment with LPS significantly up-regulated COX-2, CXCL1 (IL-8 equivalent) and CCL2 in myometrium and this is significantly suppressed after co-administration of D-JNKI.

CONCLUSION

We conclude that specific inhibition of JNK signaling may have a potential of controlling preterm labor and preventing fetal brain damage as a result of infection/inflammation.

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The Prostaglandin 15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2 Delays LPS-Induced Preterm Delivery and Reduces Mortality in the Mouse. Grisha Pirianov,[1] Vasso Terzidou,[1] Simon Waddington,[2] Tamsin Lindstrom,[1] Huseyin Mehmet,[1] Phillip R Bennett.[1] [1]*Department of Reproductive Biology, Imperial College London, London, United Kingdom;* [2]*Department of Haematology, Royal Free and University College, London, United Kingdom.*

BACKGROUND & OBJECTIVES

Intrauterine infection is a common trigger for preterm birth, and is also a risk factor for the development of neurodevelopmental abnormalities in the neonate. Bacterial lipopolysaccharide (LPS) binds to toll-like receptor-4 (TLR4) to activate pro-inflammatory signaling pathways. The transcription factor nuclear factor kappa B (NF-kB) is a key player in the orchestration of the inflammatory response and has a central role in parturition. We have previously shown that exposure to the anti-inflammatory cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) inhibits IL-1b-induced NF-kB activity and COX-2 expression in human myometrial and amnion epithelial cells *in vitro*. In the present study, we used a mouse model of intrauterine infection-associated preterm labor to determine whether the administration of 15d-PGJ_2 can (i) inhibit NF-kB *in vivo*, (ii) delay LPS-induced preterm delivery, and (iii) improve neonatal outcome in the presence of intrauterine inflammation.

METHODS & RESULTS

Intrauterine administration of TLR4 specific LPS to CD1 pregnant mice at 16 day of pregnancy caused preterm delivery after 18 to 24 h with 70% pup mortality. Co-administration of 15d-PGJ_2 with LPS to pregnant mice delayed significantly ($p<0.008$) LPS-induced preterm delivery and conferred protection from LPS-induced fetal mortality up to 5%. We have looked at the expression profile of several labor associated genes in myometrium 6 hours after LPS administration. (OTR, connexin 23 and 46, COX-1, COX-2, CXCL1 (IL-8 equivalent) and CCL2). We have found that treatment with LPS significantly up-regulated COX-2, CXCL1 and CCL2 and this is significantly suppressed after with co-administration of 15d-PGJ_2. Western analysis for Ser536-phosphorylated p65 and IKKb *in-vitro* kinase assay has demonstrated that LPS induced activation of NF-kB at both 1 h and 6 h. Co-administration of 15d-PGJ_2 was associated with inhibition of NF-kB activation in both the maternal uterus and the fetal brain.

CONCLUSION

15d-PGJ_2 may have potential as a therapeutic agent in the management of preterm labor and, by targeting the player NF-kB, may have the added advantage of preventing detrimental effects to the fetus that may result from infection/inflammation.

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Synergistic Macrophage Response to Co-Activation of TLR-2 and TLR-3: Mechanisms and Implications for Bacterial/Viral Co-Infection. Vladimir Ilievski,[1] Emmet Hirsch.[1,2] [1]*Department of Ob/Gyn, Evanston Northwestern Healthcare, Evanston, IL;* [2]*Department of Ob/Gyn, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

Background: Toll-like receptors (TLRs) recognize structural components of pathogens and initiate host defenses. TLR-2 responds to Gram-positive organisms and peptidoglycan (PGN), a Gram-positive cell wall constituent. TLR-3 is activated by viral infection in response to double-stranded RNA. Polyinosinic-cytidylic acid (poly(I:C)) is a TLR-3 ligand. We have shown that PGN and poly(I:C) have a synergistic effect on the expression of downstream genes for both TLR-2 and TLR-3. Here we identify mechanisms underlying this synergy.

Methods: Mouse peritoneal macrophages or a mouse macrophage cell line (RAW 264.7) were treated in tissue culture with either PGN (1μg/ml), poly(I:C) (10μg/ml) or both PGN and poly(I:C) either simultaneously or sequentially for 5-10 hours. Total RNA was extracted and duplex RT-PCR was performed for inducible nitric oxide synthase (iNOS), interleukin 1 β (IL-1β), tumor necrosis factor (TNF), the chemokine RANTES and TLR-2, normalized to the housekeeping gene GAPDH.

Results: Compared to stimulation with either PGN or poly(I:C) alone, co-stimulation of RAW 264.7 cells with both PGN and poly(I:C) resulted in synergistic expression of iNOS, IL-1β, TNF and RANTES ($p<0.05$ for all) at 5 and 10 hours . Sequential stimulation with either PGN or poly(I:C) for 5h followed by incubation for an additional 5h with the alternate ligand also induced synergistic expression of the same RNAs, albeit at lower levels than were elicited by simultaneous stimulation. In contrast, incubation with either PGN or poly(I:C) for 5h followed by medium for 5h induced minimal to no gene expression. Both PGN and poly(I:C) induced TLR-2 mRNA after 5h but not 10h. TLR-3 mRNA was not detectable by RT-PCR. In primary peritoneal macrophages, similar synergy due to PGN and poly(I:C) was seen.

Conclusions: Simultaneous or sequential exposure to PGN and poly(I:C) exerts a synergistic effect on the expression of inflammatory mediators in macrophages. Interestingly, either one of these two TLR pathways can prime cells for activation of the other pathway. A possible mechanism for this effect may be induction of TLR-2 by either TLR-2 or TLR-3 activation. These observations have implications for bacterial/viral co-infection.

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Antibiotics Appear To Suppress Maternal Serum Interleukin-6 Levels in Women with Preterm Premature Rupture of Membranes. Bernard J Canzoneri, Chad A Grotegut, Geeta K Swamy, Leo R Brancazio, Amy P Murtha. *Maternal Fetal Medicine, Obstetrics & Gynecology, Duke University Medical Center, Durham, NC, USA.*

Objective

Patients diagnosed with preterm premature rupture of membranes (PPROM) are often managed expectantly with hospitalization, bed rest, and administration of steroids and antibiotic therapy. Despite the use of broad spectrum antibiotics, approximately one third of patients with PPROM will develop clinical chorioamnionitis. Recently, maternal serum interleukin (IL)-6 levels were reported as biomarkers in the identification of women with PPROM likely to develop funisitis. The objective of this investigation is to determine if antibiotic administration suppresses maternal serum IL-6 levels in women with PPROM.

Study design

This is a secondary analysis of a prospective cohort study. After IRB approval, daily blood samples were obtained from PPROM subjects and analyzed for IL-6 by ELISA. Paired maternal serum IL-6 levels from 34 subjects were divided into 2 groups: IL-6 levels obtained 12-120 hours prior to completion of antibiotics and those obtained 12-120 hours after completion of antibiotics. The Wilcoxon signed rank test was used to perform the data comparison on the Analyze-it statistical software program. Statistical significance was defined as $P<0.05$.

Results

Of the 34 PPROM subjects, the maternal age was 26.8 yrs; gestational age at admission was 26.7 weeks; latency was 13.1 days; gestational age at delivery was 30 weeks; and infant birth weight was 1409 grams. Median maternal serum IL-6 levels obtained off antibiotics were significantly higher when compared to those on antibiotics (4.9 vs. 2.7 pg/mL, $P<0.02$).

Conclusions

The results of this investigation suggest that maternal serum IL-6 levels rise after discontinuation of antibiotics. The optimal duration of antibiotics

administration in the setting of PPROM is unknown. This data suggests a role for continuation of antibiotics in women with PPROM in order to prolong the latency period and potentially decrease neonatal morbidity.

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Histologic Chorioamnionitis: Clinical and Pathologic Factors Associated with Fetal Inflammatory Responses in the Term Parturient. William M Curtin,[1] Heather Florescue,[1] Leon A Metlay,[2] Philip J Katzman.[2] [1]*Obstetrics & Gynecology, University of Rochester School of Medicine & Dentistry, Rochester, NY, USA;* [2]*Pathology & Laboratory Medicine, University of Rochester School of Medicine & Dentistry, Rochester, NY, USA.*

OBJECTIVE: To identify clinical and pathologic factors associated with fetal inflammatory responses in the placenta from term parturients.

METHODS: Retrospective cohort study of consecutive term parturients with submitted placentas in 2005. Placentas with histologic chorioamnionitis were divided into two cohorts: group 1- maternal inflammatory responses only, and group 2- maternal and fetal inflammatory responses. Maternal and fetal inflammatory responses in the placenta were classified according to guidelines established by the Amniotic Fluid Infection Nosology Committee of the Perinatal Section of the Society of Pediatric Pathologists. Selected demographic, intrapartum, newborn and placental characteristics were analyzed with t-tests and chi-square tests as appropriate. Multiple logistic regression was used to identify independent predictors of fetal inflammatory responses in the placenta.

RESULTS: Of 351 consecutively submitted placentas, 210 had histologic chorioamnionitis: 155 with maternal inflammatory responses only (group 1) and 55 with both maternal and fetal inflammatory responses (group 2). Fetal inflammatory responses observed in group 2 were associated with higher stages of maternal inflammatory responses (p<.001). 80% of fetal inflammatory responses were Stage I (acute chorionic vasculitis or phlebitis).Group 2 patients were more likely to have had amnioinfusion (29% v 13%, p=.006) and less likely induction of labor (22% v 39%, p=.02). Group 2 was more likely to have had intrapartum fever (56% v 40%, p=.04) and maternal tachycardia (58% v 39%, p=.02). Newborns from group 2 were more likely to have been observed for sepsis (69% v 37%, p <.001) and have an Apgar score ≤ 6 at 5 minutes (7% v 1%, p=.02). A logistic regression model showed that stage II or greater maternal inflammatory responses (OR 6.3) and amnioinfusion (OR 2.9) were independent predictors of fetal inflammatory responses.

CONCLUSION: Higher stages of maternal inflammatory responses in the placenta and amnioinfusion were independent predictors of fetal inflammatory responses in term parturients with histologic chorioamnionitis.

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Does Sulfasalazine Prevent Interleukin-8 (IL-8) Production by Endocervical Cells Stimulated with Bacteria Associated with Preterm Birth? Morgan R Peltier, Siew C Tee, John C Smulian. *Obstetrics, Gynecology and Reproductive Sciences, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, New Brunswick, NJ, USA.*

Objective: Previous studies have shown that Sulfasalazaine (SASP) inhibits lipopolysaccharide (LPS)-induced NF-κB activation and decreases LPS-stimulated interleukin-8 (IL-8) production by cultured explants of placenta, amnion and choriodecidua with no effect on cell viability. Bacteria-induced IL-8 production in the cervix is a potential mechanism for premature cervical ripening that may lead to preterm birth. It is unclear, however, whether SASP can inhibit IL-8 production by endocervical cells. Therefore, we performed a series of *in vitro* studies to determine if SASP inhibits IL-8 production by endocervical epithelial cells stimulated with bacterial pathogens associated with preterm birth.

Methods: Human endocervical epithelial cells were incubated with 0-1.6 mM SASP overnight and then stimulated with 9 CCU/ml *Ureaplasma parvum*, 10^8 CFU/ml *Escherichia coli*, or 2 x 10^6 CFU/ml *Gardnerella vaginalis* for another overnight incubation in 96-well cultures. Conditioned medium was then harvested and production of IL-8 was quantified by ELISA. Viability of the cells was ascertained at the end of the experiment with the MTT-assay. Each treatment was applied in quadruplicate wells and experiments were repeated 3 times using different batches of cells for each pathogen. Results were evaluated using the general linear models procedure of SAS for a randomized block design.

Results: SASP had no detectible effect on IL-8 production by endocervical cells not treated with bacteria. At the highest concentration tested (1.6 mM), SASP significantly inhibited IL-8 production by cultures stimulated with *E. coli* (P<0.001), *U. parvum* (P<0.001), and *G. vaginalis* (P<0.001). Viability of the cells, however, was significantly reduced by SASP at 0.8 and 1.6 mM in the both the presence and absence of bacteria for all experiments.

Conclusion: Although high concentrations of SASP inhibit IL-8 production by cultures of endocervical cells stimulated with pathogens associated with preterm birth, this effect may be due to toxicity of the antibiotic on the cells.

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The Effect of 17α-Hydroxyprogesterone Caproate on Lipopolysaccharide Stimulated Peripheral Blood Mononuclear Cells from Pregnant Women. Richard H Lee, Aimin Li, Frank Z Stanczyk, Jinwen I Lin, T Murphy Goodwin. *Obstetrics and Gynecology, University of Southern California, Los Angeles, CA, USA.*

INTRODUCTION: 17α-hydroxyprogesterone caproate (17-OHPC) reduces the rate of recurrent preterm birth in high-risk women. However, there are lines of evidence to suggest that 17-OHPC alters inflammatory response in the setting of gram-negative infection. In a mouse model, 17-OHPC decreased the rate of preterm birth but was associated with significantly increased maternal morbidity when mice were exposed to lipopolysaccharide (LPS). In non-pregnant women, 17-OHPC pre-treatment of whole blood exposed to LPS significantly increased the production of TNF-α.

OBJECTIVE: To determine if 17-OHPC has an effect on the production of proinflammatory cytokines from peripheral blood mononuclear cells (PBMC) in pregnant women.

METHODS: PBMC were isolated from fresh whole blood samples of 10 pregnant women between 16 and 24 weeks. Pregnant women had no prior history of preterm birth. Cells were treated with 17-OHPC (1μM) and *Escherichia coli* lipopolysaccharide (LPS, 1μg/mL) alone or in combination. After 6 and 24 hours of culture, supernatants were collected and tested for TNF-α and IL-6 levels by enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed using non-parametric tests. $P < 0.05$ was considered significant.

RESULTS: PBMC exposed to LPS significantly increased TNF-α and IL-6 secretion compared to untreated controls (P=0.008 and P=0.008).

TNF-α concentrations were not significantly different between LPS and LPS+17-OHPC treated cells at both 6 and 24 hours (P=0.86 and P=0.17).

IL-6 production was significantly increased after 6-hour treatment with LPS+17-OHPC compared to LPS alone (2,730 [2,150-5,090] pg/ml vs. 1,504 [1,497-3,090] pg/ml, P=0.01), but this effect was not seen after 24-hours (13,029 [7,710-24,876] pg/ml vs. 10,256 [5,652-20,311] pg/ml, P=0.17).

CONCLUSION: In our study, 17-OHPC may potentially augment IL-6 production in LPS stimulated monocytes from gravid humans.

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Alternative Signalling Pathways Contribute towards Mediation of Anti-Inflammatory Properties of Progestins. Marie C Smith, Alison J Tyson-Capper, Steve C Robson. *School of Surgical and Reproductive Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, Tyne & Wear, United Kingdom.*

Background. Recent clinical trials indicate that progestins reduce the incidence of preterm birth in some high risk pregnancies. It has been proposed that progesterone promotes uterine quiescence, in part, via its anti-inflammatory properties with inhibition of pro-inflammatory gene expression. It is intriguing that progestins are clinically effective given the considerably increased background circulating levels of the hormone during pregnancy. We hypothesised that non-classical progesterone signalling pathways contribute towards mediation of the anti inflammatory effects of progestins.

Methods. Human myometrial cells (both pregnant and non-pregnant) respond to the endotoxin lipopolysaccharide (LPS) by activation of inflammatory pathways. Myometrial cell cultures were treated with LPS (1μg/ml)+/- progestin (50nM). Two progestin compounds were investigated. Natural progesterone (P) is known to have a strong affinity with progesterone receptor (PR) analogues in contrast to 17-hydroxyprogesterone (17-HP) which, in the absence of esterification with caproate or acetate, has been reported to have no progestogenic activity at PR. The effect of P and 17-HP on the activation of two inflammatory genes known to be associated with labour (cycloxygenase 2 [COX-2], Toll-like Receptor 4 [TLR-4]) was evaluated. COX-2 and TLR-4 were detected at the protein and mRNA levels using SDS-PAGE and RT-PCR.

Results. LPS-induced expression of COX-2 and TLR-4 proteins were significantly inhibited by both P (P<0.01 and P<0.05, respectively) and 17-HP

(P<0.01 and P<0.05, respectively). Furthermore, preliminary results indicate that co-incubation with the anti-progesterone mifepristone, fail to abrogate the anti inflammatory effect associated with progestin treatment.
Conclusion. Non-classical progesterone signalling pathways have a role in mediating the anti-inflammatory properties of progestins. Further elucidation of the molecular actions of progestins may allow novel approaches to ameliorate the inflammatory response associated with preterm labour.

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Detection and Transcriptional Expression of TLR-2, TLR-4 and TLR-9 at the Maternal-Fetal Interface. Jacqueline P Tilak, Karen Zakharian, Alexandra Tungol, Gabriel O Schubiner, David M Svinarich. *Patient Care Research, Providence Hospital, Southfield, MI, USA.*
Preterm labor and delivery remains a leading cause of neonatal morbidity and mortality and bacterial infection is considered to be the most common etiology. Toll-like receptors (TLR's) and the associated components of the innate immune system may represent a first line of defense against these pathogens. TLR's belong to a family of pattern-recognition receptors that bind to highly conserved pathogen-associated molecular patterns (PAMPS) including lipopolysaccharide (LPS), lipotechoic acid (LTA) and CpG DNA, and are a key component of the innate immune system. This study was undertaken to characterize the transcriptional expression and regulation of TLR-2, TLR-4 and TLR-9 within gynecologic and gestational tissues.
Human first trimester trophoblasts, endometrial mesoderm, ectocervix, choriocarcinoma and neonatal epithelium, were cultured in media alone or in the presence of either LPS (1mg/ml) or LTA (10 mg/ml) for 0, 2, 4, 6, 8 and 24 hours. Total RNA was isolated and semi-quantitative RT-PCR was performed using intron-spanning amplimers to TLR-2, TLR-4, TLR-9 and GAPDH. Following gel electrophoresis, the integrated optical densities were determined for the corresponding PCR products and normalized with respect to GAPDH levels.
Inducible transcription of TLR-2 with LTA was observed in choriocarcinoma cells (6-fold, 2h), and endometrial mesoderm cells (19-fold, 4h). TLR-4 induction with LPS was observed in ectocervical cells (4-fold, 4h), choriocarcinoma cells (4-fold, 4h) and endometrial mesoderm cells (7-fold, 24h). TLR-9 induction with LPS was observed in choriocarcinoma cells (32-fold, 4h) and neonatal epithelial cells (11-fold, 4h). All cell lines showed at least constitutive levels of transcription for TLR-2, TLR-4 and TLR-9.
These data demonstrate that TLR-2, TLR-4 and TLR-9 are transcriptionally expressed either constitutively or inducibly in both gynecologic (endometrial mesoderm, ectocervix) and gestational (chorion, trophoblast), tissues. Translation of these receptors suggests that the innate immune system may function at the maternal-fetal interface to help protect the fetus against infection and prevent or diminish the likelihood of prematurity.

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Effects of Chronic Intra-Amniotic *Ureaplasma parvum* (Serovar 3 and 6) Colonization in the Ovine Fetus. Ilias Nitsos,[1] Christine Knox,[2] Samantha Dando,[2] Alan H Jobe,[3] Timothy JM Moss,[4] John P Newnham.[1] *[1]School of Women's and Infants' Health, The University of Western Australia, Perth, Western Australia, Australia; [2]School of Life Sciences/IHBI, Queensland University of Technology, Brisbane, Queensland, Australia; [3]Division Of Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [4]Department of Physiology, Monash University, Melbourne, Victoria, Australia.*
Objective: Further to our development of a sheep model of intrauterine *Ureaplasma* spp infection, we aimed to examine the capability of ureaplasma colonization in the amniotic fluid to infect the fetus and alter lung and brain development.
Methods: At 50 days of gestation (d, term=150 d) ewes bearing single fetuses were given a single ultrasound-guided intra-amniotic injection of (a) $2x10^7$ colony forming units (cfu) of *U parvum* (serovar 3, n=7; serovar 6, n=8), (b) $2x10^4$cfu of *U parvum* (serovar 3, n=6; serovar 6, n=8) or (c) media control (n=6). At 125 d, fetuses were delivered by Cesarean section. Amniotic fluid and umbilical arterial blood samples were collected. Fetal body weight was recorded, fetal cerebrospinal fluid (CSF) collected and a descending pressure-volume curve constructed after inflation of the lungs to 40 cmH_2O. Fetal membranes were immersion fixed and the fetal brain was perfusion fixed with 4% paraformaldehyde. The fetal brain and membranes were blocked, paraffin embedded, stained and viewed under the light microscope.
Results: Chronic intra-amniotic colonisation with *U parvum* serovar 3 or 6 (ureaplasmas) did not result in fetal abortion or death. Amniotic fluid ureaplasma titers at delivery were not dose-dependent. Chronic ureaplasma exposure did not affect fetal body or brain weights, or result in a fetal systemic inflammatory response. Umbilical arterial blood gases at delivery were similar between ureaplasma- and media-exposed fetuses. Chronic intra-amniotic exposure to ureaplasmas resulted in higher inflammatory cell scores in the fetal membranes compared to media controls (p<0.05). Lung compliance was increased in ureaplasma-exposed fetuses compared to controls (p<0.05). There were no gross anatomical changes observed in the white or grey matter in the cerebral hemispheres of fetuses that had been exposed to ureaplasmas; even in animals (n=3) that had CSF ureaplasma colonisation.
Conclusion: Chronic ureaplasma colonisation enhances fetal lung compliance without gross anatomical changes in the fetal brain.

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Reduced Vitamin D Levels in African American Pregnancies Are Associated with Increased Rate of Infection. Adegoke Adeniji,[1] Chander P Arora,[1] Lilit Baldjyan,[1] Olga Miadel,[1] Priya Arora,[1] Maria Ortega,[1] Amy Le,[1] Michelle Koenig,[1] Calvin J Hobel.[2] *[1]Ob-Gyn, Cedars-Sinai Medical Center Suite # 160 W, Los Angeles, CA, USA; [2]University of California Los Angeles, Los Angeles, CA, USA.*
BACKGROUND: An important source of vitamin D is its synthesis by skin from UV solar radiations. The skin pigment melanin absorbs UV photons thus reducing the vitamin D synthesis by more than 90% making African Americans at high risk for vitamin D deficiency. Low maternal vitamin D status during pregnancy has been linked to molecular pathways for adverse outcomes.
OBJECTIVE: To assess the role of Vitamin D deficiency in the occurrence of maternal infection and compare rates in Caucasian and African-American women at term.
HYPOTHESIS: Racial disparity in Vitamin D metabolism could affect the rate of infection during pregnancy.
STUDY DESIGN: Plasma levels of 25(OH)D were determined by ELISA and the rate of infection was recorded in a Behavior in Pregnancy Study. In this study, 626 ethnically diverse women were followed up at 18-20 weeks (T1), 28-30 weeks (T2) and 34-36weeks (T3). Maternal infections were documented at each stage as well as at baseline visit with history of infection in current pregnancy. Of these subjects who delivered at term two groups (101 Caucasians, 86 African Americans) were matched further for non-smoking status, non-diabetics and maternal age. Plasma from these were assayed for 25(OH)D and analyzed on the basis of ethnicity and their relationship with the rate of maternal infection using Fisher´s exact test or Chi-square test.
RESULTS: The proportion of infection percent in the Caucasian and African American groups is significantly different at all the time periods as well as their levels of 25(OH)D. African Americans show a trend to have higher proportion of infections (26.9 at T1, 30.2 at T2 and 28.7 at T3) than the Caucasian group at all three visits (17.3 at T1, 17.9 at T2 and 17.8 at T3) and had significantly lower 25(OH) D (p<.001) at these visits respectively (27.4±3.8 vs 46.2±6.8 nmol/l at T1, 12.6±2.6 vs 28.4±4.1 nmol/l at T2 and 18.7±2.1 vs 30.2 ±3.6 nmol/l at T3).
CONCLUSION: The results reveal a positive association between 25(OH) D concentrations and elevated risk of infection. Vitamin D insufficiency may, therefore be involved in the pathogenesis of maternal infection during pregnancy. Vitamin D levels could modulate the maternal susceptibility to infection during pregnancy.

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Nuclear Localization of NFκB in Full-Thickness Fetal Membranes, Decidua, and Myometrium in Term and Preterm Labor. Sonali Vora,[1] William E Ackerman,[1] Douglas A Kniss,[1] Asad Abbas,[2] Chong Jai Kim,[2] Roberto Romero.[2] *[1]Obstetrics & Gynecology, Ohio State University, Columbus, OH, USA; [2]Perinatology Research Branch, NICHD, NIH, DHHS, Detroit, MI, USA.*
Objective: The NFκB transcription factor regulates genes involved in inflammation and immune processes, and is proposed to play a major role in the successful outcome of pregnancy and labor. Prior immunohistochemical (IHC) and biochemical studies have been conflicting regarding NFκB activation in human intrauterine tissues. The aim of this study was to quantify nuclear localization of p65, the major tranactivating NFκB subunit, in full-thickness fetal membranes (FM) and myometrium using IHC.
Methods: A retrospective analysis of paired FM, decidua, and myometrial samples was conducted using tissues collected from women in 4 cohorts: preterm no labor (PNL, N=20), preterm labor (PTL, N=20), spontaneous term labor (STL, N=19), and term no labor (TNL, N=22). Subjects were delivered

between gestational ages 26-36 weeks (preterm) and 37-44 weeks (term) by cesarean. Paraffin sections were stained with polyclonal (rabbit) anti-human p65 and microscopically examined. Myometrial samples were categorized in a blinded fashion to 3 groups of staining: no nuclear p65 (-), rare nuclear p65 (+), and >50% nuclear p65 (++). A p65 nuclear labeling index (NLI; % nuclear p65/total cells) was calculated for each histological layer in full-thickness FM specimens using a blinded targeted randomization scheme consisting of 5 non-overlapping low-magnification fields.

Results: Nuclear p65 labeling was rare in amnion and inconsistently present in chorion. In decidua, p65 nuclear labeling was observed in all cases; however, decidual NLI did not vary significantly across cohorts [PNL-40% (24-55%); PTL-45% (39-57%); TNL-37% (25-43%); STL-41% (30-59%); all values are median (IQR)]. In myometrium, ++ p65 nuclear labeling was significantly associated with labor, but present only in a portion of cases (PTL-26%; STL-50%). Despite a trend, decidual NLI was not significantly correlated with myometrial nuclear p65 labeling: myometrial specimens classified as -, +, and ++ corresponded with decidual NLI of 40% (32-55%) [median (IQR)], 37% (28-47%), and 56% (40-58%), respectively.

Conclusions: In a comprehensive IHC analysis, significant nuclear p65 immunolabeling was observed in myometrial cells following labor. Nuclear p65 labeling in decidua was present in all cases, and did not vary significantly among the cohorts.

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The Inflammatory Cytokines Interleukin-1β and TNF-α Increase G-CSF Expression in Term Decidual Cells. Felice Arcuri,[1] Paolo Toti,[1] Lynn F Buchwalder,[2] Alessandra Casciaro,[1] Marcella Cintorino,[1] Frederick Schatz,[2] Charles J Lockwood.[2] *[1]Human Pathology & Oncology, University of Siena, Siena, Italy; [2]Obstetrics/Gynecology & Reprod. Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objectives: Chorioamnionitis (CAM) elicits premature rupture of the membranes and pre-term delivery. Previously, we found that the decidua from CAM patients contains much higher neutrophil numbers than control decidua, but macrophage numbers are similar in both groups. Granulocyte colony-stimulating factor (G-CSF) enhances granulocyte colony formation chemoattraction and activation. The amniotic fluid during CAM contains elevated TNF-α and IL-1β levels. To account for the marked influx of neutrophils infiltration in CAM-complicated decidua, TNF-α and IL-β effects were assessed on G-CSF expression in term decidual cell (DC) monolayers.

Methods: Confluent leukocyte-free term DCs from normal term deliveries were primed with 10^{-8} M estradiol (E2) + 10^{-7} M medroxyprogesterone acetate (MPA) for 7 days, then switched to serum- free defined medium (DM) with steroids ± TNF-α or IL-1β. After 24h, aliquots of conditioned DM supernatants, cell lysates and extracted RNA from 6h parallel incubations were frozen. Secreted G-CSF was measured by ELISA in conditioned DM and normalized to cell protein and mRNA was assessed by quantitative real time RT-PCR and normalized to β-actin mRNA.

Results: In cultured first trimester DCs, basal G-CSF levels in conditioned DM were 0.13± 0.06 pg/ml/ug cell protein [mean ± SEM, n=8] and did not differ from E2+MPA basal levels. The addition of 1ng/ml of TNF-α or IL-1β significantly elevated G-CSF output to 2.22 ± 0.97 and 454 ± 122 respectively ($p<0.05$), representing more than a 10-fold and 4,000-fold change; respectively. Western blotting confirmed the ELISA results. Quantitative RT-PCR demonstrated corresponding changes in G-CSF mRNA levels as found for the ELISA measurements. Concentration-dependent effects for both TNF-α and IL-1β from 0.01 to 10.0 ng/ml were observed.

Conclusions: When extrapolated to the placental milieu of CAM, the marked increase in G-CSF elicited in term DCs by TNF-α and IL-1β may provide a mechanism to account for the selective increase in decidual neutrophils versus macrophages.

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Semi-Quantitative Analysis of TLR2 and TLR4 Expression in the Non-Pregnant and Pregnant Cervix and the Potential Role of Oestrogen. Nicola L Hardman, Neil R Chapman, Victoria J Cookson, Dilly O Anumba. *Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Sheffield, South Yorkshire, United Kingdom.*

Background. The immunological mechanisms of the female reproductive tract are unclear. Toll-like receptors (TLRs), innate immune receptors that combat microbial infections and establish adaptive immunity, may play a role. Infection in pregnancy has been associated with preterm birth and TLRs may modulate the host response to such infections. We hypothesised that the distribution of TLR2 and TLR4 in cervical epithelial cells may differ with pregnancy, and that oestrogen may contribute to the modulation of these receptors.

Aims and Objectives. 3. To compare TLR2 and TLR4 protein expression, using immunocytochemistry, in the cervical epithelium of pre-menopausal non-pregnant women with pregnant women at term.

Methods. Fresh non-pregnant (n=18) and pregnant (n=11) human cervical cells were obtained by smear and TLR2 and TLR4 expression investigated by immunocytochemistry. Cervical epithelial cells from nonpregnant women obtained by smears were then coincubated with varying concentrations of estradiol, and TLR2 and TLR4 protein expression quantified by immunocytochemistry.

Results. Using pixel-based image analysis software, we demonstrated a reduction in TLR2 expression in late pregnant compared with non-pregnant cervical epithelium (p=0.0001), whilst TLR4 did not appear to differ. There was an apparent up-regulation of TLR2 protein expression in response to 17-beta-oestradiol (n=5) (p=<0.05). **Conclusions.** A putative role for oestradiol in regulating female reproductive tract immune responses during pregnancy is likely.

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Effect of Daily Cranberry Exposure on In Vitro Antimicrobial Activity of Common Uropathogens. Deborah Wing,[1] Christine Preslicka,[2] Sheryl Barron,[3] Yee-Lean Lee,[4] Cesario Thomas.[5] *[1]Obstetrics-Gynecology, University of California, Irvine, Orange, CA, USA; [2]Long Beach Memorial Medical Center, Long Beach, CA, USA; [3]Medicine, University of California, Irvine, Orange, CA, USA; [4]Medicine, University of California, Irvine, Orange, CA, USA; [5]Medicine, Univeristy of California, Irvine, Orange, CA, USA.*

Objective: To evaluate in vitro antimicrobial activity of cranberry-exposed urine against common urinary pathogens. Subjects were pregnant women enrolled in a clinical trial evaluating the effect of daily cranberry juice cocktail or matching placebo on asymptomatic bacteriuria and other urinary tract infections.

Methods: 4-hour urine samples from 28 pregnant women who were randomized to cranberry or placebo in three treatment arms: A. Cranberry two times daily with meals (C, C; n=10), B: cranberry in the AM, then placebo at dinner (C, P; n=10), C: placebo two times daily with meals (P, P; n=8). We identified 15 non-pregnant, reproductive-aged controls, randomized them to the same treatment groups and collected 4-hour urine specimens from them. The pH of all urine specimens was adjusted to pH=7 and filtered. Aliquots of each were independently inoculated with overnight culture of 10^{2-3} cell/mL each of single strains of E. coli with fimbriae type I and type II, K. pneumoniae, and C. albicans. After 24 hours of incubation for E. coli and K. pneumoniae, and 48 hours for C. albicans CFU/mL of each specimen were enumerated by subculture with quantitative plate counts in duplicate.

Results: There were no differences for any of the antimicrobial activities between pregnant and non-pregnant groups, or based on treatment allocation.

	Pregnant	Non-pregnant	*P* (between pregnant and non-pregnant groups)*	*P* (within treatment groups)*	
				Pregnant	Non-pregnant
E. coli					
Group A (C, C)	$1.2 \times 10^8 \pm 8.5 \times 10^8$	$1.2 \times 10^8 \pm 3.0 \times 10^8$	.33	.81	.83
Group B (C, P)	$2.3 \times 10^8 \pm 2.7 \times 10^8$	$9.4 \times 10^7 \pm 4.8 \times 10^8$			
Group C (P, P)	$1.1 \times 10^8 \pm 7.5 \times 10^8$	$1.1 \times 10^8 \pm 1.0 \times 10^8$			
K. pneumoniae					
Group A (C, C)	$5.3 \times 10^7 \pm 3.4 \times 10^7$	$9.2 \times 10^7 \pm 6.7 \times 10^7$	.88	.87	.91
Group B (C, P)	$6.0 \times 10^7 \pm 4.8 \times 10^7$	$6.3 \times 10^7 \pm 3.0 \times 10^7$			
Group C (P, P)	$4.7 \times 10^7 \pm 2.3 \times 10^7$	$6.9 \times 10^7 \pm 4.6 \times 10^7$			
C. albicans					
Group A (C, C)	$1.1 \times 10^5 \pm 1.8 \times 10^7$	$1.9 \times 10^6 \pm 1.0 \times 10^6$	.75	.87	.60
Group B (C, P)	$1.5 \times 10^6 \pm 1.0 \times 10^7$	$2.4 \times 10^6 \pm 1.1 \times 10^6$			
Group C (P, P)	$4.7 \times 10^7 \pm 2.3 \times 10^7$	$2.1 \times 10^7 \pm 5.9 \times 10^5$			

Data presented as mean±SD; *ANOVA; Kruskall Wallis test.

Conclusions: We were able to perform antimicrobial assays on the urine of women exposed to cranberry juice or placebo, but unable to demonstrate differences based on treatment allocation or pregnancy. This may be due to beta-error. Further studies are planned.

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Evaluation of Bacterial Anti-Adhesion Activity of Urinary Cranberry Metabolites Following Daily Ingestion for Asymptomatic Bacteriuria Prevention in Pregnancy. Deborah A Wing,[1] Pamela J Rumney,[2] Amy Howell.[3]
[1]Obstetrics-Gynecology, University of California, Irvine, Orange, CA, USA; [2]Obstetrics-Gynecology, University of California, Irvine, Orange, CA, USA; [3]Phillip E. Marucci Center for Blueberry & Cranberry Research, Rutgers University, Chatsworth, NJ, USA.

Objective: To measure compliance, we sought to evaluate the use of a bioassay for cranberry in the urine of women enrolled in a clinical trial designed to determine the effect of daily dosing of cranberry juice cocktail or matching placebo on the incidence of asymptomatic bacteriuria (ASB) and other urinary tract infections (UTI) during pregnancy.

Methods: We collected 4-hour urine specimens from 34 pregnant women who were randomized to ingest cranberry or placebo in three treatment arms: A: Cranberry two times daily with meals (n=11), B: cranberry in the AM, then placebo at dinner (n=12), C. Placebo two times daily with meals (n=11). We identified 14 non-pregnant, reproductive-aged controls, randomized them to the same treatment regimens (Group A: n=6, Group B: n=3, Group C: n=5), and collected 4-hour urine specimens from them. Bacterial anti-adhesion effects of the cranberry-exposed urine were evaluated utilizing a human red blood cell hemagglutination assay specific for uropathogenic P-fimbriated *E. coli*. Activity was quantified as 0, 50, and 100%.

Results: When combining all subjects and dosing regimens, the sensitivity of the assay was 25%, range 17% in the pregnant group assigned single daily dose of cranberry to 100% in the non-pregnant group assigned to multiple daily doses. The specificity ranged from 63% to 100%.

Conclusions: These data indicate that the bioassay can be applied to the pregnant patient population, although the sensitivity of the assay is variable. Higher daily dosing appears to confer greater sensitivity. Further studies are needed to evaluate the utility of this bioassay for compliance.

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Up-Regulation of Pro-Inflammatory Cytokine Expression in Circulating Leukocytes during Term and Preterm Labour. Meifang Yuan,[1] Fiona Jordan,[1] Iain B McInnes,[2] Margaret M Harnett,[2] Jane E Norman.[1] *[1]Reproductive and Maternal Medicine, University of Glasgow, Glasgow, United Kingdom; [2]Inflammation, Infection and Immunity, University of Glasgow, Glasgow, United Kingdom.*

Objective: Increasing evidence suggests that inflammation plays a crucial role in initiation of labour both at term and preterm. We have previously shown upregulation of pro-inflammatory cytokines in myometrium, cervix and membranes at term labour. We have also shown that myometrium and cervix is invaded by leukocytes at the time of labour, and these leukocytes express pro-inflammatory cytokines. In this study, we aimed to test the hypothesis that pro-inflammatory cytokines and Toll-like receptor 2 and 4 (TLR2 and 4) mRNA expression are up-regulated in circulating leukocytes during term and preterm labour.

Methods: Peripheral blood samples were taken from 37 pregnant women: 28-36 weeks gestation (w) preterm not in labour (PTNL) n=10; 28-36 w preterm in labour (PTL), n=7; 37-42 w term not in labour (TNL) n=10; and 37-42 w term in labour (TL) n=10. Leukocytes were isolated using dextran sedimentation. Total RNA was isolated and reverse transcribed using High Capacity cDNA Archive Kit, and mRNA expression determined by Real-time PCR (ABI, TaqMan). Student's t-test was used to compare outcomes between groups.

Results: Messenger RNA expression of IL-8, ICAM-1, MCP-1, TLR2 and TLR4 was significantly increased in TL compared to gestation matched TNL. The expression levels of IL-1b, IL-8 and TLR-2 were significantly greater in PTL compared with gestation matched PTNL. (Table I).

Conclusions: We show up-regulation of pro-inflammatory cytokine production in circulating leukocytes in both term and preterm labour. Thus, the pathophysiology of labour seems to involve the upregulation of pro-inflammatory cytokine production in peripheral blood leukocytes, followed by their invasion into the myometrium and cervix. This work further contributes to our understanding of the pathophysiology of parturition, and suggests that peripheral blood leukocytes may be potential targets for therapeutic agents aimed at modifying the time course of parturition.

Gene Expression

	IL-1β	IL-8	ICAM-1	MCP-1	TLR2	TLR4
PTNL	269±73	405±134	214±61	414±160	166±42	58±9
PTL	4329±1612**	1216±418*	1855±1397	996±367	1179±529*	131±45
TNL	268±49	572±165	170±48	167±49	224±62	50±11
TL	5122±2552	2289±606*	525±134*	905±359*	1365±352**	173±138**

Data expressed as mean±SEM. *P<0.05, **P<0.01, PTL/PTNL, TL/TNL.

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Distribution of Natural Killer Cells in Peripheral Blood during Term and Preterm Parturition. Meifang Yuan,[1] Iain B McInnes,[2] Margaret M Harnett,[2] Jane E Norman.[1] *[1]Reproductive and Maternal Medicine, University of Glasgow, Glasgow, United Kingdom; [2]Inflammation, Infection and Immunity, University of Glasgow, Glasgow, United Kingdom.*

Objective: Natural killer (NK) cells are a subset of lymphocytes with unique phenotypic characterization and functional properties. During normal human pregnancy, peripheral blood NK cells are reported to decrease in number, activation status, and cytotoxicity. Activated NK cells secrete large amounts of various cytokines such as IFN-g and TNF-a. In this study, we aimed to test the hypothesis that distribution of NK cells in peripheral blood is increased during term and preterm parturition.

Methods: Whole peripheral blood was analysed by flow cytometry to assess expression of NK cell markers, CD16 and CD56 on the surface of lymphocytes using CD16-, CD56- and CD3-specific antibodies. Samples were taken from 36 pregnant women: 28-36 week gestation (w) preterm not in labour (PTNL) n=10; 28-36 w preterm in labour (PTL) n=6; 37-42 w term not in labour (TNL) n=10 and 37-42 w term in labour (TL) n=10. NK cells were defined as the following: $CD16^+$ ($CD3^+$, $CD3^-$, resting NK cells); $CD56^+$ ($CD3^+$, $CD3^-$, resting and activated NK cells); $CD3^+CD16/56^+$ (cytotoxic T cells); $CD3^-CD16/56^+$ (NK cells). Student's t-test was used to compare outcomes between the groups.

Results: The proportion of NK cells amongst lymphocytes was significantly increased in PTL compared with PTNL (P<0.05, Table I). Additionally, the mean fluorescence (MF) intensity of CD16 and CD56 expression on NK cells and $CD3^+$ T cells was significantly greater in PTL (Table II).

Conclusions: NK cell and NK/T cell numbers were significantly greater in PTL women. As NK cells provide a first-line defence mechanism against inflammation and infection, the results of this study further support the association between inflammation and preterm parturition

Percentage Distribution of NK Cells in Circulating Lymphocytes

	CD3+ CD16+	CD3+ CD56+	CD3+ CD16/56+	CD3- CD16+	CD3- CD56+	CD3- CD16/56+
PTNL	1.12	4.66	0.54	36	30	27
PTL	2.80*	5.36	1.45	49*	42*	41*
TNL	1.72	4.10	0.59	41	34	31
TL	1.94	5.25	0.99	35	31	32

*P<0.05, PTL/PTNL, TL/TNL.

Expression of CD16 and CD56 on the Surface of Lymphocytes

	CD3+ CD16+	CD3+ CD56+	CD3+ CD16/56+	CD3- CD16+	CD3- CD56+	CD3- CD16/56+
PTNL	29	40	65	143	125	203
PTL	46**	57*	91*	494***	166	616***
TNL	47	48	111	216	76	281
TL	61	47	100	286	165**	387

Data expressed as intensity of MF. *P<0.05, **P<0.01, ***P<0.001, PTL/PTNL, TL/TNL.

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Expression of Toll-Like Receptor 2 in Human Uterine Cervical Epithelial Cells and Their Cytokine Responses to Toll2 Ligands. Mariyum I Hyrapetian, Susan Newton, Dilly O Anumba. *Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Sheffield, South Yorkshire, United Kingdom.*

Introduction. The mechanisms of innate immunity and tolerance in the female reproductive tract (FRT) are ill-understood but involve the pattern recognition toll-like receptors (TLRs). We have previously demonstrated high expression levels of TLR2 gene and protein in fresh human cervical epithelium.

Aims. In order to gain insight into the immunological role of cervical epithelial cells (CECs), we sought to determine CEC responses to the following TLR-2 agonists: macrophage-activating lipopeptide (MALP-2), Pam_3CSK_4, and peptidoglycan.

Methods. CECs were isolated by smears and compared between 7 pregnant (3rd trimester) and 7 nonpregnant women. Following cell preparation, flow cytometry was performed using a direct staining procedure with mouse anti-Human TLR2 primary antibody and its $IgG_{1,}$ κ isotype control, to determine TLR-2 protein expression. A further 7 nonpregnant smear samples were each prepared, and seeded at a concentration of 100,000 cervical epithelial cells/ml into 24-well cell plates. Cells were incubated at 37°C in 5% CO_2 overnight with the TLR2 agonists MALP-2 and Pam_3CSK_4 (at concentrations of 10 and

1000ng/ml), peptidoglycan(50ng/ml), IL-1β (10ng/ml, positive control) and RPMI 1640 + 1-Glutamine media only (vehicle). Following centrifugation, all resulting supernatants were stored at -80°C until the concentration of IL-8 was determined by ELISA and an array of cytokines by bead assays.

Results. Both pregnant and nonpregnant CECs expressed TLR2 (specific mean fluorescence 4.7 vs 3.3 respectively) but did not differ (P = 0.2). Unstimulated cells incubated with buffer alone demonstrated high IL-8 levels (6602 pg/ml), which did not differ following stimulation with MALP-2 (6217 pg/ml), Pam_3CSK_4 (6449 pg/ml) or peptidoglycan (4867 pg/ml). Results of an array of pro- and anti-inflammatory cytokines following incubation of cells stimulated with TLR2 agonists are pending.

Conclusion. High basal IL-8 levels suggest constitutive expression by CECs but the physiological relevance of this intriguing observation is unclear. That CEC stimulation with TLR-2 agonists did not lead to further release of IL-8 may epitomise the resistance of these cells to antigenic challenge by the vaginal commensal flora. CECs may play a pivotal role in modulating the immunological tolerance necessary to minimise inappropriate inflammation in the cervix.

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Omega-3 Fatty Acids Reduce LPS-Induced Inflammation Responses in Human Umbilical Vascular Endothelial Cell (HUVEC). Soon Ae Lee,[1] Hye Jung Kim,[2] Won Young Paik,[1] Jong Hak Lee,[1] Won Jun Choi.[1] *[1]Obstetrics and Gynecology, Gyeongsang National University, Jinju, Korea; [2]Pharmacology, Gyeongsang National University, Jinju, Korea.*

There are several epidemiological and clinical studies that Omega-3 fatty acids and related fish oils can decrease the premature labor and birth. However, the scientific mechanisms are not well elucidated. This study was carried out to investigate the effects of omega-3 fatty acids on producing PGE2 and IL-6, in human umbilical vascular endothelial cell(HUVEC) media with artificial inflammation as an infection-induced premature labor tissue model. Also, if there are some significant effects with omega-3 fatty acids, we want to investigate the possible mechanisms. Materials and Methods; Human umbilical vascular vascular cell primary culture was done. In the adequate cell confluence in each cell dish, pretreatment with various concentrations of EPA, DHA and Troglitazone (another PPAR-γ ligand) and incubation were done for 24 hours in 37°C, CO_2 incubator. After that, 10μg/ml conc. of lipopolysaccharide(LPS) was treated to the each cell dishes and incubated for 8 hours. The cell media were collected, and PGE_2 and IL-6 concentration were checked by ELISA. The each cell lysates were collected and Western blot anaysis for Cyclooxygenase(COX)-2 were done. On the other hand, Nuclear factor kappa B (NFκB) luciferase vector was transfected and then did the same pretreatment with EPA, DHA and troglitazone and LPS treatment to each cell dishes. After that, NFκB luciferase activity was checked with luminometer. Statistical analysis was done by Student t-test. Results: EPA, DHA and Troglitazone decreased the PGE2 ($p<0.05$) and IL-6($p<0.01$) significantly in ELISA. COX-2 expression revealed the significant reduction with pretreatment of EPA, DHA and Troglitazone in higher concentration (50, 100μM) in the Western blotting ($p<0.01$). NFκB luciferase activity were also significantly decresed with pretreatment of EPA, DHA and Troglitazone in higher concentration ($p<0.01$).

Conclusion; This study offers some scientific mechanisms, by which omega-3 fatty acids (EPA, DHA) and Troglitazone may be one kind of the preventive medicine for infection-induced preterm labor and delivery. Also, these effects may come from the common mechanism of EPA, DHA and Troglitazone, PPAR-γ ligands. The next study would be how the COX-2, NFκB and PPAR-γ are related.

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Effects of Medroxyprogesterone Acetate and 2-Aminopurine on LPS Induced Cytokine Production in Human Myometrium *In Vitro*. Refaat E Youssef, Fiona Jordan, Shrikant S Bollapragada, Scott M Nelson, Jane E Norman. *Division of Developmental Medicine, University of Glasgow, Glasgow, Scotland, United Kingdom.*

Progestational agents (17-Hydroxyprogesterone & MPA) have recently been shown to decrease cytokine production and infection induced preterm birth in a mouse model. We evaluated the effect of MPA and the PKR inhibitor 2-AP on LPS induced cytokine release in an *in vitro* human myometrial model.

Methods:

Myometrial samples obtained from women having caesearean section were treated with purified P.gingivalis LPS (TLR2 ligand) or S.minnesota LPS (TLR4 ligand) (100 ng/ml) (Invivogen) with or without pre-treatment with the PKR inhibitor 2-Aminopurine (Invivogen) (10mM) or MPA (Pharmacia & Upjohn) (2mg/ml). Cytokine expression was measured in medium using the Bio-Plex suspension array system (Bio-Rad). Mean expression was compared between the two groups using t test.

Results:

2-Aminopurine significantly inhibited LPS stimulated cytokine production by human myometrium. In contrast, there were no significant differences in expression after MPA treatment, although a trend towards inhibition of pro-inflammatory cytokine and an increase in IL-10 production was noted.

Conclusion:

MPA only weakly suppressed LPS stimulated cytokine production. Further experiments are required to determine if larger doses of MPA or alternative progestogens would be more effective. These studies do not support a major anti-inflammatory effect of progestogens on human pregnant myometrium at term.

MPA and cytokine expression (n= 5 samples)

	P.gingivakis LPS	LPS + MPA	P.value	S.minnesota LPS	LPS +MPA	p.value
IL1-β	545.8	332.4	0.13	1367	1139	0.68
IL-6	771.3	309.9	0.58	654.7	552.2	0.23
IL-8	364.8	78.9	0.06	1049.6	678.1	0.31
IL-10	376	404	0.79	1478	1511	0.9
TNF-α	1245	822	0.07	1156.1	388	0.06
INF-γ	4124	2579	0.06	4533	2612	0.05

(pgm/ml)

2 Aminopurine and cytokine expression (n = 5 samples)

	P.gingivalis LPS	LPS + 2-AP	p.value	S.minnesota LPS	LPS + 2-AP	p.value
IL-1 β	345	137	0.02	1367	106	0.05
IL-6	771.26	6.13	0.008	654.7	3.21	0.001
IL-8	982.7	336	0.002	1049.6	257	0.03
IL-10	862	19.98	0.004	1478	14.62	0.002
TNF-α	1245	67.21	0.001	1998	76.14	0.06
IFN-γ	4124	64.8	0.001	4533	10.63	0.002

(pg/ml)

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A Fetal Sheep Model of Infection-Related Preterm Brain Injury. Mhoyra Fraser, Kimiora Henare, Peter Shang, Roger J Lins, Murray D Mitchell. *Liggins Institute, University of Auckland, Auckland, New Zealand.*

Introduction: Intrauterine infection is now recognised as a major antecedent of white matter injury (WMI) in the preterm infant brain, which can manifest later as cerebral palsy or as varying degrees of cognitive dysfunction. WMI in these infants is characterized by damage to immature oligodendrocytes, and has been linked both to microglial activation and to elevated levels of TNFa, IL-1b and IL-6. We have developed a fetal sheep model for diffuse and focal WMI, based on repeated administration of *E. coli* lipopolysaccharide (LPS) as the stimulus for an inflammatory response.

Methods: Surgery to implant fetal vascular catheters was performed on pregnant ewes carrying single fetuses at d89-90 of gestation. Fetuses received repeated IV injections of LPS (100ng/kg estimated fetal weight/day) between d95 and d99. Plasma levels of pro-inflammatory cytokines were determined in fetal arterial blood samples taken between d94 and d104. At d105 ewes and fetuses were euthanized and fetal brain tissue samples collected for histological analysis.

Results: Five days after final administration of LPS to fetuses we observed a pattern of WMI similar to that seen clinically, ranging from focal to diffuse injury within the periventricular region, impairment of white matter (CNPase; marker for immature/mature oligodendrocytes) tracts, and thinning of the corpus callosum, characterised by oligodendrocyte disruption and microglial proliferation in the surrounding and sub-cortical white matter. We also found a progressive rise in fetal plasma TNFα levels between days 97 and 103 (day two of treatment to third day following final dose of LPS).

Conclusion: We have established and validated a clinically relevant fetal sheep model of infection-related WMI in the preterm infant. An LPS-induced inflammatory response in the model results in the same pattern of WM damage as is seen clinically, and is accompanied by increases in plasma levels of pro-inflammatory cytokines. This model represents a valuable tool for the investigation of the roles of various inflammatory mediators in the etiology of infection-related brain injury in the preterm infant, and of the effectiveness of new neuroprotective strategies.

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A Fetal Inflammatory Response to Repetitive Umbilical Cord Occlusions (UCOs) with Worsening Acidosis in the Ovine Fetus near Term. Andrew P Prout,[1] Martin G Frasch,[1] Ruud Veldhuizen,[1] Robert Gagnon,[1] Michael G Ross,[2] Bryan S Richardson.[1] *[1]Depts ObGyn, Physiology and Pharmacology, CHRI, The University of Western Ontario, London, Canada; [2]Dept ObGyn, UCLA, Los Angeles, USA.*

Objective: Uterine contractions with restrictions in uterine and/or umbilical blood flow and leading to fetal hypoxic-acidemia may activate inflammatory pathways increasing the release of inflammatory cytokines and further aggravating adverse outcome in the newborn. We have therefore determined the change in maternal and fetal plasma IL-1β and IL-6 levels in response to repetitive UCOs with worsening acidemia in the ovine fetus near term.

Methods: Near term fetal sheep (n=10, 125±2 dGA) were chronically prepared with arterial catheters and placement of an inflatable umbilical cord occluder. Following a baseline recording period, animals underwent a series of mild (1min every 5min), moderate (1min every 3min) and severe (1min every 2min) UCOs each lasting 1h or until fetal arterial pH decreased to <7.0. Maternal and fetal blood samples were taken at selected time points during the baseline, UCO, and recovery periods for blood gases/pH, metabolites, and cytokines with plasma IL-1β and IL-6 levels measured by ELISA.

Results: Repetitive UCOs as studied resulted in worsening acidosis over 3 to 4h and eventually a severe degree of acidemia, fetal pH 7.36±0.01 (SEM) to 6.91±0.03, p<0.001. Maternal IL-1β and IL-6 levels at baseline were 379±64 and 328±33pg/mL, respectively, and remained unchanged when again measured at 3h recovery. Fetal IL-1β level at baseline was 672±171pg/mL, and increased to 1134±210pg/mL after the severe UCO series (p<0.05), and remained elevated at 860±171pg/ml after 1hr recovery (p<0.02), but was largely normalized when again measured at 24h recovery. Fetal IL-6 level at baseline was 356±99pg/mL, and remained unchanged when again measured during the UCO and recovery periods. There was no correlation between the fetal and maternal cytokine levels either with the baseline or the recovery measurements.

Conclusion: Fetal IL-1β plasma levels are increased in response to repetitive UCOs leading to severe fetal acidemia, likely due to altered placental perfusion with increased placental release, which might then contribute to the increased risk for adverse outcome with severe acidemia at birth.

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Fatty Acids Modulate Placenta Inflammatory Responses. Oonagh Dowling,[1] Stefanie Hong,[1] Burton Rochelson,[2] Christine Metz.[1] *[1]Center for Patient Oriented Research, The Feinstein Institute, Manhasset, NY, USA; [2]OB-GYN, NSUH-NS-LIJ Health System, Manhasset, NY, USA.*

Background: Plasma fatty acid (FA) levels are associated with altered host inflammatory responses, blood pressure regulation, and insulin resistance, characteristic features of pregnancy-induced hypertension (PIH). Most studies compare the n-3 and n-6 polyunsaturated fatty acids (PUFAs). In addition, recent data demonstrate that saturated and trans-FAs promote inflammation. Based on the immunomodulatory activity of various FAs, we explored their effects on placenta inflammatory responses *ex vivo*.

Methods: Placenta cells were isolated from fresh human (anonymous), term placentas and treated with/without lipopolysaccharide (LPS) with various FAs, including saturated FAs, trans-FAs, and n-3 and n-6 PUFAs (at physiological concentrations). After an overnight treatment, TNF-alpha (TNF) production was determined. Data were analyzed by analysis of variance (ANOVA) followed by the Dunnett's Test.

Results: Oleic acid (18:1n-9), a cis-monosaturated FA common in the Mediterranean diet, did not induce significant placenta TNF production (Fig. A). By contrast, elaidic acid (18:1n-9), a trans-monosaturated FA, induced TNF production by 300-fold compared to vehicle (Fig. A). Likewise, palmitic acid (18:0), a saturated FA, induced placenta TNF production by 800-fold (Fig. A).

To mimic inflammation, placenta cells were treated with LPS *ex vivo* in the presence/absence of FAs. Docosahexanoic acid (22:6n-3, DHA) reduced placenta TNF production by up to 90% following stimulation (Fig. B). Similarly, treatment of placenta cells with linoleic acid (18:2n-6, LA) or arachidonic acid (n20:4n-6, AA) suppressed TNF production by up to 87% and 98%, respectively (Fig. B).

Conclusions: Both saturated FAs and trans-FAs, which positively correlate with hypertension, induce placental TNF production. The n-6 FA precursors to prostaglandins, AA and linoleic acid, reduce placental TNF production following stimulation. Likewise, DHA, a product of n-3 FA metabolism commonly consumed in fish oil and associated with improved blood pressure, suppresses TNF production by stimulated placenta cells.

Fig. A

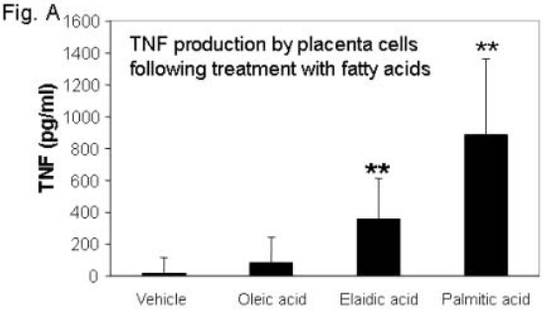
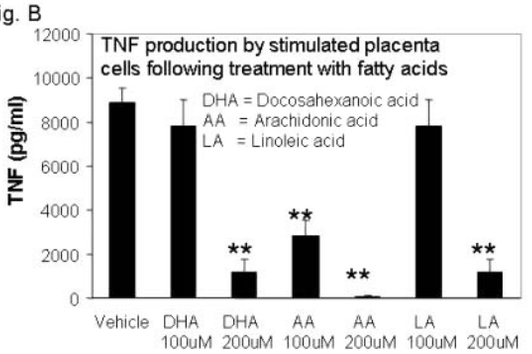


Fig. B



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VEGF and Flk1 Mediated Glomerulogenesis in Offspring Exposed to Maternal Hypernatremia. Roy Z Mansano, Mina Desai, Ahmed Abdel-Hakeem, Thomas R Magee, Tazmia Q Henry, Cynthia Nast, John S Torday, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Growth restricted human and animal offspring, resulting from maternal nutrient restriction or uteroplacental insufficiency, consistently demonstrate reduced glomerular number and a predisposition to adult systemic hypertension and renal compromise. In contrast, maternal water restriction (WR) produces newborns with a unique programmed phenotype of increased glomerular number. Glomerulogenesis is dependent, in part, on appropriately timed and quantified vascular development. As VEGF and its receptor Flk1 have crucial stimulatory effects on renal vasculogenesis, we hypothesized that maternal WR-induced morphologic renal changes are secondary to VEGF-mediated vasculogenic effects.

Methods: From day 10 to term gestation, pregnant rats received either ad libitum food and water (Control, n=7), or WR to produce an increment of ~6 mEq/l in plasma sodium (n=7). Following delivery, all dams received ad libitum food and water. At d1 after birth, offspring kidneys were extracted for mRNA. VEGF and its receptor, Flk1, mRNA levels were determined using real-time RT-PCR (presented as fold difference normalized to 18s). At d21 after birth, offspring glomerular number were determined in formalin fixed 5μm sections using histomorphometric analysis. Values are means±SE.

Results: WR offspring (day 21) had higher glomerular number than control (WR 25±1, Control 22±1 per mm2, p<0.01). Flk1 mRNA expression was increased in WR offspring kidneys (WR 6.4±2.3, Control 1.0±0.3, p<0.05) as compared to controls. In contrast, VEGF mRNA expression in WR offspring kidney was comparable to control (WR 0.9±0.1, Control 1.0±0.2, p=0.8).

Conclusion: Prenatally WR offspring demonstrate significantly increased glomerular number. As VEGF recruits angioblasts to the developing glomerulus via its receptor Flk1, increased receptors (Flk1) with the same level of the ligand (VEGF) suggest that enhanced vasculogenesis may represent a putative mechanism for increased nephrogenesis in WR offspring. Modulation of newborn vasculogenesis via VEGF and Flk1 expression may represent a therapeutic option for growth restricted offspring with decreased glomerular number.

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Maternal Food Restriction Reduces Kidney Branching Early in Fetal Nephrogenesis. Ahmed K Abedl-Hakeem, Thomas R Magee, Tasmia Q Henry, Mina Desai, Cynthia Nast, John S Torday, Roy Z Mansano, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Maternal food restriction (MFR) during gestation (embryonic day 10 to birth) reduces rat kidney glomerular number by 19% at 3 weeks of age. Undernutrition during human gestation leads to similar impaired nephrogenesis and increased hypertension in adults. Renal development may be delineated into stages including ureteric bud branching, mesenchymal to epithelial transformation and glomerulogenesis. Previously, we detected dysregulation of genes controlling nephrogenesis at gestational ages, e16-e20, suggesting that MFR has significant effects on ureteric bud branching. In the present study we evaluated the effect of this dysregulation on early nephron development in e16 fetal kidney explants from dams with MFR.

Methods: e16 fetal kidneys were collected from 50% MFR pregnant rats and ad libitum control rats (n=3 per group and time point), incubated in DMEM/F12K medium for 0, 1, 2, 3, and 4 days, and fixed with 4% paraformaldehyde. Kidneys were stained with fluorescein-labeled Dolichos biflorus agglutinin (DBA), images captured and terminal ureteric buds quantitated. All values are presented as mean ± SEM. Differences were considered significant at $p<0.05$.

Results: MFR ex-vivo kidneys at day 0 demonstrated an initial moderate reduction in terminal branches versus control (C) samples (MFR: 78±9 vs C: 86±3 terminal branch ends per kidney). Both MFR and control (C) kidneys demonstrated significant ($p<0.05$) increases in branching in explant culture to maximum values at 4 days (MFR: 147±16 vs C: 217±16). With increasing culture days, the percent reduction in MFR branching increased with terminal branch point decreases of 9% at day 0, 14% at day 1, 24% at day 2, 20% at day 3, and 32% at day 4 ($p<0.05$, MFR vs C at day 4).

Conclusion: Kidney explant cultures from MFR treated fetuses display basal and culture-based decreased branching compared to controls. This decrease in terminal ureteric buds, in combination with our previous findings of dysregulation of branching-associated kidney transcription and growth factors, suggests MFR induces early (e16) dysregulation of branching as a major mechanism of the associated nephropenia. These results indicate that early programming events in kidney development induce nephropenia and renal disease in adults.

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G-Protein Coupled Receptor 91 and 99 Are Elevated in Kidneys from Growth Restricted Rats: A Novel Mechanism for Developmental Causes of Hypertension. Christy-Lynn M Cooke,[1,3] Lin Zhao,[1] Edith Arany,[2] Timothy R Regnault.[1,3] *[1]Obstetrics & Gynecology, University of Western Ontario, London, ON, Canada; [2]Medicine, University of Western Ontario, London, ON, Canada; [3]Physiology and Pharmacology, University of Western Ontario, London, ON, Canada.*

Intrauterine growth restriction (IUGR) has important implications for the neonate not only at the time of birth, but also as an adult. In humans and animals with IUGR, the elevated risk of developing hypertension is thought to involve an upregulation of the renin-angiotensin system (RAS). However, the link between the intrauterine insult and enhanced RAS is not known. A novel mechanism leading to hypertension has emerged recently involving two orphan G-protein coupled receptors (GCR91 and GCR99) and their endogenous ligands, succinate and α-ketoglutarate. Infusion of succinate into adult mice induces hypertension, an effect which was eliminated in GCR91 knockout mice or by pretreatment with ACE inhibitors. Interestingly, succinate levels have been shown to increase in the circulation under conditions of oxidative stress, one of the hypothesized mediators of developmental programming of hypertension. Thus, we hypothesized that GCR91 and GCR99 are upregulated in a rat model of IUGR and may contribute to *in utero* programming of hypertension. Timed-pregnant rats were fed either control (C, 20% casein) or low protein (LP, 8% casein) diet throughout gestation. Kidneys were collected on embryonic day 19 (E19), post-natal day 30 (d30) or 130 (d130), $n \geq 3$. Real-time PCR was used to compare GCR91, GCR99 and renin expression. Data were standardized to a housekeeping gene (S15) and are expressed as fold increase/ decrease compared to control. A Student's t-test was used to determine significance between groups ($P<0.05$). Offspring from LP dams were significantly smaller at birth and did not display catch-up growth. In kidneys from LP fetuses, both GCR91 and GCR99 were significantly elevated compared with controls (3.6 fold and 4.7 fold respectively; P=0.02), whereas renin was 0.78 fold lower. On post-natal d30, there was a trend towards increased expression of both GCR91 (1.7 fold; P=0.07) and renin (1.6 fold; P=0.09) in offspring from LP dams. At d130, there were no significant differences between groups. In conclusion, these preliminary data suggest that in IUGR offspring from LP rats, the GCR91/ 99 pathway may be enhanced, indicating a novel mechanism for the programming of hypertension.

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Non-Alcoholic Steatohepatitis in Intrauterine Growth Restricted Offspring: Transcription Factor (PPAR) Mediated Mechanism of Lipid Dysregulation and Inflammation. Thomas Magee, Bindu Cherian, Guang Han, Glenda Calvario, Michael G Ross, Mina Desai. *Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: In addition to excess adipose tissue, obesity is accompanied by increased fat storage in organs such as the liver. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors involved in the regulation of lipid metabolism, and lipid-associated inflammatory response. Obesity represents a state of chronic low-level inflammation, with PPARγ and PPARα implicated in this process. We have previously shown that nutrient restriction in pregnancy results in intrauterine growth restricted (IUGR) newborns which develop adult obesity with elevated C-reactive protein (CRP) levels. As CRP is derived from the liver, we hypothesized that IUGR-induced obesity inhibition of hepatic PPARγ and PPARα is associated with an increased inflammatory response.

Methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21 to produce IUGR newborns. All pups were nursed by Control dams and weaned at 3 weeks to ad libitum feed. At 1 day and 9 months of age, male offspring were analyzed for hepatic PPARγ, PPARα and CRP mRNA (real time RT-PCR) and protein (Western Blot) expression. Data was normalized to β-actin and presented as fold change for protein levels. At 9 months, hepatic triglyceride content was determined enzymatically.

Results: At 1d of age, IUGR pups showed significant downregulation of PPARγ (0.2-fold) and PPARα (0.4-fold) expression, though CRP expression was significantly upregulated (4-fold). Findings persisted at 9 months of age, with continued downregulation of PPARγ and PPARα (0.5-fold) and upregulation of CRP expression (5-fold). Furthermore, IUGR adults had significantly increased hepatic triglyceride content (600±60 vs. 373±28 mg/g liver, $p<0.05$).

Conclusions: Reduced expression of hepatic PPARγ and PPARα in IUGR offspring may contribute to elevated hepatic CRP levels and triglyceride content. Thus, developmental hepatic dysregulation leads to programmed obesity-induced inflammation in IUGR offspring.

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Programmed Alteration of Hypothalamic Leptin and Insulin Signaling Pathways Contributes to Reduced Anorexigenic Responses in IUGR Offspring. Mina Desai,[1] Ederlen Casillas,[1] Guang Han,[1] Darran N Tosh,[1,2] Michael G Ross.[1] *[1]Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Dept. of Physiology, Univ. of Adelaide, Australia.*

Objective: Leptin and insulin mediate central anorexigenic signaling responses via different receptor molecules: leptin binds to the ObRb receptor activating JAK-STAT3 and PI3K pathways, whereas insulin activates PI3K pathway by binding to its receptor (IR) and substrate (IRS2). Maternal food restriction in pregnancy results in IUGR newborns that develop hyperphagia and adult obesity. The IUGR newborns have significantly decreased plasma leptin levels with increased hypothalamic expression of basal ObRb, STAT3 and decreased expression of IR and IRS2, suggesting altered anorexigenic pathways. We studied the response of hypothalamic leptin/insulin signal molecules to peripheral leptin in IUGR newborns.

Methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21. 1 day old male offspring were given saline or leptin (1μg/g, i.p). Hypothalamus was dissected at 15, 30 and 45 minutes and protein expression of total STAT3, phosphorylated STAT3 (p-STAT3), inhibitor of leptin signal (SOCS3), IR, IRS2, total AKT and phosphorylated AKT was determined by Western Blot (normalized to β-actin). Data is compared between leptin and saline treatments in IUGR and Controls.

Results: In response to peripheral leptin, IUGR newborns showed marked dysfunction in stimulated hypothalamic leptin and insulin signaling responses. (1) JAK-STAT3: Leptin-treated Controls show progressively increased pSTAT3 (4-fold) with initial suppression of SOCS3 (0.5-fold) as compared to saline-treated Controls. Conversely in leptin-treated IUGR, the pattern is reversed such that there is sustained decline in pSTAT3 expression (0.3-fold) with failure to downregulate SOCS3. (2) PI3K pathway: Leptin-treated Controls showed a

significant reduction in IRS2 (0.3-fold) and pAKT (0.5-fold) as compared to saline-treated Controls. However, leptin-treated IUGR newborns exhibited a paradoxical increase expression of IRS2 (3-fold) and pAKT (2-fold).
Conclusion: The IUGR offspring demonstrate persistent upregulation of leptin receptor, a reduced phosphorylated STAT3 (p-STAT3) response in conjunction with an enhanced SOCS-3 response. The persistent increase in insulin responses indicates a dysfunction in dynamic signaling, leading to altered anorexigenic response and development of programmed obesity.

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Upregulation of Vascular VEGF Induces Inflammation: Implications for Mechanism of Programmed Hypertension. Omid Khorram, Guang Han, Thomas Magee. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*
Objective: We have previously demonstrated that maternal food restriction (MFR) in rats induces a marked increase in the expression of VEGF protein in the aorta and mesenteric arterioles, accompanied by an increase in TGF-β and collagen in both vessel types in adult rat offspring (Am J Physiol, 2007). The aim of this study was to determine if this in vivo finding could be reproduced in an in vitro preparation.
Methods: Two types of preparations were used in this study. We isolated endothelial cells from 3 week old male control rat aortas. These cells were used after the third passage. Staining with Von Willerbrand factor demonstrated that these cells were pure endothelial cells. The second type of preparation was aortic explants from 3 week old male control rats. Endothelial cells and aortic explants were transfected with a VEGF adenovirus (106-109 viral infective particles) or a β-galactosidase-adenovirus as a control. After 24 hours of culture protein was isolated from cells and explants for Western blot analysis using rat specific antibodies. Culture media was assayed for VEGF by ELISA.
Results: Transfection of VEGF adenovirus induced a dose-dependant increase in the expression of VEGF protein in primary endothelial cells and aortic explants. The transfection of VEGF into the endothelial cells showed a bell shaped curve, and was accompanied by an increase in media levels of VEGF protein. Maximal secretion of VEGF was found with 107 viral infective particles. VEGF adenovirus transfection induced a dose-dependant increase in C-reactive protein (CRP) (inflammatory marker), and TGF-β protein in both aortic explants and primary endothelial cells.
Conclusion: These results indicate that upregulation of VEGF in blood vessels induces inflammation and TGF-β expression which in turn can induce collagen synthesis. Thus the increased collagen expression and reduced compliance previously reported by us in vessels of MFR offspring can be explained by the over-expression of VEGF which we reported. Therapeutic intervention aimed at prevention of the increase in vascular VEGF expression in MFR offspring could potentially prevent programmed hypertension.

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Maternal Regulation of High Fat Nourishment during Lactation Period Reduce a Hypertension of Male Offspring. Hidenori Takahashi, Toshiaki Okawa, Keiya Fujimori, Akira Sato. *OBGYN, Fukushima Med.Univ., Fukushima, Fukushima, Japan.*
OBJECTIVE:
Exposure to undernutrition or high fat nourishment during fetal life has been proposed as an underlying cause of adult hypertension, but the effect of maternal feeding regulation during lactation period on blood pressure of offspring is unclear.
Our objective was to investigate the effects of either high-fat diet (HFD) during gestation to lactation period or restrictive fed a HFD during lactation period on blood pressure in male rat offspring.
STUDY DESIGN:
We use 3 types pregnant Wistar rats as fed with normal nutrition (Group A), with a high fat diet (HFD) during gestation to lactation period (Group B) and with HFD nutritionally restricted by feeding with 30% of the normal lactation-matched dietary intake from the day of delivery to the end of lactation period (Group C).
The male offspring was measured blood pressure at 12, 24 and 60 weeks by using indirect tail-cuff method. Statically analysis was performed using one-way ANNOVA.
RESULTS: Body weight was significantly reduced in C offspring compared to A and B in male offspring at day 28 after delivery ($p<0.01$). At 12 weeks old, the body weight of C offspring was no difference to catch up compared to A and B offspring. Systolic and Diastolic blood pressures were significantly elevated at all 12, 24 and 60 weeks in offspring of B > C >A. ($p<0.01$, vs. A)
CONCLUSIONS: Under high-fat nutrition during gestation to lactation period induced hypertension in male rat offspring. Maternal high fat environment make a hypertensive offspring, but regulation of fat feeding during lactation period may reduce adulthood hypertension.

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Does First Trimester Uterine Artery Doppler Velocimetry Correlate with Adverse Obstetric Outcome in the Multiparous Patient? Kimberly W Hickey,[1] Joy Vink,[1] Yesmean Hassan,[3] Sarah H Poggi,[2] Alessandro Ghidini.[2] *[1]Maternal Fetal Medicine, Georgetown University Hospital, Washington, DC, USA; [2]Perinatal Diagnostic Center, Inova Alexandria Hospital, Alexandria, VA, USA; [3]Georgetown University Medical School, Washington, DC, USA.*
Background: Uterine artery (UtA) Doppler velocimetry has been validated in populations of heterogeneous parity in the second trimester for prediction of obstetric outcomes requiring preterm delivery to include: fetal growth restriction, fetal demise, hypertensive disorders of pregnancy, abruption, and indicated preterm delivery. Understanding that parity may affect UtA Doppler indices in subsequent pregnancies, we sought to validate these predictive values in the first trimester in a homogeneous population of multiparous women.
Study Design: Multiparous women undergoing first trimester screening of singleton pregnancies were enrolled and followed prospectively until delivery (n=91). These women were divided into controls, RI < 0.78 (n=74) and cases, RI ≥0.78 (n=17), based on prior studies. Demographic, clinical, and sonographic data (including UtA indices and assessment of notching) were obtained. Statistical analysis included Student's t test and Chi Square.
Results: Cases were not significantly different from controls in terms of maternal age, ethnicity, BMI, or medical history. UtA Doppler indices were significantly different between the two cohorts in terms of the presence of unilateral or bilateral notching (48% vs, 55% $p<0.001$ and 22% vs. 68%, $p<0.001$, respectively). In contrast to that observed in patients of heterogenous parity previously, RI ≥ 0.78 was not associated with adverse pregnancy outcomes despite an average RI of 0.86, significantly above this threshold.
Conclusions: In this multiparous cohort RI was not predictive of adverse obstetrical outcome, in contrast to that observed in cohorts including nulliparous patient. Parity may affect UtA vasculature and obscure the ability of Doppler velocimetry to predict adverse obstetric outcome in multiparous women. Presence of UtA Doppler notching in the first trimester remained a robust predictor of adverse obstetric outcomes in multiparous patients.

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Age and Gender Differences in Airway Reactivity in Offspring of Mice with a Preeclampsia-Like Syndrome Induced by sFlt-1. Egle Bytautiene, Fangxian Lu, Monica Longo, Gary DV Hankins, Garland D Anderson, George R Saade. *Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
Objective: Epidemiological studies have shown that offspring exposed to preeclampsia during fetal development are more susceptible to airway disease later in life. We have shown previously that gender, but not sFlt-1 over-expression during pregnancy determines higher reactivity in the offspring airways at 6 months of age. The objective of this study was to examine the effect of preeclampsia on the trachea from female and male offspring in our model of sFlt-1 induced preeclampsia at 1 year of age and compare responses between the two age groups.
Methods: CD-1 mice at day 8 of gestation were injected via the tail vein with adenovirus carrying sFlt1 (AdsFlt1, 10^9 PFU/100 μL) or mFc (AdmFc, 10^9 PFU/100 μL). Mice were allowed to deliver. Tracheas were isolated from female and male offspring at 6 months and 1 year of age, and rings were mounted in organ chambers for isometric tension recording. Responses to potassium chloride (KCl, 60 mM), the mast cell degranulating agent compound 48/80 (48/80, 100 μg/mL), and concentration-responses curve to acetylcholine (10^{-9}–10^{-5} M) were obtained.
Results: There was no significant difference in responses to acetylcholine, KCl, or compound 48/80 between 1 year old offspring born to the sFlt1 and mFc groups. When comparing offspring within the same pregnancy exposure groups, responses to acetylcholine in AdsFlt1-treated group were significantly higher in 1 year old females than males. Comparison between age groups by pregnancy exposure revealed that in the mFc group, 1 year old male offspring had higher responses to compound 48/80 and acetylcholine than 6 months old males. Responses to KCl were significantly higher in 6 months old males than 1 year olds independent of maternal treatment during pregnancy. In females,

the only difference between age groups was observed in the mFc group, where 6 months old offspring demonstrated significantly higher responses to acetylcholine compared to 1 year old offspring.
Conclusions: Our findings did not show that airways of 1 year old offspring born to mice with a preeclampsia-like syndrome induced by sFlt-1 over-expression have airway hyperreactivity. However, sex and age differences in airway responses dependent on maternal exposure during pregnancy were observed, and needs to be explored further to elucidate underlying mechanisms.

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Delayed Postnatal Catch-Up Growth Prevents Changes in Hepatic IGF-1 mRNA and Chromatin Structure: Epigenetic Mechanisms for the Prevention of Obesity. Darran Tosh,[1,2,3] Robert H Lane,[4] Qi Fu,[4] Christopher W Callaway,[4] Robert A McKnight,[4] Isabella C McMillen,[3] Michael G Ross,[2] Mina Desai.[2] *[1]Dept. of Physiol., Univ. of Adelaide, Australia; [2]Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [3]Sansom Res. Inst., Univ. of S. Australia, Australia; [4]Dept. of Peds., Univ. of Utah, UT, USA.*
Objective: Maternal food restriction (FR) results in IUGR newborns that when normally nursed exhibit rapid catch-up growth and adult obesity. Continued FR during nursing delays catch-up growth and prevents adult obesity. IGF-1, which modulates growth and is secreted by the liver, may contribute to these morbidities. IGF-1 is epigenetically regulated involving two promoters, alternative exon splicing and multiple transcription termination sites. We determined if hepatic IGF-1 mRNA levels correlate with obesity, and whether these changes are due to programmed epigenetic modification.
Methods: Control pregnant rats received ad libitum food from gestation day 10 to 21 and lactation, whereas study dams were 50% FR. FR pups were nursed by either control (FR/AdLib) or FR dams (FR/FR) and weaned to ad libitum feed. At 1 day and 9 months, male livers were analyzed for IGF-1 mRNA variant levels (real time RT-PCR). Chromatin immunoprecipitation (ChIP) was performed using the antibody for H3K4 trimethyl, and associated levels of each IGF-1 species were measured by PCR.
Results: At 9 months, obese FR/AdLib males showed increased mRNA levels of IGF-1A, IGF-1B, IGF-1 Exon 1, and IGF-1 Exon 2 as compared to Controls (134±5, 165±6, 149±6, and 146±7 %). Comparing FR/AdLib 9 month to newborn offspring, H3/K4 was increased at IGF1-Promoter 1, Promoter 2, Exon 5, UTR#3 and UTR#4 (610±157, 731±167, 345±66, 1597±412, and 794±208 %), though there was no differences between Control 9 month and newborns. In contrast, 9 month FR/FR males had comparable mRNA levels to the Controls except for IGF-1B (% of control: 147±19). Further, FR/FR 9 month H3/K4 was only different from newborns at UTR#4 (% of newborn: 289±69).
Conclusion: IUGR newborns with rapid catch-up growth and adult obesity have increased postnatal hepatic IGF-1 mRNA levels, likely a result of IGF-1 histone and chromatin structure modifications to H3K4 trimethylation. Conversely, IUGR with delayed catch-up growth and absence of adult obesity have levels similar to that of Controls. Thus, modulation of the rate of IUGR newborn catch-up growth may protect against IGF-1 epigenetic modifications.

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Effect of Nicotine Exposure during Pregnancy and Lactation on Maternal, Fetal and Postnatal IGF-II Processing. Carolyn E Cesta,[1] Mike Bell,[2] Qing Qiu,[2] Maria A Petre,[1] Andree Gruslin,[2,3] Alison C Holloway.[1] *[1]Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada; [2]Hormones Growth & Development Program, Ottawa Health Research Institute, Ottawa, ON, Canada; [3]Obstetrics and Gynecology, The Ottawa Hospital, Ottawa, ON, Canada.*
Introduction: IGF-II is synthesized as a pro-hormone (proIGF-II; 156-amino acid peptide) which is then processed into its active forms: "big" IGF-II (1-87) and mature IGF-II (1-67). These active forms are essential for placental and fetal development and have also been shown to persist into postnatal life. Since maternal smoking is known to adversely affect feto-placental growth and postnatal development, we postulated that these effects might be mediated through nicotine-induced alterations in IGF-II processing.
Methods: In the present study, nulliparous female Wistar rats (200-250g) were given nicotine (1mg/kg/day) or saline for 14 days prior to mating, during pregnancy, and throughout lactation. At gestational day 15, 18 and 21, dams were euthanized and we collected serum (fetal and maternal), amniotic fluid and recorded fetal body weight. A subset of dams were allowed to deliver at term. Following parturition, serum samples from the offspring were collected at birth (PND1) and weaning (PND21). Body weight was recorded weekly from birth to weaning. Pro, "big" and mature IGF-II levels were determined by Western blot analysis.
Results: Maternal nicotine exposure during pregnancy resulted in a significant reduction in fetal body weight by gestational day 21. However, there was no effect of nicotine on fetal serum or amniotic fluid IGF-II levels at any gestational age examined. In maternal serum, mature IGF-II in control animals decreased with advancing gestational age such that IGF-II levels were lowest at gestational day 21. Nicotine administration prevented this decline, which resulted in significantly higher mature IGF-II levels in nicotine-exposed mothers at gestational day 21. In postnatal life, nicotine exposed offspring had significantly lower levels of "big" IGF-II expression at weaning (PND21).
Conclusions: These data demonstrate that nicotine can alter the amount of the active forms of IGF-II in the mother and the newborn. Dysregulation of maternal IGF-II occurs concomitantly with suboptimal fetal growth. Results from this study suggest a mechanism by which maternal smoking causes impaired fetal growth and adverse postnatal health outcomes.

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Response of IUGR Primary Cell Culture Adipocytes to PPARγ Activator-Ligand and Repressor-Ligand: Mechanisms of Programmed Obesity. Mina Desai,[1] Robert H Lane,[2] Guang Han,[1] Michael G Ross.[1] *[1]Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Dept. of Pediatrics, Univ. of Utah, SLC, UT, USA.*
Objective: Maternal food restriction in pregnancy results in IUGR newborns which develop adult metabolic syndrome. Programming of both increased appetite-mediated hyperphagia and enhanced adipogenesis contribute to the development of obesity. Transcription factors, peroxisome-proliferator-activated-receptor (PPARγ2), CCAAT/enhancer binding-protein (C/EBPα), and sterol regulatory element binding-protein (SREBP1c) regulate adipogenesis and lipogenesis. Although IUGR offspring exhibit acute upregulation of the adipogenesis signaling cascade prior to the development of obesity, we determined if this increased adipogenic potential was an intrinsic cellular response, and thus maintained in cell culture. We further examined the responses to adipocyte stimulators (PPARγ *activator*-ligand rosiglitazone) and inhibitors (PPARγ *repressor*-ligand BADGE).
Methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to term. Adipocytes from 1 day old IUGR and Controls were isolated and cell proliferation rate was determined (MTT). Primary adipocyte cell cultures were established and following 100% confluence, IUGR and Control adipocytes were treated to two doses (1 and 10μm) of either rosiglitazone or BADGE for 24h. mRNA and protein was extracted for expression of PPARγ, C/EBPα, SREBP1c. Data was normalized to β-actin and compared to the respective untreated cells.
Results: IUGR adipocytes had significantly increased protein expression of PPARγ (3.5-fold) and C/EBPα (2-fold) as compared to Control adipocytes, though SREBP1c levels were unchanged. mRNA levels showed similar changes in IUGR newborns. Importantly, IUGR adipocytes exhibited increased cell proliferation (125% of Control, $P<0.05$) and showed greater response to rosiglitazone (2.5-fold), though similar response to BADGE, as the Control adipocytes
Conclusion: IUGR primary adipocytes cell culture exhibit basal phenotypic characteristic of programmed upregulation of adipogenic transcription factors which promote adipose cell proliferation. The enhanced response to the adipogenic stimulant is further evidence of the predisposition to obesity. In contrast, the normal suppressive response to the inhibitor suggests that IUGR adipocytes may respond to pharmacologic approaches to prevent obesity during this period.

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Epigenetic Mechanisms for Diet-Induced Versus Programmed Obesity: Effect of High-Fat Diet on Hepatic IGF-1 mRNA and Chromatin Structure. Darran Tosh,[1,2,3] Robert H Lane,[4] Qi Fu,[4] Christopher W Callaway,[4] Robert A McKnight,[4] Isabella C McMillen,[3] Michael G Ross,[2] Mina Desai.[2] *[1]Dept. of Physiol., Univ. of Adelaide, Australia; [2]Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [3]Sansom Res. Inst., Univ. of S. Australia, Australia; [4]Dept. of Peds., Univ. of Utah, UT, USA.*
Objective: Maternal nutrient restriction results in intrauterine growth restricted (IUGR) newborns which develop programmed obesity despite a normal post-weaning diet. The epidemic of obesity has been attributed in part to programmed "thrifty phenotype" and exposure to "Western" diets. Hepatic IGF-1 is epigenetically regulated involving two promoters, alternative exon splicing, and multiple transcription termination sites. IUGR offspring with normal post-weaning diet have increased postnatal hepatic IGF-1 mRNA levels, likely a result of IGF-1 histone and chromatin structure modifications

to H3K4 trimethylation. We hypothesized that IUGR newborns that develop programmed obesity would demonstrate discernable hepatic IGF-1 changes which are distinct from diet-induced obesity. We determined IGF-1 hepatic mRNA levels and epigenetic characteristics in programmed (IUGR) and diet-induced (DIO) offspring.

Methods:: Control pregnant rats received ad libitum food whereas study dams were 50% maternal food restricted from day 10 to 21. All pups were nursed on ad libitum fed dams. Controls were weaned to high-fat (fat, 16%) diet whereas IUGR were weaned to normal ad libitum diet (fat, 9%) to produce diet-induced (DIO) and programmed obese groups, respectively. At 9 months, male hepatic IGF-1 were analyzed for IGF-1 mRNA variant levels (real time RT-PCR). Chromatin immunoprecipitation (ChIP) was performed using the antibody for H3K4 dimethyl and H3K4 trimethyl, and associated levels of each IGF-1 species were measured by PCR.

Result: Relative to DIO control males, IUGR had increased mRNA of IGF-1A, Exon 1 and Exon 2 (336±4, 267±4, 143±5%). ChIP with H3K4 dimethyl showed increased IGF-1 Exon 6 (215±20%) and with H3K4 trimethyl, increased IGF-1 Promoter 1 and Promoter 2 (282±26, 1183±23%) as compared to DIO controls.

Conclusion: Adult obese IUGR males exposed to normal postweaning diet have increased hepatic IGF-1A mRNA and H3K4 dimethylation and trimethylation of IGF-1 than DIO controls. Changes in IGF-1 in adulthood from a prenatal insult thus suggest that IGF-1 is programmed during the fetal period and may be associated with programmed adult obesity.

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In Utero Low Protein Diet Exposure Induces Hypertension in Adult Offspring; Gender Differences and Possible Mechanisms in Vasculature. Rebekah Elkins,[1] Pandu Gangula,[2] Chandra Yallampalli.[1] *[1]Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA; [2]Internal Medicine, University of Texas Medical Branch, Galveston, TX, USA.*

OBJECTIVES: We previously reported that the offspring of rats fed 6% protein during gestation develop hypertension at two to four months and that the hypertension is exacerbated in males. This study is to evaluate: 1) changes in estrogen receptor (ER) angiotensin II subtype receptor 1 (AT_1-R) and endothelial nitric oxide synthase (eNOS) in the mesenteric artery and aorta of offspring and assess if these changes, if any, are gender specific.

METHODS: Pregnant Sprague Dawley rats were fed either 20% protein (CTRL) or 6% protein (LPD) from day 1 of gestation. The offspring were evaluated for hypertension by means of systolic blood pressure measurements. At four months for the males and nine months for the females, mesenteric artery and aorta were collected in RNAlater. Expression of estrogen receptor a (ER-a) and b (ER-b), AT_1-R, and eNOS were analyzed by Western immunoblotting and RT-PCR and expressed relative to b-actin or 18s.

RESULTS: Mesenteric artery shows no differences between CTRL and LPD female offspring in AT_1-R, eNOS, ER-a or ER-b. Similarly mesenteric artery shows no diet exposure related changes in AT_1-R, ER-a or ER-b in male offspring. However, eNOS expression was lower in mesenteric artery of LPD male offspring. On the other hand, in the aorta both ER-a and ER-b levels are lower in LPD female offspring while there were no changes in AT_1-R or eNOS. No changes in AT_1-R, ER-a or ER-b were observed in male offspring aorta of CTRL and LPD rats.

CONCLUSION: The in utero exposure to LPD results in adult hypertension in both male and female offspring. Some mechanisms for hypertension include the decrease in ER-a and ER-b but not AT1-R or eNOS in females, and the decrease in eNOS but not AT_1-R or ER in males indicating gender related differences.

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Maternal Severe Undernutrition during Both Late Gestation and Lactation Period Induce Hypertension in Male Rat Offspring. Hidenori Takahashi, Toshiaki Okawa, Keiya Fujimori, Akira Sato. *OBGYN, Fukushima Med. Univ., Fukushima, Fukushima, Japan.*

OBJECTIVE:

Our objective was to investigate the effects of either severe undernutrition during late gestation or lactation period on blood pressure and the development of vascular function in male rat offspring.

STUDY DESIGN:

We use normal pregnant Wistar rats (Group A), nutritionally restricted by feeding with 30% of the normal gestation-matched dietary intake from day 17 of gestation to delivery (Group B) and 30% restricted after delivery to the end of lactation period (Group C).

The offspring was measured blood pressure at 12 and 24 weeks by using indirect tail-cuff method.

Rings of thoracic aorta with intact endothelium from the male offspring of A and B at 8 weeks, were equilibrated at 2 g passive tension in organ chambers filled with Krebs-Henseleit solution continuously bubbled with 5%CO2 in air (37°, pH 7.4) for isometric tension recording. Concentration-response relationships to Norepinephrine (NE) and angiotensinII(ATII) were obtained in the absence or presence of N(omega)-nitro-L-arginine methyl ester (L-NAME) or a selective ATII type-1 receptor blocker (Valsartan). Responses to cumulative concentrations of sodium nitroprusside (SNP) and to 10-5M oxyhemoglobin (Hb, nitric oxide scavenger) were also determined. Contractions were expressed as a percent of the reference contraction induced by potassium chloride (60 mM). Statically analysis was performed using one-way ANNOVA.

RESULTS: Body weight was significantly reduced in B offspring compared to A and C in male offspring at day 1 ($p<0.01$). At 12 weeks the body weight of offspring of B was no difference to catch up compared to A and C offspring. Systolic and Diastolic blood pressures were significantly elevated at both 12 and 24 weeks in offspring of B > C >A.

NE concentration-dependently stimulated tension of aortic rings from in A and B offspring, which was not significantly (n=6). Maximal contractions to NE were significantly stimulated by L-NAME in A ($p<0.05$), but not B offspring. Valsartan significantly inhibited aortic contractions by NE in R ($p<0.05$), but not A offspring. There was no significant difference on responses of aortic rings by ATII, SNP and Hb in A and B offspring. **Conclusions**: Severe under nutrition during not only late gestation but also lactation period induced hypertension in male rat offspring in adulthood. Fetal origin of adult hypertension might be vascular endothelial dysfunction.

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Regulation of Maternal Feeding during Lactation Period May Control Adulthood Hypertension. Hidenori Takahashi, Toshiaki Okawa, Keiya Fujimori, Akira Sato. *OBGYN, Fukushima Med. Univ., Fukushima, Fukushima, Japan.*

OBJECTIVE: Exposure to undernutrition during fetal life has been proposed as an underlying cause of adult hypertension, but the effect of either high fat nourishment or undernutrition during lactation period on blood pressure is unclear. Our objective was to investigate the most effective maternal nourishment and feeding period for offspring induced adulthood hypertension in using high-fat diet (HFD).

STUDY DESIGN: We use 5 types pregnant Wistar rats as fed with normal nutrition (Group A), nutritionally restricted by feeding with 30% of the normal gestation-matched dietary intake from day 17 of gestation to delivery (Group B), 30% restricted after delivery to the end of lactation period (Group C), with a high fat diet (HFD) during gestation to lactation period (Group D) and with HFD nutritionally 30% restricted from the day of delivery to the end of lactation period (Group E). The offspring was measured Body Weight (BW) and measured blood pressure at 12, 24 and 60 weeks by using indirect tail-cuff method. Statically analysis was performed using one-way ANNOVA.

RESULTS: BW was significantly reduced in B offspring compared to another (A, C, D, E) male offspring at day 1 ($p<0.01$). At day 28 after delivery, BW was significantly reduced in C, E offspring compared to A, D in male offspring ($p<0.01$). At 12 weeks old, BW of all type offspring was no difference. Systolic and Diastolic blood pressures were significantly elevated at 12 weeks in offspring of D> B > E > C >A. ($p<0.01$, vs. A). At 24 weeks, hypertensive offspring as B>D>E>C>A ($p<0.01$, vs. A). At 60 weeks, D> E > B > C >A ($p<0.01$, vs. A).

CONCLUSIONS: Maternal high fat environment make a hypertensive offspring, but regulation of fat feeding during lactation period may reduce adulthood hypertension. In case with normal food, restrictive feeding during late gestation is more effective than lactation period for inducing hypertensive male offspring. Regulation of maternal feeding not only during late gestation but also lactation period may control adulthood hypertension. The strongest epigenetical factor of maternal nutrition is high fat feeding during pregnancy to lactation period for F1 blood pressure, respectively.

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Impact of Prenatal Hypoxia on Offspring Growth and Cardiac Development. Jann Rhodes,[1,2] Katherine C McBroom,[1] Adam J Chicco,[3] Russell V Anthony.[1,2] *[1]Biomedical Sciences, Colorado State University, Fort Collins, CO; [2]Pediatrics, University of Colorado Health Sciences Center, Aurora, CO; [3]Integrative Physiology, University of Colorado, Boulder, CO.*

Objective: Hypoxia as a result of placental insufficiency, maternal smoking,

or residence at high altitude, impacts fetal growth and development. In a preliminary study, we observed a significant decrease in birth weight, subsequent compensatory postnatal growth, and an increase in relative right ventricular (RV) weight at postnatal (pn) day 30 in female offspring of rats exposed to hypoxia (14,000 ft; ~11.6% PO_2) from days 12 thru 15 of gestation (dga). Thus, our objective was to further elucidate the impact of prenatal hypoxia on fetal growth and postnatal development. **Methods:** Pregnant dams (Hx, n=15) were hypoxic from 12-15 dga with additional control dams either fed *ad libitum* (AL, n=10), pair-fed with the Hx dams throughout gestation (PG, n=12), or only pair-fed during the window of hypoxia (PH, n=12). Female offspring from Hx, PG, and PH dams were cross-fostered onto additional AL dams (n=8/litter) by 48 h after birth. **Results:** At birth, there was no difference in litter size; however, body weight (bw) of the Hx, PG, and PH pups was lower ($P<0.05$) than that of AL pups, and Hx pups were lighter ($P<0.05$) than PH pups. Weight of Hx offspring remained lower ($P<0.05$) than AL pups until the termination of the study at pn120, while the PG and PH pups reached weights comparable to the AL offspring by pn90. Relative to bw, heart weight and left ventricular/septal (LVS) weight was not different among groups; however, right ventricular weight (RV/bw) was greater ($P<0.05$) in the Hx offspring at pn120 as was RV/LVS ($P<0.05$). Cardiac function was evaluated by echocardiography at pn174. RV wall thickness was 47% greater ($P<0.05$) in Hx pups as compared to AL pups, confirming the significantly higher relative RV weight observed at necropsy. PEP, PEP/AT, and PEP/ET were 36%, 14%, and 31% higher respectively in the Hx offspring relative to the AL offspring. LV end diastolic and end systolic diameters were smaller ($P<0.05$) in Hx and PH offspring relative to the AL group. Myosin heavy chain (MHC) α and β mRNA concentrations in the RV were evaluated by qRT-PCR, and the MHC β/α mRNA ratio was greater ($P<0.05$) in the Hx pups. **Conclusion:** Prenatal hypoxia from 12-15 dGA impacted both fetal and postnatal growth, altered postnatal heart development and function, with the primary impact being on the RV. Supported by NIH HD48902.

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Effect of Prenatal Nicotine and Intrauterine Hypoxia on Matrix Metalloproteinase Expression in Fetal Guinea Pig Hearts. Loren P Thompson,[1] Hongshan Liu,[1] Jessica A Mong.[2] *[1]Obstetrics, Gynecology & Reproductive Sciences; [2]Pharmacology & Experimental Therapeutics, University of Maryland, Baltimore, MD, USA.*

Background: Exposure to nicotine (NIC) is a significant risk to normal fetal development. Fetal NIC, which readily crosses the placenta, can be acquired from pregnant mothers by smoking or nicotine replacement therapy. The impact of NIC on fetal organs may be mediated directly and/or via intrauterine hypoxia (HPX) via constriction of the uterine circulation. In adult hearts, both NIC and hypoxia stimulate gene expression of matrix metalloproteinases (MMP), although the study of NIC and hypoxia on gene expression in fetal organs remains incomplete. Because MMPs are involved in the regulation of extracellular matrix turnover and cardiac remodeling, we tested the hypothesis that prenatal NIC and intrauterine HPX upregulate protein expression and activity of MMP2 in the fetal guinea pig heart.

Methods: Pregnant guinea pigs were placed in either normoxia (NMX) or HPX (10.5%O2 in chamber) for 14d prior to term (65d). In two separate groups, NIC was also added to the drinking water (~18 mg/kg/d) for 10d at a dose that generates fetal NIC levels (88ng/ml cotinine) equivalent to a moderate smoker. Anesthetized near-term fetuses (~63d) were excised and weighed. Left ventricles of hearts were obtained and frozen at -80C for storage. MMP2 protein levels and enzymatic activity were measured by Western analysis and gel zymography, respectively.

Results: NIC alone (NMX+NIC) decreased ($P<0.05$) fetal body wt by 12%, increased ($P<0.05$) the relative fetal brain wt (brain wt/fetal body wt ratio) by 14.2% and had no effect on relative placental or fetal heart wts. HPX alone decreased ($P<0.05$) fetal body wt by 20.3%, increased the relative fetal brain wt by 12% but, in contrast to NIC alone, increased relative placental wt by 33.8%. Both MMP2 protein levels (MMP2/a-actin density values) and activity (clear band density) were increased ($P<0.05$) by NIC alone (by 1.6 and 1.8 fold, respectively) and HPX alone (by 1.5 and 1.3 fold, respectively). In addition, both protein and activity levels of HPX hearts were further increased by NIC (by 1.6 and 1.3 fold) compared to HPX alone.

Conclusion: Prenatal NIC upregulates MMP2 expression in NMX fetal hearts and is potentiated by HPX. This suggests that under conditions of intrauterine stress cardiac remodeling by MMP activation may be an important mechanism by which NIC and HPX affect fetal heart function.

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Suppressed Hypothalamic Insulin Signaling Via PI3K and MAPK in IUGR Newborns: Mechanism of Impaired Anorexigenic Responses and Programmed Obesity. Ederlen Casillas,[1] Mina Desai,[1] Guang Han,[1] Darran N Tosh,[1,2] Michael G Ross.[1] *[1]Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Dept. of Physiology, Univ. of Adelaide, Adelaide, S. Australia, Australia.*

Objective: In addition to peripheral hypoglycemic effects, insulin induces central anorexigenic responses via stimulation of the phosphoinositide-3 kinase (PI3K) pathway and cellular growth by mitogen activated protein kinase (MAPK) pathway. The PI3K signaling cascade is activated by insulin binding to its receptor (IR), recruiting IR substrate 2 (IRS-2), and phosphorylating PI3K. Activated PI3K in turn causes phosphorylation of protein kinase B/AKT which subsequently modulates hypothalamic anorexigenic responses. In contrast, the MAPK (ERK1/ERK2) signaling pathway likely involves IRS-1. Further, insulin signaling is inhibited by the lipid phosphatase PTEN. We have previously shown that maternal food restriction (MFR) during pregnancy results in IUGR newborns that develop hyperphagia, obesity and insulin resistance as adults. We sought to determine if altered hypothalamic basal insulin signaling expression of PI3K and MAPK pathways contribute to reduced satiety responses and thus enhanced growth in IUGR newborns

Methods: Pregnant control dams received ad libitum (n=5) food, whereas study dams were 50% MFR (n=5) from pregnancy day 10 to 21 to produce IUGR newborns. At day 1, hypothalamic region was dissected and analyzed for mRNA levels (real time RT-PCR) of insulin signaling components via PI3K (IR, IRS2, PI3K and AKT) and MAPK (IRS1, ERK1, ERK2) pathways, and PTEN. Data is presented as fold difference normalized to beta-2-microglobulin.

Results: At 1d of age, IUGR pups exhibited downregulation of the entire PI3K pathway with significantly decreased ($p<0.05$) mRNA levels of IR (0.6-fold), IRS-2 (0.5-fold), PI3K (0.6-fold) and AKT (0.6-fold). Further, IUGR pups showed similar decreased mRNA expression of ERK1 (0.6-fold) and ERK2 (0.5-fold). However PTEN expression was similar to the Controls.

Conclusion: Reduced insulin-mediated PI3K signaling likely contributes to the suppressed anorexigenic responses and development of obesity in IUGR offspring. Reduction of central MAPK signaling suggests a potential maldevelopment of additional neuronal pathways in IUGR offspring.

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Being Born Small Impairs and Extends Nephrogenesis Which Can Be Altered by Improving Postnatal Nutrition. Mary E Wlodek,[1] Andrew L Siebel,[1] Jeanette Qianyu Zhao,[1] Karen M Moritz.[2] *[1]Physiology, University of Melbourne, Melbourne, Victoria, Australia; [2]School of Biomedical Sciences, University of Queensland, St Lucia, Queensland, Australia.*

Objectives: In the rat, uteroplacental insufficiency restricts fetal growth and impairs mammary development further compromising postnatal growth. Both male and female growth-restricted offspring have a reduced nephron endowment but only males develop hypertension with glomerular hypertrophy, which can be reversed by improving the lactational environment. This study used cross-fostering to assess the influence of the prenatal and postnatal environments on renal development and nephrogenesis.

Methods: Bilateral uterine vessel ligation (Restricted, R) or sham surgery (Control, C) was performed on day 18 of gestation in WKY rats. Control and Restricted pups were cross-fostered onto C or R mothers on postnatal day 1 (PN1). Post mortem was carried out on PN1 (C and R) and PN7 (C-on-C, C-on-R, R-on-C, R-on-R).

Results: Body and kidney weights were decreased in R and R-on-R pups on PN1 and PN7 ($p<0.05$). There was some evidence of accelerated pup growth for R-on-C relative to R-on-R on PN7. Male, but not female, relative BMP4 mRNA expression on PN1 was higher in R than C ($p<0.05$) while GDNF, TGFβ1 and AT1 receptor were not different. On PN7, Wnt4 (but not AT1R, VEGF-A) mRNA expression (males only) was relatively higher in R-on-R ($p<0.05$) when compared to C-on-C ($p<0.05$). This and the histological analyses suggests an up-regulation of nephrogenic activity with more immature nephrons (males and females) in R-on-R ($p<0.05$) when compared to C-on-C, while R-on-C remained intermediate.

Conclusions: Intrauterine growth-restricted pups were born lighter and with smaller kidneys. This was partially rescued by improving lactational nutrition (R-on-C) at PN7. Higher BMP4 mRNA expression indicates impaired branching morphogenesis in PN1 R male, but not female kidneys, suggesting the timing and/or molecular mechanisms underlying the nephron deficit may be sex specific. At PN7 there was evidence of extended and increased nephrogenic

activity in R-on-R, however, this was unable to restore the later nephron deficit. Improved lactation for R-on-C, which prevented the adult nephron deficit and hypertension, increased and extended nephrogenesis to a lesser degree than R-on-R suggesting that the restoration of nephron endowment was likely to have occurred prior to PN7.

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Maternal Ghrelin Deficiency Compromises Reproduction in Female Offspring Via a Ghrelin Receptor Independent Mediated Mechanism. Amy M Tetrault, Sarah B Lieber, Marya Shanabrough, Tamas L Horvath, Hugh S Taylor. *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objective: Classically recognized for its role in energy balance, body weight and appetite, ghrelin has also been implicated in reproduction. Ghrelin (-/-) mice are infertile while administration of ghrelin to wt mice results in decreased litter size and constrained embryonic growth. Here we investigate the effect of maternal ghrelin deficiency on in utero developmental programming of the female reproductive tract. HOX genes determine developmental identity of the paramesonephric duct. We determined that HOXA10 is regulated by ghrelin in vitro and that in utero ghrelin deficency alters F2 HOXA10 gene expression and reproductive success.

Methods: Wild-type females mice parented by ghrelin +/- B6D2F1 (ghrellin deficient) mice were analyzed for litter size, oocyte, and corpus luteum number. RNA was extracted from the uterus of mice exposed to ghrelin deficiency in utero. Ishikawa cells were treated with ghrelin with/without receptor (GHSR) antagonist, or saline and RNA extracted. In both HOXA10 expression was analysed by real time RT-PCR normalized to β-actin and also determined by IHC. Experiments were repeated in triplicate and mRNA expression compared by Student's T-test.

Results: Wild-type female offspring of ghrelin deficient dams had smaller litter sizes than controls (n=5, 4.7±3.3 pups; n= 4, 8.7±2.3 pups, respectively; $p<0.01$). No differences were seen in oocyte or corpus luteum number suggesting a uterine defect. HOXA10 mRNA and protein expression were decreased in the uterus of the F2 females. GHSR was expressed in uterine endometrium. Treatment of Ishikawa cells with 1nM to 100nM ghrelin resulted in a 30 to 80% increase ($p<0.01$) in HOXA10 expression. Treatment with ghrelin and GHSR antagonist resulted in similar increases in HOXA10 expression indicating a non-receptor mediated mechanism.

Conclusion: Ghrelin contributes to reproductive tract developmental programming; in utero ghrelin deficiency compromises reproduction in female offspring. The developmental effects of ghrelin were mediated by alteration in HOX gene expression and not through the classic GHSR receptor. Obesity and decreased ghrelin may lead to defects in developmental programming of the reproductive tract. These findings demonstrate the importance of nutrition, energy utilization and appropriate ghrelin levels on normal uterine development.

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Attenuation of Hepatic Histopathologic Damage in NOS3 Deficient Dams from the Initial to the Subsequent Pregnancy. Nima Goharkhay, Elena Sbrana, Joan E Moss, Nnaja Okorafor, Esther H Tamayo, Phyllis Gamble, George R Saade, Monica Longo. *Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

OBJECTIVE:

We have previously studied the deleterious effects of lack of the endothelial nitric oxid synthase (NOS3) in mouse dams and their offspring. Our laboratory demonstrated that adaptive responses in subsequent pregnancies may offset the harmful effects of the genetic deficiency of NOS3. In this study we aimed to determine hepatic and renal histopathologic damage in NOS3 deficient pregnant mice comparing animals carrying their first versus their second pregnancy.

STUDY DESIGN:

Gravid $NOS3^{+/+WT}$ and $NOS3^{-/-KO}$ mice during their first (P0) or second (P1) pregnancy were sacrificed at day 18 of gestation. Livers and kidneys were stained and analyzed for the presence and extent of histopathologic lesions.

RESULTS:

$NOS3^{+/+WT}$ dams displayed a low incidence of significant renal or hepatic lesions in either the first or the second pregnancy. In $NOS3^{-/-KO}$ mice the incidence of liver necrosis and inflammation during the first pregnancy was 44% and 49%, respectively. In $NOS3^{-/-KO}$ dams sacrificed during the second gestation the incidence rates for the same lesions were 6% and 19%, respectively ($p<0.05$). This correlation persisted when we analyzed the relative severity of hepatic lesions between P0 and P1 animals. Although a similar trend was observed, the difference between P0 and P1 animals with regards to kidney lesions did not reach the level of statistical significance in our study.

CONCLUSIONS:

A second pregnancy in this animal model of hypertension was associated with a significantly improved hepatic histopathology compared with the first pregnancy. This observation is consistent with our previous studies showing a decrease in systemic vascular resistance in P1 versus P0 $NOS3^{-/-KO}$ mice. The beneficial effects of a prior pregnancy may partially underlie the phenomenon of a decreased risk of preeclampsia in multiparous versus nulliparous women. Further studies are required to delineate the counterregulatory mechanisms leading to improved cardiovascular function in subsequent pregnancies in these genetically modified animals.

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Maternal Hypomethylation Is Associated with Congenital Heart Defects in Down Syndrome. LMJW van Driel,[1,2] R de Jonge,[3] WA Helbing,[2] BD van Zelst,[3] J Lindemans,[3] EAP Steegers,[1] RPM Steegers-Theunissen.[1,2,4,5] *[1]Obstetrics&Gynecology; [2]Pediatrics; [3]Clinical Chemistry; [4]Epidemiology&Biostatistics; [5]Clinical Genetics; [6]Erasmus University MC, Rotterdam, Netherlands.*

Background: Maternal age and hyperhomocysteinemia are risk factors for having a child with Down syndrome (DS) and congenital heart defects (CHDs), respectively. Evidence is rising that ageing is associated with a state of hypomethylation.

Objectives: To investigate whether the risk of a child with DS and CHD is associated with maternal hypomethylation.

Methods: We conducted a case-control triad study at 16 months after the index-pregnancy. Case-children (*n*=24) were included if they had DS and CHD. Children (*n*=251) without a major congenital malformation served as controls. The concentrations of S-adenosyl methionine (SAM), S-adenosyl homocysteine (SAH), SAM/SAH ratio, and homocysteine in maternal blood were measured as biomarkers for methylation. The data were analyzed using the Mann-Whitney U test and a logistic regression model.

Results: Maternal age was included in the model as potential confounder. The levels and the crude and adjusted OR(95%CI) of the biomarkers are shown in table 1. An increase of the SAM/SAH ratio with 1 unit decreases the risk of a child with DS and CHD with 30 percent. Moreover, every increase of 1 µmol/L of homocysteine 1.1 fold increases this risk.

Conclusions: Maternal hypomethylation is significantly associated with an increased risk of having a child with DS and CHD. Since, the effects are confounded by maternal age, hypomethylation can be considered as feature of ageing.

Biochemistry: levels and risk estimates

	Cases (n=24)	Controls (n=251)	P-value	OR (95% CI)[1]	P-value	OR (95% CI)[2]	P-value
SAM (nmol/L)	79.6 (72.1-83.7)	79.6 (72.8-88.0)	0.781	1.0 (0.97-1.04)	0.691	1.0 (0.97-1.0)	0.855
SAH (nmol/L)	15.7 (12.7-17.6)	14.1 (12.0-16.3)	0.052	1.1 (0.98-1.2)	0.096	1.1 (0.97-1.2)	0.185
SAM/ SAH ratio	5.37 (4.56-6.19)	5.80 (5.10-6.67)	0.052	0.7 (0.5-0.96)	0.029	0.7 (0.5-1.04)	0.080
Homo-cysteine (µmol/L)	11.3 (10.3-15.0)	10.3 (8.5-12.5)	0.004	1.1 (1.02-1.2)	0.009	1.1 (1.04-1.2)	0.002

Values are median (interquartile range) [1] Crude estimates. [2] Adjusted for maternal age.

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Dietary Protein Restriction of Pregnant Mouse Induces Altered Methylation of Oct-4 and Sphk-1 Gene Promoters in the Liver of Offspring. Jun Murotsuki,[1] Kunihiro Okamura,[1] Hiroi Kaku,[2] Yoshihiko Araki,[3] Hiroshi Yoshitake.[3] *[1]Obstetrics and Gynecology, Tohoku University School of Medicine, Sendai, Miyagi, Japan; [2]Obsterics and Gynecology, Iwate Medical University School of Medicine, Morioka, Iwate, Japan; [3]Institute for Environmental and Gender-Specific Medicine, Juntendo University Graduate School of Medicine, Urayasu, Chiba, Japan.*

The developmental origins hypothesis postulates that during critical ontogenetic periods, transient environmental stimuli perturb developmental pathways and induce permanent changes in gene expression, metabolism, and chronic disease susceptibility. One likely mechanism is via early nutritional influences on epigenetic gene modification consisting of the presence of a methyl group on the carbon 5 of a cytosine residue. This modification is responsible for an important form of gene regulation in eukaryotes. In the present study, we have

tested the hypothesis that maternal low-protein diet altered epigenetic regulation of specific gene of the offspring. C57BL/6 female mice were mated and on the day the plug was detected, these females were then randomly allocated to be fed isocaloric diets consisting 18% protein or 9% protein. At delivery, offspring were killed and the livers were removed immediately, frozen in liquid nitrogen and stored at -80C. Genomic sequencing after bisulfite modification is used to study site-specific DNA methylation. DNA methylation status of *Oct-4* and *Sphk-1* gene upstream regions in the mouse liver was analyzed. Hepatic Oct-4 or Sphk-1 promoter methylation was not significantly different between both groups. However, DNA methylation pattern of the genomic DNA is specific in low-protein diet group. Aberrant *Oct-4* and *Sphk-1* gene expression may cause perturbations in cell differentiation. We suggest that the epigenetic mechanism consisting of DNA methylation underlies the fetal programming theory.

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Cytokine Profile Dysregulation in Early Infection of Cytotrophoblasts by Human Cytomegalovirus. Jessica A Warner,[1,3] Seth B Coffelt,[1] Kenneth F Swan,[1,2] Deborah E Sullivan,[1] Gabriella Pridjian,[2] Cindy A Morris.[1] *[1]Department of Microbiology and Immunology, Tulane University Health Sciences Center, New Orleans, LA, USA; [2]Department of Obstetrics and Gynecology, Tulane University Health Sciences Center, New Orleans, LA, USA; [3]Physician Scientist Program, Tulane University Health Sciences Center, New Orleans, LA, USA.*
Primary human cytomegalovirus (HCMV) infection during pregnancy can have devastating consequences for both the mother and fetus. HCMV infection has been implicated in the development of pre-eclampsia and intrauterine growth retardation (IUGR), as well as congenital CMV syndrome in newborns exposed in utero. Previously, we have shown that HCMV infection of placental cytotrophoblasts inhibits their normal invasion, proliferation, and migration. However, the mechanisms occurring during early establishment of placental infection are largely unknown. We assessed the impact of HCMV infection on cytotrophoblasts by performing immuno-based assays for various cytokines and cellular growth factors. We detected significant cytokine dysregulation at both 24 and 48 hours after in vitro HCMV infection of cytotrophoblast cells. Soluble cytokines involved in recruitment of monocytes and macrophages (Gro-a, MCP-3) were downregulated at both 24 and 48 hours after infection. SDF-1, which is chemotactic for lymphocytes during early inflammation, was also decreased. These results suggest that recruitment of cells involved in the anti-viral immune response is being interrupted early in the course of infection by HCMV. Additionally, a large decrease in the amount of soluble HGF was seen. HGF normally induces migration of cytotrophoblasts along the invasive pathway, and downregulation of this factor could severely affect these processes. Finally, we saw increased amounts of soluble ICAM-1, contrasted by decreased amounts of VCAM-1, indicating dysregulation of adhesion molecules that are necessary for successful placental invasion to occur. All together, these data indicate significant alterations in cytokine profiles as early as 24 hours after HCMV infection, which could provide important clues to the pathogenesis of HCMV in placental invasion and inflammation. Additional studies will further elucidate this dysregulation, and determine whether these effects are due to alterations in pre-existing cellular factors or if transcriptional alterations are involved.

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Ovine Maternal and Fetal Plasma Corticotrophin Releasing Factor (CRF) Levels: Contribution to In Utero Meconium Passage. Jayaraman Lakshmanan,[1] Chander P Arora,[2] Calvin J Hobel,[2] Glenda Calvario,[1] Sharon K Sugano,[1] Michael G Ross.[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Dept. of Ob/Gyn, Cedars-Sinai Med. Ctr, Burns & Allen Res. Inst., Los Angeles, CA, USA.*
Background: Overwhelming evidence in adult rats indicates that CRF mediates gastrointestinal stress responses. Utilizing a maternal hypoxic-stress paradigm we recently reported rat in utero meconium passage may be mediated by the CRF pathway, with peripheral CRF likely a result of placental CRF synthesis and release. To evaluate the potential for peripheral CRF to participate in maternal-fetal neurovisceral stress responses, we examined gestation-dependent changes in plasma CRF levels in blood collected from un-instrumented pregnant ewes and their fetuses.
Method: Studies were performed in pregnant ewes (n=6 in each group) with twin fetuses at 118-120 days (very preterm) and 140-142 days (near-term). Neither mother nor fetuses were instrumented prior to the time of blood collection. Maternal blood was collected from the jugular vein before sedation. Anesthesia was then induced with isofluorane, the abdomen was opened, fetuses exteriorized and blood collected from umbilical cords. Blood samples were transferred to plastic tubes containing ethylene-diamino tetraacetic acid and reduced glutathione, plasma separated and stored at -80C. Commercial radioimmunoassay kits for ovine-CRF (Phoenix Pharmaceuticals, B90: 7.2 ± 1.5 pg/ml) and cortisol (Diagnostic Laboratory, B90: 1.2 ± 0.4 ng/ml) were used according to the manufacturer's instructions.
Results: In very-preterm gestations, maternal and fetal plasma CRF levels were undetectable. Maternal, but not fetal, plasma cortisol levels were measurable (33±4 ng/ml). In near-term gestations, both cortisol and CRF were measurable in maternal (CRF: 86±6 pg/ml; cortisol: 55±1 ng/m) and fetal plasma (CRF: 730±68 pg/ml; cortisol: 5±2 ng/ml). Plasma CRF levels were higher in near-term fetuses than in their maternal ewes ($p < 0.001$).
Conclusion: Ovine plasma CRF levels are measurable in maternal and fetal plasma in near-term but not very-preterm gestations. The absence of CRF in preterm plasma, perhaps due to reduced placental expression and/or placental CRF release, may contribute to the rarity of in utero meconium passage in preterm gestations.

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Interleukin-6 and Pre-Pregnany BMI Are Associated with Infant Birth Weight. Wanda K Nicholson,[1] Lori Bash,[2] FL Brancati,[1] Neil R Powe.[1] *[1]Gynecology and Obstetrics; [2]Epidemiology; [3]Internal Medicine, Johns Hopkins Medical Institutions.*
Objectives: Interleukin-6 (IL-6) is a pro-inflammatory cytokine produced in adipose cells. Recent studies suggest IL-6 may be a marker of maternal obesity and in utero fetal programming. Our hypotheses were 1) IL-6 correlates with maternal obesity and 2) IL-6 mediates the effect of maternal obesity on infant birth weight.
Methods: *The Parity, Inflammation, and Diabetes (PID) Study* is a longitudinal study of adipokine levels in a diverse sample of pregnant women. We present a cross-sectional analysis of first trimester IL-6 levels from 173 non-diabetic women who underwent a live birth. The independent variable was IL-6 (pg/ml), measured with monoclonal antibody ELISA assays. The dependent variable was infant birth weight (gms). Maternal BMI categories were: normal/underweight (< 25 kg/m^2); overweight (25-30 kg/m^2); and obese (> 30 kg/m^2). Data on demographic and clinical factors, nutrition and physical activity were collected at baseline. Average IL-6 levels were compared across BMI categories using ANOVA. The association of IL-6 levels with infant birth weight was estimated using multiple linear regression, adjusting for covariates.
Results: Average IL-6 levels were significantly higher in obese women (2.6± 1.6) compared to overweight (1.2± 0.4) and normal/underweight women (1.1± 0.7) [P< 0.001]. After adjustment, IL-6 levels was positively correlated with pre-pregnancy BMI [Regression coefficient (RC)0.12; 95% CI: (0.05, 0.2)]. As shown in the table below, elevated levels of IL-6 were statistically significantly associated with a 0.5 gm higher infant birth weight after full adjustment. Each 1 unit increase in pre-pregnancy BMI was associated with a 8 gm higher infant birth weight.

Table 1. Association of IL-6 with infant birth weight

Characteristic	Regression coefficient	95% Confidence Interval
Interleukin-6	0.5	0.02, 0.7
Pre-pregnancy BMI	8	0.7, 38
Gestational weight gain	-3	-15, 10

BMI = body mass index; Coefficients adjusted for demographics, clinical factors, pre-pregnancy BMI, gestational weight gain, non-fasting first trimester glucose levels, gestational age, nutritional intake and physical activity

Conclusion: IL-6 and pre-pregnancy BMI were associated with infant birth weight after adjustment for covariates. Our findings suggest that the effect of maternal obesity on infant birth weight may be mediated through IL-6 or an alternative independent pathway.

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The Endocannabinoid, Anandamide, Inhibits Trophoblast Cell Line Proliferation by Increasing the Expression of Histone Deacetylase 3. Anthony H Taylor, Muna S Abbas, Stephen C Bell, Justin C Konje. *Cancer Studies and Molecular Medicine, Leicester University, Leicester, United Kingdom.*
Background:
We have demonstrated that Δ^9-tetrahydrocannabinol (THC), in physiologically relevant concentrations, inhibits the growth and tight transcriptional control of the BeWo trophoblast cell line[1]. The mechanism involved the decreased

expression of the transcriptional regulator histone deacetylase 3 (*HDAC3*). In these experiments we sought to answer the question, 'Does anandamide (AEA) work in the same manner as THC?'

Methods:

The first trimester human trophoblast cell, BeWo were plated at 1 or 4 x 10^5 cells /well to 96-well and 6-well plates for growth and RNA experiments, respectively. After growing to 70-80% confluence, cultures were treated with varying concentrations of AEA up to a maximum of 30μM for 48hr. Cell numbers were determined using the XTT apoptosis/proliferation assay. Total cellular RNA was prepared and the relative levels of *HDAC3* and GAPDH determined by end-point RT-PCR.

Results:

AEA exhibited an inhibitory effect on the BeWo cell cultures, but only at concentrations in excess of 3μM where confluency was significantly reduced from ~75% at 3μM to ~50-60% at 15μM and 30μM (*P<0.05; one-way ANOVA with Tukey's HSD test; *n*= 4). Cultures treated with 15μM and 30μM AEA did not exhibit increased cell death or failure to attach to the substratum, as evidenced by the lack of increase in the shedding of cells into the spent medium. BeWo cells treated with AEA showed a dose-dependent decrease in *HDAC3* mRNA expression with a significant effect at 0.3μM (**P*<0.05; one-way ANOVA with Tukey's HSD test; n=9). At this dose, the effect of AEA had reached an effective maximum decrease in *HDAC3* mRNA levels (48%) because *HDAC3* mRNA levels were not decreased further by either 3μM AEA (45%) or 30μM AEA (49%).

Conclusion:

The alteration of *HDAC3* gene expression by AEA in BeWo cells with its associated decrease in cell number suggest that the trophoblast cell may be an important target for circulating endocannabinoids during the 1st trimester of pregnancy. The data indicates that although exocannabinoids and endocannabinoids both inhibit BeWo cell growth, they do so using different transcriptional mechanisms. Further understanding of the mechanism(s) by which AEA alters placental physiology may lead to new strategies for the prevention of pregnancy complications such as 1st trimester miscarriages.

References:

(1) Taylor, A.H., et al. (2007) *Reprod. Sci.* **14:** 261A, 720.

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Expression of the Endocannabinoid System in Human First Trimester Placenta. Anthony H Taylor, Osama MH Habayeb, Stephen C Bell, David J Taylor, Justin C Konje. *Endocannabinoid Research Group, Department of CSMM, University of Leicester, Leicester, United Kingdom.*

Background: Anandamide (AEA) exerts its effects by acting on two cannabinoid receptors, CB1 and CB2 with the main regulator for AEA levels being the metabolising enzyme, fatty acid amide hydrolase (FAAH). AEA, CB1, CB2 and FAAH constitute the endocannabinoid system and previous studies have shown that FAAH and CB1 are expressed in term human placenta(1) suggesting that the endocannabinoid system might be present earlier in gestation. This study aimed to document changes in FAAH, CB1 and CB2 expression in the placenta during the first trimester of pregnancy.

Methods: First trimester samples (7 to 12 weeks gestation) were fixed in 10% neutral-buffered formalin for 4 days before embedding into paraffin wax or frozen in liquid nitrogen for RNA analysis. For immunohistochemistry, FAAH polyclonal antibodies were used at an optimal dilution of 1:2000 in PBS, CB1 at 1:4000 and CB2 at 1:500. Transcripts were measured using Q-PCR with gene-specific primers.

Results: Immunoreactive FAAH, CB1 and CB2 were detected in all samples. FAAH immunoreactivity in the syncytiotrophoblast increased between the 7th and 10th gestational week and by week 11 FAAH was barely detectable within large parts of the placenta. Simultaneously, FAAH immunoreactivity increased in the mesenchymal core of the developing villi. Immunoreactive CB1 and CB2 localised to the syncytiotrophoblast, cytotrophoblast and mesenchymal core with CB1 immunoreactivity showing diminished intensity after week 10, although this did not reach significance at the transcript level. CB1 immunoreactivity was absent from fetal blood cells and infiltrating maternal plasma cells, whereas CB1 and CB2 immunoreactivity was detected in endothelial cells but not in the vascular smooth muscle cells of blood vessels. The intensity of CB2 immunoreactivity in the syncytiotrophoblast differed from that of CB1 and FAAH in that it remained constant throughout.

Conclusion: The data suggest that placental FAAH and CB2 levels do not alter significantly during the first trimester, but alter their cellular distribution from the syncytiotrophoblast to the mesenchymal core. The significant loss of CB1 expression from the syncytiotrophoblast after the 10th week of gestation, a point of critical alteration in the developing placenta, suggests that its retention may be detrimental to normal placental development.

Reference: Park, B. *et al.*, (2003) *Placenta* **24:** 473-478.

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Effect of CGRP on In Vitro Angiogenesis by Human Microvascular Endothelial Cells. Yaun-Lin Dong, Hong Y Wen, Manubai Nagamani, Gary Hankins, Chandra Yallampalli. *Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Background: Numerous angiogenic proteins synthesized in the placenta are thought to be involved in placental vascularization and development; however, the molecular mechanism modeling the angiogenic process in early pregnancy remains elusive. Calatonin gene-related peptide (CGRP) is a multifunctional peptide expressed at the human implantation site; but its influence on in vitro angiogenesis by human micro vascular endothelial cells is not known.

Objective: The present study was designed to determine the influence of CGRP on angiogenesis by human dermal microvascular endothelial cell (HDMVEC) in vitro.

Methods: HDMVECs (VEC Technologies) were cultured in MCDB-131 complete solution containing CGRP (10^{-8}M), CGRP plus its antagonist CGRP 8-37 (10^{-7}M), in 6 well plates with 2x10^5 cells per well. The existence of CGRP receptor components calcitonin receptor-like receptor (CRLR) and receptor activity modifying protein 1 (RAMP1) was determined using immunofluorescent staining. Cell proliferation was examined using methylthiazoltetrazolium (MTT) assay. The pro-angiogenic bioactivity of CGRP was evaluated using cell migration and capillary like tube formation on the Matrigel.

Results: 1) Immunofluorescent staining showed that CGRP receptor components CRLR and RAMP1 are abundantly expressed by HDMVECs. Replacement of the primary antibodies with preimmune serum resulted in a negative staining; 2) CGRP dose-dependently (10^{-10} to 10^{-7}M) stimulated HDMVEC proliferation, and this effect was totally blocked by CGRP antagonist, CGRP 8-37; 3) Quantitative analysis for cell migration revealed that CGRP increases HDMVEC migration in a dose and time-dependant manner; and 4) CGRP promotes HDMVEC capillary like tube formation, and the length of capillary tube induced by CGRP (10^{-8}M) was significantly increased over that of the untreated controls. This increase was observed at 12 hours of treatment and further increase was noted at 24 hours of culture.

Conclusion: CGRP induces in vitro angiogenesis by promoting microvascular endothelial cell proliferation, migration and capillary like tube formation. Therefore, trophoblast derived CGRP at the implantation site may play a role in placental angiogenesis and fetal growth.

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Over-Expression of SOCS-3 Gene Promotes IL-10 Production by JEG-3 Trophoblast Cells. Qin Dong,[1,2] Ruping Fan,[2] Yang Gu,[2] David F Lewis,[2] Yuping Wang.[2] *[1]Biochemistry, Harbin Medical University, Harbin, Heilongjiang, China; [2]Obstetrics and Gynecology, LSUHSC-Shreveport, Shreveport, LA, USA.*

Objective: Suppressor of cytokine signaling-3 (SOCS-3) plays an important role in negative regulation of inflammatory response in biological cells. Evidence has shown anti-inflammatory cytokine IL-10 expression was significantly reduced in trophoblasts of preeclamptic (PE) placentas. We sought to determine if over-expression of SOCS-3 in placental trophoblasts could promote IL-10 production.

Methods: Full-length SOCS-3 open reading frame (SOCS-3 cDNA) was generated by RT-PCR from total RNA samples isolated from human leukocytes and cloned into a pZsGreen1-N1 vector, which encodes a green fluorescent protein ZsGreen1. Successful SOCS-3 cloning was confirmed by sequencing. For transfection, JEG-3 cells were placed into 6 well/cluster plates at a concentration of 1.2 x105/well. The tranfection was carried out with 1.0μg of SOCS-3/ZsGreen1 plasmid (pSOCS-3/ZsGreen1) for 6 hours when cell reached 45-50% confluence. siPORT lipid were used. JEG-3 cells transfected with ZsGreen1 plasmid (pZsGreen1) only was used as control. After approximately 60 hours of transfection, cells were treated with IL-6 at 1 and 10ng/ml. Medium

was then collected and measured for IL-10 by ELISA. IL-10 production was calculated as the percentage of increase by pSOCS-3/ZsGreen1 transfected cells compared to the cells transfected with pZsGreen1 only.
Result: IL-10 production was increased by pSOCS-3/ZsGreen1 transfected JEG-3 cells compared to the cells transfected with pZsGreen1 only when stimulated by IL-6, control: 9.9% increase; 1ng/ml IL-6: 28.5% increase and 10ng/ml IL-6: 140% increase, $p< 0.05$, respectively. Data are means from three independent experiments.
Conclusion: Over-expression of SOCS-3 gene could promote IL-10 production by placental trophoblast cells.

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Reduced sIL-6R Release and Increased Ratio of sgp130/sIL-6R Production by Placental Tissues from Women with Preeclampsia. Shuang Zhao,[1] Ruping Fan,[1] Jingxia Sun,[2] Yang Gu,[1] David F Lewis,[1] Yuping Wang.[1] *[1]Obstetrics and Gynecology, LSUHSC-Shreveport, Shreveport, LA, USA; [2]Obstetrics and Gynecology, First Hospital, Harbin Medical University, Harbin, Heilongjiang, China.*
Objective: Placental tissue/trophoblasts release more inflammatory cytokines (IL-6, IL-8 and TNFα) in preeclampsia (PE) than in normal pregnancies. However, the reason for increased inflammatory cytokines released by PE placentas is not clear. Soluble IL-6 receptor (sIL-6R) and membrane receptor IL-6/gp130 complex play an important role in the negative regulation of cytokine signaling in suppressor of cytokine signaling (SOCS) pathway. In contrast, soluble gp130 (sgp130) is an antagonist for IL-6/IL-6R trans-signaling. This study was undertaken to determine sIL-6R and sgp130 production by villous tissues from normal and PE placentas.
Methods: Placentas delivered by 8 normal and 9 PE pregnant women were used in this study. Placental explants were incubated with DMEM for 48h. The culture medium was collected. Placental villous tissue productions of sIL-6R and sgp130 were measured by ELISA. All samples were assayed in duplicate. Data are expressed as mean ± SE and analyzed by Mann Whitney test. A p level <0.05 was considered statistically different.
Results: Placental tissues from PE produced significantly less sIL-6R than tissues from normal pregnancies, 3.56±0.61 vs. 5.84±0.34 pg/mg of wet tissue, $p<0.01$. Soluble gp130 production was relatively compatible between PE and normal placental tissues: 162.29±21.71 vs. 162.83± 5.56 pg/mg of wet tissue. The ratio of sgp130/sIL-6R release was significantly higher in PE than in normal placentas, 49.84±6.93 vs. 28.62±0.02, $p<0.01$.
Conclusion: Reduced sIL-6R production and/or increased ratio of sgp130/sIL-6R production by PE placentas suggest less cytokine inhibitory activity in PE placentas, which may contribute to the increased toxic cytokine production in placentas from PE.

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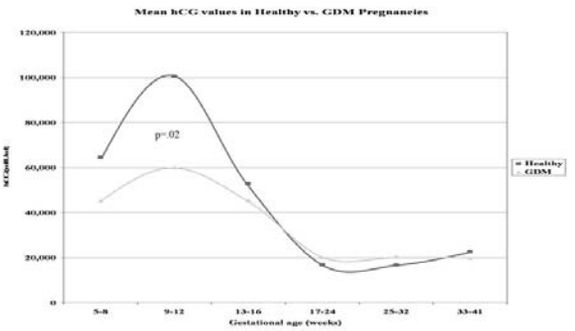
Characterization of hCG Levels during Normal and Diabetic Gestation Using Chemiluminescence Immunoassays. Peter S Uzelac,[1] Jing Dai,[2] Frank Z Stanczyk,[2] Daniel R Mishell, Jr.[2] *[1]Obstetrics and Gynecology, University of Louisville, Louisville, KY, USA; [2]Obstetrics and Gynecology, University of Southern California, Los Angeles, CA, USA.*
Objective: Characterizing human chorionic gonadotropin (hCG) levels throughout normal pregnancy is critical to its use as a bio-marker for abnormal gestations. There is a paucity of data describing hCG trends during pregnancy and most of the relevant studies use older, less specific assays. Our first objective was to characterize hCG levels throughout normal gestation using two different contemporary chemiluminescence immunoassays. Our second objective was to compare hCG patterns in healthy gestations with pregnancies affected by diabetes, a common obstetrical complication.
Methods: A single blood sample was collected from 137 healthy pregnant women and 170 diabetic pregnancies. Gestational age was confirmed by ultrasound. Serum hCG levels were quantified by chemiluminescence immunoassays using the ACS-180 and Immulite systems. Data was grouped in 4-week intervals until 16 weeks of gestation and 8-week intervals thereafter, with 21 to 27 samples in each interval for healthy women and 6 to 56 samples in each interval for diabetic women. Paired t-test and Wilcoxon rank sum test were used for statistical analysis.
Results: Using the ACS-180 system, mean hCG levels (mIU/ml) for healthy pregnant women were 64,489 at 5-8 weeks, 100,421 at 9-12 weeks, 52,724 at 13-16 weeks, 16,741 at 17-24 weeks, 16,580 at 25-32 weeks, and 22,535 at 33-41 weeks. Mean hCG levels obtained from the Immulite system were similar. For diabetic pregnancies, mean hCG levels (mIU/ml) were 45,165, 59,899, 45,448, 19,920, 20,372 and 19,525, respectively. Between 9-12 weeks of gestation, the hCG levels were significantly lower in diabetic pregnancies ($p=0.02$) compared to healthy controls.
Conclusions: 1) Previous characterization of hCG patterns throughout gestation is valid. 2) The ACS-180 and Immulite systems yield comparable hCG results. 3) Compared to healthy pregnancies, diabetic gestations have significantly lower peak hCG levels. The cause of this difference is an area deserving further investigation.



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Studies of the Placental Renin Angiotensin System (RAS) in Wild Type and Angiotensin Converting Enzyme (ACE) Knockout Mice. Lauren Wilson,[1] Kenneth Bernstein,[2] Fiona Broughton Pipkin.[1] *[1]OB/GYN, University of Nottingham at City Hospital, Nottingham, United Kingdom; [2]Pathology, Emory University, Atlanta, USA.*
Background: Mouse and human placentae share several cellular and molecular features, including a haemochorial interface, allowing the murine labyrinth to be compared with the human fetal placenta. There is an autonomous embryonic RAS from at least the time of implantation. ACE converts angiotensin I to the active angiotensin II (AngII). AngII's actions as a pro-inflammatory agent, in promoting cell migration, angiogenesis and cellular growth and apoptosis, strongly suggest a rôle in placentation. **Hypothesis:** There would be counter-regulation of the placental RAS if the effect of ACE were removed. This study investigated for the first time whether the knockout of somatic ACE affected the expression and localisation of various components of the RAS in the placentae of wild-type (wt/wt), heterozygous (wt/4) and ACE knockout (4/4) mice.
Methods: Immunohistochemistry (DAKO Envision Plus) was used to localise and semiquantify Ang type 1 receptor (AT1R), Ang type 2 receptor (AT2R), ACE, and ACE-2 in the three genotypes (n=3/group). Placental sections were blinded to genotype; a score range of 0-4 was used. The χ^2 test was used (SPSS version 15.0) to analyse the difference in staining score by genotype. **Results:** Immunoreactivity of all antigens increased in the placental labyrinth of 4/4 mice compared to wt/wt and wt/4 mice (AT1R $P<0.05$; AT2R $P<<0.001$; ACE $P<<0.001$; ACE-2 $P<<0.001$). AT2R and ACE-2 displayed increased staining in the fetal vascular endothelium of 4/4 mice (AT2R $P<<0.001$; ACE-2 $p=0.05$), and in the cells lining the maternal central artery (AT2R $P<<0.001$; ACE-2 $P<<0.001$). ACE-2 expression was very high in cytotrophoblast lining the maternal blood space in all genotypes. No gross structural differences were seen. **Comment:** The antibody used did not differentiate between ACE and the membrane-bound "testicular"-ACE (tACE). Immunohistochemically-identified ACE expression was upregulated in 4/4 placentae despite the loss of somatic ACE, suggesting that t-ACE can be expressed placentally. We believe this is the first demonstration of such expression. ACE and t-ACE are catalytically-similar in converting AngI to AngII. Furthermore, AngII acting via the AT2Rs is vasodilatory and ACE-2 catalyses production of the vasodilatory Ang (1-7); placental blood flow was presumably well-maintained and pregnancy outcome is normal in 4/4 mice.

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The Effect of 17 Alpha Hydroxyprogesterone Acetate on PGDH, COX-1, and COX-2 Expression in Human Placental Membranes at Term as Evidenced by Immunohistochemical Staining. Rebecca L Guinn,[1] Hassan M Harirah,[2] Wahiduz A Zaman.[3] *[1]Obstetrics and Gynecology, University of Texas Medical Branch at Austin, Austin, TX, USA; [2]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA; [3]Pharmacology, University of Texas Medical Branch, Galveston, TX, USA.*
It is generally accepted that prostaglandin production plays a crucial role in both term and preterm parturition, and recently the administration of 17 alpha hydroxyprogesterone acetate has been shown useful in preventing preterm labor.

What is not clear is the biochemical pathway of prostaglandin production during labor and what, if any, effect 17 alpha hydroxyprogesterone acetate has on this pathway. In order to address this question, we used immunohistochemical staining techniques to evaluate the effect of treating human placental amnion and chorion decidua with 17 alpha hydroxyprogesterone acetate. Membranes from unlabored patients were obtained at cesarean section and immediately seperated into control and drug treated specimens. Controls were from the same placenta and were subjected to all experimental procedures except for the addition of 17 alpha hydroxyprogesterone acetate to the culture media. Specimens were compared at zero, six, and twenty four hour intervals. At the appropriate time, each specimen was formalin fixed and then paraffin blocked. Tissue sections were then mounted on slides which were immunohistochemically stained using appropriate primary and secondary antibodies and standard techniques. The slides were then analyzed via light microscopy for changes in staining of three enzymes involved in prostaglandin production--cyclooxygenase 1 (COX-1), cyclooxygenase 2 (COX-2), and 15-hydroxy prostaglandin dehydrogenase (PGDH). Compared to control, the slides treated with 17 alpha hydroxyprogesterone acetate had differing amounts of enzyme expression. COX-1 was relatively unchanged and PGDH was only slightly increased, but COX-2 was noticeably decreased in the treated slides. These results were time dependent. This data suggests that 17 alpha hydroxyprogesterone acetate decreases prostaglandin production in fetal membranes primarily by downregulation of the cyclooxygenase 2 enzyme.

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The Protein Tyrosine Phosphatase, SHP-2, Is a Positive Regulator of IGF-Mediated Cell Turnover in the Human Placenta. Karen Forbes, John D Aplin, Melissa Westwood. *Maternal & Fetal Health Research Group, University of Manchester, Manchester, United Kingdom.*

Objectives: In the human placenta, proliferation, differentiation and fusion of cytotrophoblasts (CT) are essential events in the formation of the multinucleated syncytiotrophoblast, however the regulation of these processes is poorly understood. Using an explant model of human first trimester placenta we have established that both IGF-I and -II enhance CT proliferation, differentiation and survival mediated via IGF1R signalling. We have also shown that non-specific inhibition of protein tyrosine phosphatases inhibits IGF-mediated signalling in trophoblast; therefore, we have now used siRNA-mediated knockdown to investigate the role of the tyrosine phosphatase SHP-2 in this pathway.

Methods: Amaxa Nucleofector technology was used to deliver SHP-2 or scrambled siRNA (100nM) to BeWo cells or first trimester villous tissue fragments. Knockdown was confirmed by Q-PCR and western blotting. Transfected cells and tissue were maintained in culture for 72 hours, then treated with IGF-I or IGF-II (10nM) for a further 24 hours before immunohistochemical (IHC) analysis for cell proliferation (Ki67, BrdU) or apoptosis (M30).

Results: siRNA-mediated knockdown of SHP-2 in BeWo cells (95% reduction on Western blot) demonstrated that IGF-induced proliferation was reduced from 67.3±3.5% to 41.9±2.4% ($P<0.001$, n=5). IHC analysis of tissue demonstrated that SHP-2 is localised to CT. Following knockdown (93% decrease by Q-PCR), IGF-I- and IGF-II-induced CT proliferation was decreased by 60.02±7.4% and 55.9±9.6 % respectively ($P<0.001$, n=4). Furthermore, the ability of IGF-I- and IGF-II to prevent CT apoptosis (M30 staining) was reduced by 48.3±10.1% and 39.1±7.9% respectively ($P<0.05$, n=4) after SHP-2 knockdown.

Conclusions: IGF stimulation of cytotrophoblast proliferation is mediated by SHP-2. Exogenous IGF rescues cytotrophoblast from apoptosis, and this pathway is also SHP-2-dependent.

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Estradiol Mediates Genes Involved in Prostanoid Production in Placental Villous Endothelial Cells. Emily J Su, Zhi-Hong Lin, Ping Yin, Scott Reierstad, Joy Innes, Serdar E Bulun. *Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.*

Background: Within the human vascular system, estrogens have been shown to enhance vasodilatation in both normal and abnormal endothelium. Estrogenic function occurs by activation of one or both of two estrogen receptors, estrogen receptor-alpha (ESR1) and estrogen receptor-beta (ESR2). These estrogen receptors are expressed in a wide variety of tissues. Within the vasculature, estrogen receptors regulate the expression of multiple vasodilator and vasoconstrictor proteins. Specifically, ESR2 has been shown to be critical in maintaining normal vascular physiology in a murine model, where ESR2 knock-out mice demonstrate significant systolic and diastolic hypertension. We hypothesize that within placental endothelium, estrogen plays an important role in maintaining normal vascular function that is critical for normal fetal growth and development.

Methods: Term placentas from uncomplicated pregnancies were obtained, and the decidua was removed. An IV cannula was inserted into the umbilical vein, which was perfused with a collagenase/dispase solution. The perfusate was collected and subjected to further purification. These cells were cultured in complete medium, and after the initial passage of these cells, purity was confirmed via immunofluorescence and flow cytometric studies. Estrogen receptor expression was determined in these cells via western blotting. Additionally, these endothelial cells underwent treatment with varying doses of estradiol (10^{-9} M to 10^{-6} M), and quantitative real-time PCR was performed thereafter for mRNA levels of various genes important in prostanoid production.

Results: Western blotting demonstrated that ESR2 is the only estrogen receptor expressed within villous placental endothelial cells. Estradiol induced cyclooxygenase-2 (COX-2) mRNA levels 3- to 6-fold, as quantified by real-time PCR ($p<0.001$). Conversely, there was no effect of estradiol on cyclooxgenase-1 (COX-1).

Conclusion: These results suggest that estradiol and ESR2 are important in mediating the balance of prostanoid production that is essential in maintaining placental vascular health. Future studies will further delineate estrogenic effects on prostanoid production within placental endothelial cells in health and disease. Supported by the SMFM/AAOGF Scholarship Award and the NIH grant U54-HD40093.

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EGF Receptor Signaling Mediates Extravillous Trophoblast Differentiation. Julie K Wright,[1,2] Caroline E Dunk,[1] Elke Winterhager,[3] John CP Kingdom,[1,2] Stephen J Lye.[1,2] *[1]Mount Sinai Hospital, Samuel Lunenfeld Research Institute, Toronto, ON, Canada; [2]Physiology; Obstetrics and Gynecology, University of Toronto, Toronto, ON, Canada; [3]Institute of Anatomy, University of Essen, Essen, Germany.*

Previously we established that proteins secreted by the decidua promote the differentiation of extravillous trophoblasts (EVT) from a proliferative phenotype (characterized by Cx40, HER-1 and alpha5 integrin protein expression) to an invasive phenotype (characterized by HER-2 and alpha1 protein expression). The ability of decidua-conditioned media (DCM) to induce trophoblast differentiation was inhibited in the presence of the HER-1 receptor antagonist, AG1478. Furthermore, DCM-induced JAR cell migration was also attenuated in the presence of AG1478. Thus, the purpose of this study is to define the role of HER signaling in EVT differentiation and invasion.

Methods: EVT differentiation was assessed in placental villous explant outgrowths and JAR cells using antibodies against markers of the proliferative and invasive phenotypes. Trophoblast migration was assessed using JAR cells in transwell migration assays.

Results: Treatment of placental villous explants with EGF, a HER-1 ligand, resulted in the downregulation of HER-1 and an upregulation of HER-2 expression, as well as an induction of alpha1 integrin expression. Pre-treatment of placental villous explants with AG1478 blocked this effect. In the JAR cell line, EGF treatment mimicked the differentiation-promoting effects of DCM by downregulating HER-1 and upregulating HER-2 expression, effects that were both blocked when JAR cells were pre-incubated with AG1478. In contrast to DCM however, EGF stimulation did not induce JAR migration. Stimulation of JAR cells with HB-EGF, a HER-1/HER-4 heterodimer ligand, induced JAR migration in a dose-dependent manner. Analysis of DCM using antibody arrays confirmed the presence of many members of the EGF family including HB-EGF. Immunohistochemical assessments of placental villous explants verified the expression of HER-4 in EVT outgrowths and in JAR cells; HER-4 expression was not affected by stimulation with either EGF or HB-EGF.

Conclusions: HER signaling is an important and necessary component of the invasive EVT differentiation cascade. Our data supports a role for HER-4 signaling in the induction of the invasive EVT phenotype.

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PTHrP Silencing: Effects on Placental Trophoblast Growth Factors. Chandrasekhar Thota, Chandra Yallampalli. *Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

Background: Parathyroid hormone related peptide (PTHrP) is expressed in

trophoblast cells and may play a role in placental growth and function. Studies conducted in pregnant rats using PTHrP antagonist showed decreases in feto-placental growth during mid gestation.
Objective: To assess the effects of PTHrP silencing on the expression of growth factors in immortalized first trimester trophoblast cells (HTR-8/SVneo cells).
Methods: HTR-8/SVneo cells cultured at 37ºC and 5%CO2 in RPMI-1640 medium supplemented with 5% FBS were transiently transfected with 100nM of three different siRNA sequences of PTHrP, si1, AUACCUAACUCAGGAAACUUU; si2, GAGCUGUGUCUGAACAUCAUU; and si4, CAAGAUUUACGGCGACGAUUU. For control, a scrambled siRNA sequence was used. At 90% confluency, cells from each well were split and transfected again in triplicates with respective siRNA sequences. Total RNA was isolated using TRIzol reagent 24 h after transfection, and protein was extracted using lysis buffer 72 h after transfection. The isolated RNA and protein were subjected to reverse transcription and polymerase chain reaction (RT-PCR) and western analysis, respectively, using primers and antibodies specific for PTHrP, PLGF, VEGF and LIF. The results are expressed relative to either 18S for changes in mRNA expression or β-actin for changes in protein expression.
Results: RT-PCR of total RNA obtained from HTR cells subjected to double transfection with siRNAs for PTHrP showed a significant decrease in PTHrP expression. Expression of growth factors PLGF, VEGF and LIF showed decreases with all the three siRNA sequences used compared to the scrambled sequence. Western analysis of cell lysates obtained from HTR cells subjected to transfection with siRNAs for PTHrP showed a significant decrease in protein expression of PTHrP and VEGF. However the protein expression for PLGF, and LIF decreased in cells transfected with only si4 sequence of PTHrP.
Conclusions: Our studies showed that transient transfection of HTR cells with siRNA for PTHrP caused decreases in both mRNA and protein expression of PTHrP. Our results further suggests that decrease in PTHrP peptide in transfected cells resulted in a decrease in VEGF, PLGF and LIF suggesting that PTHrP may play role in regulating trophoblast cell functions.

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Maternal Caloric Excess: Placental TGF Beta and DNA Methylation Pathway Gene Expression Changes and Epigenesis. Ciprian P Gheorghe,[1] Joshua D Holweger,[1] Lucilla Poston,[2] Anne-Maj Samuelsson,[2] Paul D Taylor,[2] Lawrence D Longo.[1] *[1]Center for Perinatal Biology, Depts. of Physiology and Obstetrics and Gynecology, Loma Linda University, School of Medicine, Loma Linda, CA, USA; [2]Division of Reproduction and Endocrinology, King's College London, London, United Kingdom.*
Objective. Maternal obesity poses an increased risk to the fetus during pregnancy, and has long term consequences for the progeny. We tested the hypothesis that maternal caloric excess effects growth-related gene expression changes in the murine placenta.
Methods. Female C57BL/65 mice were fed a hypercaloric diet (20% fat, 38% sugar) or standard chow for six weeks prior to mating and throughout pregnancy. Near-term (day 18 gestation) the dams (4 controls, 4 overfed) were sacrificed. Following placental RNA extraction, we used the Affymetrix Mouse 430A_2.0 array to measure gene expression changes. We performed pathway analysis on regulated genes.
Results. Maternal overfeeding was associated with a two-fold increase in body fat mass. 41 probe sets, corresponding to 37 genes showed differential expression ($p < 0.01$); twenty-seven of which were up-regulated, and ten down-regulated, as compared to the placenta of control fed dams. Of note, several genes related to obesity, diabetes, DNA methylation, and the TGF Beta pathway were differentially expressed.
Conclusions. Diet-induced obesity in mice was associated with altered placental gene expression, including genes involved in TGF Beta signaling and DNA methylation pathways. These findings may have important implications for placental growth and epigenetic regulation. (Supported by USPHS HD-03807, EARNEST EU Framework 6, Tommy's the Baby Charity, UK).

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Maternal Protein Restriction: p53 Pathway Placental Gene Expression and Intrauterine Growth Restriction. Ciprian P Gheorghe, Joshua D Holweger, Lawrence D Longo. *Center for Perinatal Biology, Depts. of Physiology and Obstetrics and Gynecology, Loma Linda University, School of Medicine, Loma Linda, CA, USA.*
Objective. Maternal dietary protein restriction has been shown to have deleterious effects on placental development, and has long-term consequences for the progeny. To comprehend more completely stress responses to maternal protein restriction, we measured gene expression changes in the mouse placenta.
Methods. Pregnant FVB/NJ mice were fed an isocaloric diet containing 50% less protein than normal chow (10% vs. 20% protein content) from embryonic day 10.5 (E10.5) to E17.5. Following placental RNA extraction, we used the Affymetrix Mouse 430A_2.0 array to measure gene expression changes. We performed pathway analysis on the regulated genes, and used both qRT-PCR and immunohistochemistry to verify the results.
Results. The weights of the E17.5 pups were decreased ~15% ($p<0.05$). 244 probe sets, corresponding to 235 genes, were regulated by protein restriction ($p<0.001$); ninety-one being up-regulated and 153 down-regulated. Of particular note, several genes related to the p53 pathway were up-regulated. Along with p53 itself, positive regulators of p53 (Zmiz1, Jmy, Hipk2) and genes activated by p53 (Inpp5d, Cebpa) were induced. For selected genes we confirmed these results using qRT-PCR and immunohistochemistry.
Conclusions. By microarray analysis, we have described the genetic response to maternal protein deprivation in the mouse placenta. We observed that pups were growth restricted, and genes related to the p53 pathway were regulated. We propose a model through which intrauterine growth restriction is triggered, in part, by activation of the p53 pathway. (Supported by USPHS HD-03807 and the SGI Medical Student grant to CPG).

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Formation and Efflux of ATP-Binding Cassette (ABC) Transporter Substrate 2,4-Dinitrophenyl-S-Glutathione in Cultured Human Placental Villous Tissue. Soniya S Vaidya,[1] Scott W Walsh,[2] Phillip M Gerk.[1] *[1]Pharmaceutics, Virginia Commonwealth University, Richmond, VA, USA; [2]Obstetrics and Gynecology, Virginia Commonwealth University, Richmond, VA, USA.*
Purpose: Human placental villous tissue cultures have been underused in the study of placental drug disposition. Thus we assessed the utility of this model by studying the effect of time in culture on the viability and integrity of the tissue and the, expression and function of proteins involved in the formation and efflux of 1-chloro-2,4-dinitrobenzene (CDNB) conjugate 2,4-dinitrophenyl-S-glutathione (DNP-SG) as a model system for phase II metabolism and cellular efflux.
Methods: Placental tissue samples were obtained within 30 minutes of cesarean deliveries following normal pregnancies in three patients. Villous tissue was cultured in M199 medium to 48hr. At 2, 4, 6, 10, 24, and 48hr post culture, villous tissue was preincubated without or with ATPase inhibitor sodium orthovanadate, exposed to 100 µM CDNB, rinsed and incubated in buffer at 37°C to determine formation and efflux of DNP-SG, which was assayed by HPLC. Changes in expression of GSTP1-1, ABC transporter isoforms B1, C2 and G2 (ABCB1, ABCC2, and ABCG2, resp.) were assessed by immunoblotting. Lactate dehydrogenase (LDH) release, methyl tetrazolium thiazolyl blue (MTT) incorporation, and total tissue glutathione content were monitored up to 48hr. Villous tissue morphology was assessed by immunohistochemistry.
Results: Villous tissue structure and protein expression of glutathione-S-transferase isoform P1-1 (GSTP1-1) and ABCG2 remained unchanged over 48hr in culture. Expression of ABCB1 and ABCC2, and total tissue glutathione decreased with culture time. LDH release was unchanged up to 24hr and increased at 48hr, while MTT incorporation remained constant to 10hr and decreased at 24 and 48hr suggesting a decline in tissue integrity and viability at 48hr. However, DNP-SG formation, DNP-SG buffer/tissue ratio, and the extent of inhibition of DNP-SG efflux by sodium orthovanadate remained unchanged through 48hr. Sodium orthovanadate decreased the DNP-SG buffer/tissue ratio by 70.5 ±6.90% ($p<0.05$), consistent with inhibition of apical ABC transporters.
Conclusions: These results support the use of this model to study the coordinated function of metabolizing enzyme GSTP1-1 and apical ABC transporters in the formation and efflux of the model substrate DNP-SG. The model may be useful to study metabolism and transport of other compounds.

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Regulation of Protein Synthesis by Hypoxia in Term Human Syncytiotrophoblast. Shauna F Williams, Ewa Fik-Rymarkiewicz, Stacy Zamudio, Nicholas P Illsley. *Obstetrics, Gynecology and Women's Health, UMD-New Jersey Medical School, Newark, NJ, USA.*
Introduction: Multiple inputs influence placental protein synthesis. Nutritional, endocrine and metabolic factors have been implicated but its regulation has not

been investigated. One of the factors shown to be associated with the inhibition of protein synthesis is hypoxia. The goal of this study was to determine the effects of hypoxia on a marker of placental protein synthesis. Eukaryotic initiation factor 2α (eIF2α) is a subunit of eIF2 which is required for initiation of translation however when phosphorylated, eIF2 is unable to participate in the assembly of the initiation complex. Hypoxia has been shown previously to cause increased phosphorylation of eIF2α. We hypothesized that hypoxia would increase the levels of phosphorylated eIF2α in term syncytiotrophoblast, thus inhibiting protein synthesis. **Methods**: Primary syncytiotrophoblast cultured from term cytotrophoblast were incubated for 18 hr in atmospheres of 1, 3, 5 or 10% O_2 or in the presence of the hypoxia-mimetic, dimethyloxalylglycine (DMOG, 0.02-2.0 mM) in 20% O_2. Cell extracts were analyzed by Western blotting to determine the degree of eIF2α phosphorylation. **Results**: Incubation in 1, 3, or 5% O_2 did not increase eIF2α phosphorylation relative to the 10% O_2 control (n=4, separate placental preparations). Incubation in DMOG concentrations up to 0.5 mM did not affect eIF2α phosphorylation however incubation in 2.0 mM DMOG increased eIF2α phosphorylation by 58 ± 15% ($p < 0.05$, n=3). **Conclusions**: Contrary to our expectations, inhibition of protein synthesis via the eIF2 regulatory pathway was not apparent except when induced by the highest concentration of DMOG, consistent with severe hypoxia. Thus while eIF2α phosphorylation does occur, we did not observe changes at dissolved oxygen levels of ≥ 1%. These data suggest that a reduction in syncytial protein synthesis via the eIF2 pathway takes place only under severe hypoxic stress. *(Supported by NIH HD46982)*.

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Maternal Obesity and Over-Nutrition down-Regulates System A Amino Acid (AA) Transporter2 (SNAT2) in Ewe Placentomes. Mei J Zhu,[1] Guoyao Wu,[2] Min Du,[1] Bret W Hess,[1] Gary E Moss,[1] Peter W Nathanielsz,[1,3] Stephen P Ford.[1] *[1]Center for the Study of Fetal Programming, Department of Animal Science, University of Wyoming, Laramie, WY, USA; [2]Department of Animal Science, Texas A & M University, College Station, TX, USA; [3]Department of Obstetrics and Gynecology, University of Texas Health Sciences Center, San Antonio, TX, USA.*

Introduction: The increasing prevalence of overweight and obese women of childbearing age is a growing public health concern. The impact of maternal obesity on placental AA transport, which is essential for normal fetal development, remains poorly defined. There are three sub-types of the placental Na-dependent system A transporter, SNAT1, 2 and 4 which mediate neutral AA transport. SNAT2 is ubiquitously expressed in mammalian tissues and is likely responsible for the majority of placental system A activity.

Objective: To examine the impact of maternal obesity and over-nutrition on the fetal: maternal (F:M) AA ratio and placental protein abundance for SNAT2.

Methods: Nonpregnant ewes were randomly assigned to a control (C, 100% of NRC recommendations) or obesogenic (OB, 150% of NRC) diet from -60 to 75 days of gestation (dG). Under isofluorane anesthesia, maternal and fetal blood samples were collected for AA analysis by HPLC from five twin bearing ewes in each dietary group. After euthanasia, placental cotyledonary (COT) tissue was separated from caruncular tissue, frozen in liquid nitrogen and stored at -80^{0}C for western blot analysis.

Results: Fetuses from OB ewes were ~26% heavier ($P<0.05$) than those from C ewes at 75 dG (234± 7 vs. 186±7g). Blood concentrations of Asn, Thr, Cit, Arg, Tau, Tyr, Trp, Val, Phe, Leu and Orn were higher ($P<0.05$), or tended to be higher (Met and Lys, $P<0.10$) in OB than C ewes. In contrast, F:M ratios, for Asn, Ser, Gln, His, Gly, Thr, Cit, Arg, b-Ala, Tau, Ala, Tyr, Trp, Met, Val, Phe, Leu, Orn and Lys were reduced ($P<0.05$) in OB compared to C ewes. SNAT2 content in COT tissue was reduced in OB when compared to C ewes (0.5±0.1 vs. 1.0±0.2 Arbitrary Units; $P<0.05$).

Conclusions: Maternal obesity in pregnancy reduced expression of placental SNAT2 protein and efficiency of placental AA transport in ewes, providing a mechanism whereby fetuses may mitigate excessive delivery of AA under conditions of maternal obesity and over-nutrition. Decreased AA transport to the fetus may play a role in altered cellular structure and function. NIH INBRE 1P20RR16474.

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Early Gestation Utero-Placental Hemodynamics in an Ovine Model of Fetal Growth Restriction. Lucia Dohnal, James S Barry, Henry L Galan, Randall B Wilkening, Russell V Anthony. *Perinatal Research Center, University of Colorado Health Sciences Center, Aurora, CO.*

Objective: Fetal growth restricted (FGR) pregnancies, during late gestation, exhibit altered placental hemodynamics, and reduced capacity for O_2 and nutrient transfer. It was our objective to examine utero-placental hemodynamics and O_2 uptake during early gestation in an ovine model of FGR. **Methods**: Singleton-bearing ewes were instrumented with uterine artery flow probes, uterine venous and femoral artery catheters before being placed into a high-ambient temperature (FGR; n=9) or normothermic (CON; n=6) environment at 40 days of gestation (dGA). Maternal arterial and venous blood, uterine artery flow, heart rate, arterial pressure and respiration rate was collected until 55 dGA, at which time umbilical venous blood, fetal weight, placental weight and tissue were harvested. Data reported here were analyzed by Students T-test. **Results:** Maternal respiration rate (153.3±5.4 vs 91.6±9.2 breaths/min) and arterial PO_2 (91.0±1.2 vs 85.8±1.0 mmHg) were increased ($P\leq0.01$), whereas maternal heart rate (74.3±1.83 vs 88.8±0.03 beats/min), blood pressure (83.7±1.3 vs 94.0±3.2 mmHg) and arterial PCO_2 (30.2±1.1 vs 35.6±0.9 mmHg) were reduced ($P\leq0.01$) in FGR pregnancies. At 55 dGA, fetal weight was not different ($P\geq0.10$), but placental (total placentome) weight (85.5±15.1 vs 146.7±27.0 g) was reduced ($P\leq0.05$) in FGR pregnancies. While uterine artery (pregnant horn) flow (115.5±13.4 vs 178.4±54.3 ml/min) tended ($P=0.064$) to be reduced in FGR pregnancies, relative uterine artery flow (4.6±0.5 vs 5.8±0.5 ml/min/g fetus; 157.6±25.8 vs 128.1±17.3 ml/min/100g placenta) was not different ($P\geq0.10$). Uterine O_2 uptake (mmol/min), relative uterine O_2 uptake (ml/min/g fetus or ml/min/100g placenta) and uterine O_2 extraction (%) were not different ($P\geq0.10$) between FGR and CON pregnancies. At 55 dGA, umbilical vein PO_2 (mmHg), O_2 content (mM) and O_2 capacity (mM) were also not different between FGR and CON pregnancies. **Conclusions:** Reduction in absolute uterine artery flow (ml/min) did not impact utero-placental O_2 uptake or transfer to the umbilical vein, and may have resulted from reductions in maternal cardiac output. Relative uterine artery flow was not reduced, suggesting that uterine blood flow and delivery of O_2 to the conceptus does not mediate the ongoing placental growth restriction initiated during early gestation. Supported by NIH HD43089.

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Maternal Transplacental Supply of Mannose and Fetal Production of Inositol in Term Uncomplicated Pregnancies Using Stable Isotopes. Barton C Staat,[1] Anna Maria Marconi,[2] Cinzia Paolini,[2] Alex Cheung,[1] Henry L Galan,[1] Frederick C Battaglia.[1] *[1]Obstetrics & Gynecology and Pediatrics, Univ of Colorado at Denver & Health Sciences Center, Aurora, CO, USA; [2]Dept of Obstetrics and Gynecology, San Paolo Institute of Biomedical Sciences, University of Milano, Milano, Italy.*

Objective: To determine relative contributions of transplacental flux vs fetal production for myo-inositol and mannose in normal term pregnancies using stable isotopic methodolgy.

Background: Myo-inositol and mannose are important in biologic functions. An external supply of mannose may be required for glycoprotein synthesis. Low maternal myo-inositol is associated with spina bifida. Mannose concentrations are known to be higher in the mother than the fetus. In contrast, myo-inositol concentrations are higher in the fetus than the mother. What remains unknown is whether fetal levels of these polyols are a result of direct maternal transport or from conversion of glucose.

Design: Four term uncomplicated pregnancies undergoing an elective ceasaran section were infused with ^{13}C labeled isotopes of glucose, myo-inositol and mannose over 2 hours prior to delivery. Maternal samples were obtained prior to infusate being administered, and at 1 hour (H), 1.5H and 2H. Fetal concentrations were measured from umbilical artery and vein plasma. The concentrations of labeled and unlabeled glucose, mannose and myo-inositol were measured using High Pressure Anion Exchange Chromatogrpahy permitting detection of 10 polyols and sugars at concentrations in the μM range. The feto-maternal molar percent enrichment (MPE) ratio was calculated for each glucose, mannose, and myo-inositol as the ratio between fetal plasma enrichment and the maternal plasma enrichment at steady state. Steady state was calculated as the mean of the three maternal samples taken during infusion.

Results: The feto-maternal MPE ratios of mannose (0.985 ± 0.05, $p=0.03$) and glucose (0.917± 0.06, $p=0.002$) were not significantly different from 1.0, consistent with transplacental supply. The feto-maternal ratio for myo-inositol (0.1 ± 0.05, $p=0.02$) indicates little transplacental flux (10% of fetal inositol derived from maternal plasma).

Conclusion: In normal term pregnancies, fetal mannose and glucose concentrations are dependent upon maternal transplacental supply. In contrast, fetal myo-inositol concentration is not dependent upon transplacental supply, but fetal demands are met by placental conversion, likely from glucose.

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Differential Expression of Scavenger Receptor Class B Type-I between Arterial and Venous Placental Endothelial Cells Is Not the Result of Epigenetic Regulation. Christian Wadsack,[1] Manuela Augsten,[1] Christian Guelly,[2] Ursula Hiden,[1] Ingrid Lang,[3] Manfred Moertl,[1] Uwe Lang,[1] Gernot Desoye.[1] *[1]Clinic Ob/Gyn; [2]Center of Med Res; [3]Inst Cell Biol, Histol & Embryol, Med Univ Graz, Austria.*

Background

Placenta and fetus need lipids for growth and synthesis functions. Part of the lipids is supplied from maternal sources by transplacental transfer. Recently, we identified in human placenta the high density lipoprotein (HDL) receptor scavenger receptor Class B type I (SR-BI). Among other functions it mediates HDL-induced Ser1177-phosphorylation of endothelial nitric oxide synthase (eNOS) resulting in eNOS activation. This mechanism allows HDL to contribute to regulation of vasotonus in arteries. We hypothesized that term placental endothelial cells (EC) express SR-BI at levels different between arteries and veins.

Methods

SR-BI was localized by IHC and quantified by qRT-PCR in RNA isolated from arterial and venous vessels. Arterial (ECA) and venous (ECV) placental EC were rigorously characterized. SR-BI levels were measured by qRT-PCR and immunoblotting. HDL binding and uptake was measured in ECA and ECV with ^{125}I-labelled HDL. HDL from human donors was used to stimulate Ser1177 eNOS phosphorylation. Epigenetic regulation was studied by methylation-specific PCRs for 4 CpG-rich promoter regions of SR-BI. PDZK1 a key adaptor for SR-BI mediated eNOS activation was measured by sqRT-PCR.

Results

In situ analyses (IHC, qRT-PCR) showed more SR-BI in arteries than in the vein. The differential expression persisted in vitro in isolated ECA and ECV even after 7 passages and culture under same conditions suggesting epigenetic mechanisms regulating SR-BI. However, no methylation was found in ECA or ECV. SR-BI was functional since HDL cell association was 2-fold higher in ECA than in ECV (89 ± 0.4 vs 49 ± 4.1 ng HDL/mg prot). HDL did not induce Ser1177 eNOS phosporylation in ECA or ECV, which was stimulated by ionomycin about 3-fold in both cell types. PDZK1 was undetectable in ECA and ECV, whereas it was expressed in placental tissue.

Conclusion

More SR-BI is expressed in EC from arteries than from veins in situ and in vitro. This is not the result of different methylation of SR-BI promoter and, hence, unlikely an epigenetic phenomenon. Mechanism of differential expression and its functional consequences for vasotonus regulation is yet unknown. The lack of PDZK1 may account for the failure of HDL to activate eNOS. (grants 10053, 10896, 11165 OENB).

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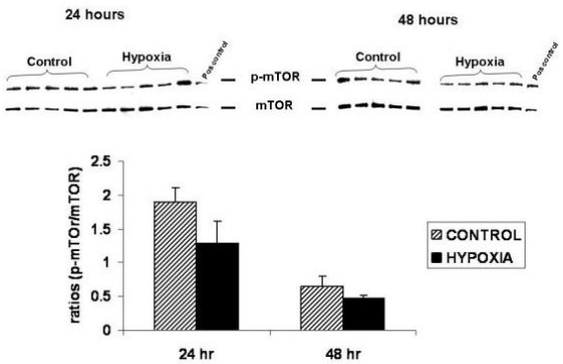
Hypoxia Does Not Affect mTOR Pathway in Cultured Human Trophoblast Cells. Juan A Arroyo, Brad Ziebell, Mi-Hye Park, Henry L Galan. *Obstetrics and Gynecology, University of Colorado andGealth Sciences Center, Aurora, CO, USA.*

Introduction: mTOR is a protein that regulates cell growth in response to nutrients and growth factors. Downstream effectors of the mTOR pathway include the p70 and the 4EBP1proteins. Activation by phosphorylation of these proteins increases protein synthesis. Given that various signaling pathways are regulated by hypoxia in human trophoblast and that mTOR is expressed in human trophoblast, our objective was to determine the effects of hypoxia in the activation of mTOR, p70 and 4EBP1 in cultured human trophoblast.

Study designs: Trophoblast cell were isolated from term uncomplicated placentas using a trypsin, DNase and disapase solution. Cytokeratin immunocytochemistry confirmed trophoblast cells culture purity. Trophoblast cells were treated with hypoxia (2% O_2) or normoxia (21% O_2) for 24 and 48 hours. Western blot for p-mTOR, mTOR, p-p70, p70, p-4EBP1, and 4 EBP1 were done for each time studied.

Results: Trophoblast cells demonstrated: 1) positive staining for cytokeratin, 2) non significant differences for mTOR at either 24 (1.5-fold; p= 0.128971) or 48 hours (1.4-fold, p= 0.13181), 3) no differences in p70 protein at 24 (1.1-fold; p= 0.128971) or 48 hours (1.3-fold, p= 0. 0.153458), 4) no differences for 4EBP1 at either 24 or 48 hour.

Conclusion: We conclude that the mTOR pathway is not regulated under hypoxic conditions in cultured trophoblast, which suggests that hypoxia does not affect protein synthesis in cultured human trophoblast. However, this may not reflect what happens *in vivo* in IUGR. (Supported by NIH grant R01 HL071990-01A1).



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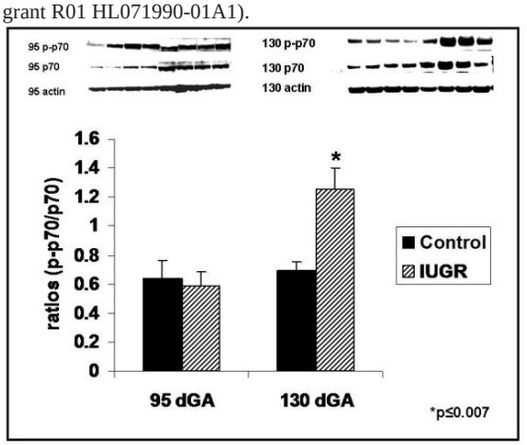
Increased Expression of Phospho-mTOR, Phospho-p70, Phospho-AKT and Phospho-ERK in an Ovine Model of Fetal Growth Restriction. Juan A Arroyo, Brad Ziebell, Henry L Galan. *Obstetrics and Gynecology, University of Colorado and Health Sciences Center, Aurora, CO, USA.*

OBJECTIVE: Both phosphorylated (p) mTOR and p70 are known to be involved in protein synthesis and are regulated by physiological conditions such as fetal growth restriction (FGR). In a hyperthermic (HT) ovine model of FGR we hypothesize that mTOR, p70, 4EBP1, ERK and AKT will be phosphorylated (activated) in the placentae of 130 age (dGA) animals.

STUDY DESIGN: 4 ewes were exposed to HT conditions for 80 days to induce IUGR and 4 were placed in ambient conditions. At necropsy (130 dGA), placentomes were separated into the maternal (caruncle) and fetal (cotyledon) components and frozen for Western blot analysis with antibodies against (p) mTOR, mTOR, (p) p70, p70, (p) 4EBP1, 4EBP1, (p) ERK, ERK, (p)AKT and AKT.

RESULTS: Compared to control animals**,** FGR animals had smaller fetuses (2914±201g v. 1718±433g; p=0.03) and smaller placentae (349±21g v. 169±22g; p=0.03) at 130 dGA. FGR cotyledon showed an increase in p-MTOR (1.8-fold; p=0.01), p-p70 (1.8-fold; p<0.008), p-ERK (1.4-fold; p<0.008) and p-AKT (2.6-fold; p<0.02). In contrast, caruncle (maternal) did not show any changes for the mTOR pathway.

CONCLUSION: In FGR ovine pregnancies, the fetal placental tissues (cotyledons) showed upregulation of the mTOR pathway for protein synthesis via phosphorylation of the p70 but not 4EBP1 while this was not seen in the maternal (caruncle) tissues. In addition neither the cotyledon or caruncle tissues at mid-gestation (95 dGA) showed changes in these endpoints, which is prior to the exponential fetal growth that starts at mid-gestation. (Supported by NIH grant R01 HL071990-01A1).



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Syncitial Uptake of Uric Acid Reduces System A Amino Acid Transport across the Human Placenta. Shannon A Bainbridge,[1,2] Denise Orlando,[1,2] James M Roberts.[1,2,3] *[1]Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, USA; [2]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA; [3]Department of Epidemiology, Univeristy of Pittsburgh, Pittsburgh, PA, USA.*

Hyperuricemia has long been recognized as a common clinical finding in preeclamptic (PE) women. To date, elevated uric acid concentrations in

these women have been considered a marker of disease severity. However preeclamptic pregnancies with hyperuricemia, are associated with an increased frequency of preterm birth and fetal growth restriction. Over the past decade several pathogenic roles for uric acid have become evident, raising the possibility of a role(s) for uric acid in the altered vascular and placental functions associated with PE.

Objective: Examine the effects of syncytial uric acid uptake on System A amino acid transport across the human placenta using a primary placental villous explant model.

Methods: Placental villous explants from placentae of healthy, term pregnancies were incubated for 2 hours with uric acid (8.3 mg/dL), corresponding to concentrations of uric acid observed in PE women. These experiments were conducted in the presence or absence of probenecid (10 μM), a uric acid cellular uptake inhibitor. System A amino acid transport was subsequently assayed using a radiochemical assay in which Na+-dependant uptake of radio-labeled System A substrate, [14C] methyl-amino-isobutyric acid, was measured over 20 minutes. Data were analyzed using a paired student's t-test and presented as mean ± SEM.

Results: Uric acid attenuated System A amino acid placental transport by 33% (±0.06%, $p<0.05$). This inhibitory effect of uric acid on System A activity was prevented by probenecid.

Conclusions: Uric acid reduces placental amino acid transport at concentrations observed in PE women. This inhibitory effect of uric acid is dependant upon syncytial uptake of uric acid, being inhibited by the uric acid transporter inhibitor probenecid. These results may be relevant to the increased frequency of fetal growth restriction observed in hyperuricemic PE. Additionally the results of this study, indicating a detrimental effect of hyperuricemia on placental function, also suggest a role for uric acid in the pathophysiology of PE.

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Uric Acid Inhibits Trophoblast Invasion through a Reconstituted Extracellular Matrix. Shannon A Bainbridge,[1,2] James M Roberts.[1,2,3] *[1]Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, USA; [2]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA; [3]Department of Epidemiology, Univeristy of Pittsburgh, Pittsburgh, PA, USA.*

Hyperuricemia, a well-documented clinical finding in preeclamptic women, is associated with pre-term birth and intrauterine growth restriction. Uric acid is higher in women destined to develop preeclampsia as early as 10 weeks of gestation at a time when cytotrophoblast are invading decidua and myometrium and remodeling uterine spiral arterioles. We propose that elevated concentrations of uric acid may have detrimental effects on placental development in part through inhibition of trophoblast invasion through the decidua.

Objective: Examine the effects of increasing concentrations of uric acid on trophoblast invasion through a reconstituted extracellular matrix.

Methods: Using the in-vitro Matrigel invasion assay, the effects of increasing concentrations of dissolved uric acid (4.5 mg/dL, 6.4 mg/dL and 8.3 mg/dL) on the ability of immortalized first trimester extravillous trophoblast cells (HTR8-svNeo) to invade through a reconstituted extracellular membrane were assessed. The concentrations of uric acid used were comparable to those measured in healthy pregnant women and preeclamptic women with an increase in uric acid of two or four standard deviations above normal. Cells that successfully invaded through the Matrigel membrane within 48 hours were fixed with methanol, stained with hematoxylin and counted. Data were analyzed using a one-way analysis of variance with fisher's post-hoc analysis.

Results: Uric acid attenuated trophoblast invasion in a dose-dependent fashion ($p<0.05$), with decreases of 29% (± 5.6%), 58% (± 3.6%) and 71% (± 2.2%) respectively compared to untreated controls.

Conclusions: Exogenous uric acid, at physiological and pathological concentrations, is capable of attenuating trophoblast invasion through a reconstituted extracellular membrane in a dose dependent fashion. These results suggest uric acid is a potential contributor to the pathophysiology of altered placental perfusion in preeclamptic pregnancies.

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Smurf2 Participates in Human Cytotrophoblast Cell Invasion during Pregnancy by Inhibiting TGF-β Type I Receptor Signaling. Baohua Zhang, Qing Yang, Haiyan Lin, Cheng Zhu, Hongmei Wang. *The State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.*

Successful embryo implantation depends on the ability of the trophoblast cells to invade the endometrium and the receptivity of the endometrium. Previous studies have shown that transforming growth factor (TGF)-β is a key inhibitory factors in the invasion of early trophoblast cells, suggesting therefore that overcoming TGF-beta signaling may be necessary for successful implantation. Smad ubiquitin regulatory factor 2 (Smurf2), a HECT type E3 ubiquitin ligase, is a key regulator of TGF-β signaling pathway, targeting TGF-β receptors and various Smads for proteasome-mediated degradation. In this context, Smurf2 has been shown to play important roles in embryonic development, cell senescence and tumor formation. As a key regulator of TGF-beta signaling, we wished to determine whether Smurf2 has a physiological role during embryo implantation, especially in trophoblast invasion. We have examined the spatio-temporal expression of Smurf2 in human placental villi during pregnancy. We have also investigated the possible function of Smurf2 in trophoblast cell migration and invasion in a model system involving a human extravillous trophoblast cell line, HTR8/Svneo. Our results showed that expression of Smurf2 in placental villi was the highest during the first trimester and the expression decreased in the 2nd trimester. Expression of Smurf2 was lowest in placental villi at parturition. Overexpression of Smurf2 in HTR8/Svneo cells reduced TGF beta type I receptor levels and attenuated the inhibitory effect of TGF-β on cell migration and invasion. Conversely, RNAi-mediated down-regulation of Smurf2 resulted in significant increase of TGF-β type I receptor protein levels. In contrast, the levels of Smad2, another potential target of Smurf2, was unchanged. In conclusion, the present study suggests that Smurf2 participates in trophoblast cell migration and invasion by down-regulating the expression of TGF-β type I receptor.

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Regulatory Effects of GnRH I and II on the Expression of MMP-26 in Human Cytotrophoblasts Involves Activation of JNK Signaling Pathway. Jing Liu,[1] Peter CK Leung,[2] Yu-xia Li,[1] Yan-ling Wang.[1] *[1]State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China; [2]Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, BC, Canada.*

Matrix metalloproteinase 26 (MMP-26) is the smallest member in MMP family. Our previous data demonstrated the extensive expression of MMP-26 in various kinds of trophoblast cells in human placenta at the early pregnancy. However, the modulation of the enzyme in trophoblasts is largely unclear. In the present study, the effects of the two types of gonadotropin releasing hormone (GnRH) on MMP-26 expression were examined in an immortalized human cytotrophoblast cell line, B6Tert-1 that has been established in this lab. Real-time quantitative PCR and Western blot analysis revealed that both types of GnRH (GnRH I and GnRH II) could increase MMP-26 mRNA and protein levels in B6Tert-1 cells in time-dependent manners. In particular, regulatory effect of GnRH I on MMP-26 expression was concentration-independent, whereas that of GnRH II was dose-dependent. Moreover, both GnRH I and GnRH II could evidently activate JNK kinase, and SP600125, an inhibitor of a JNK kinase, reversed the up-regulation of MMP-26 induced by either GnRH I or GnRH II. On the other hand, it is not likely that ERK1/2 pathway participates in the signaling of GnRH I or GnRH II. Collectively, our observations suggest that GnRH I and GnRH II elicit their modulation effects in human trophoblastic cells through JNK pathway leading to up-regulation of MMP-26.

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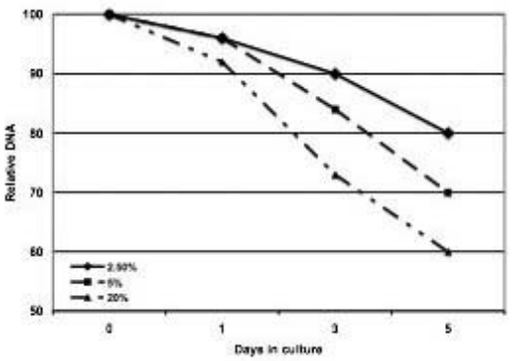
Hypoxia Restores Proliferation of Third Trimester Trophoblast Cells. Jared C Robins, Sandra A Carson. *Department of Obstetrics and Gynecology, Women and Infants Hospital/Warren Alpert School of Medicine at Brown University, Providence, RI, USA.*

During the first trimester of pregnancy, the oxygen tension of the developing trophoblast cells is less than 5%. However, the majority of studies on primary trophoblast cell development have been performed at 20% oxygen. Primary third-trimester trophoblast cells are believed to be nonproliferative syncytiotrophoblast cells. We have previously demonstrated that low oxygen tension dramatically affects the differentiation pathway of these cells. We now hypothesize that cell culture in low oxygen tension will improve cell growth and restore proliferation.

Methods: Primary trophoblast cells were purified from third-trimester placenta by enzymatic dispersion and CD-9 negative selection and cultured at 20%, 5% or 2.5% oxygen tension for up to 5 days. The number of cells in culture was assessed by cell counting and by measuring genomic DNA. Live:dead and MTT assays were used to determine viability. Proliferation was assayed with BrDU and immunohistochemistry for proliferating cell nuclear antigen. To assess cellular activity, radioactivity of protein precipitated from cells cultured in the presence of tritiated leucine was measured.

Results: There were no obvious morphologic changes in the cells cultured in different oxygen tensions. The amount of cell loss was directly proportional to oxygen tension: at 20% oxygen 60% of the cells remain in culture; at 2.5% oxygen tension 80% of the cells remained. The cells at 2.5% oxygen tension were proliferating and had a five-fold increased metabolic activity.
Conclusions: It was previously believed that third-trimester trophoblast cells are non-proliferative. We have demonstrated that low oxygen tension increases the survival of primary third-trimester trophoblast cells. This may reflect the change in the differentiation pathway of these cells. However, the cells also begin to proliferate and increase their metabolic activity.



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Repetitive Trophoblast Vessicle Assembly from Disagreggated Cells in a Novel 3-D Culture System. Jared C Robins,[1] Paula Krueger,[1] Jeffrey R Morgan,[2] Sandra A Carson.[1] *[1]Department of Obstetrics and Gynecology, Women and Infants Hospital/Warren Alpert School of Medicine at Brown University, Providence, RI, USA; [2]Department of Molecular Pharmacology, Physiology and Biotechnology, Brown University, Providence, RI, USA.*
Trophoblast cells in vivo form a three dimensional structure which promotes critical complex cell-to-cell interactions that cannot be studied with traditional monolayer cell culture. We developed a substrate-free three-dimensional trophoblast culture system capable of studying cellular interactions without a confounding artificial matrix.
Methods: Nonadhesive agarose hydrogels containing 822 cylindrical recesses 200µm in diameter were cast from molds designed using computer-assisted prototyping. TCL trophoblast cells were seeded into the gels (800,000 cells per) for up to 10 days. Viability and cellular stress were assessed and the three-dimensional structures of the spheroids were analyzed.
Results: TCL trophoblast cells formed uniform spheroids within three days of seeding. The spheroids remained intact after being removed from the mold. When placed in traditional cell culture dishes the cells adhered to the plate within one hour and rapidly proliferated into a monolayer. Repetitive reseeding allowed easy transition between monolayer and spheroid without affecting cellular morphology. Serial sectioning on days 3, 7 and 10 revealed central vacuolization forming a trophoblast vesicle with an outer rim 12.3µm (+/- 1µm) thick. This rim size remained constant for at least 20 days. Live:dead assay demonstrated that the outer cells remained viable and staining against proliferating cell nuclear antigen demonstrated that the cells were proliferating. The inner cells undergo apoptosis as demonstrated by caspase-3 staining. There is an abundance of VEGF staining in the cells remaining in the on the inside of the sphere suggesting a gradient of nutrient or oxidative stress. The formation of a vesicle has been confirmed with confocal imaging. EM imaging revealed the structure of the rim.
Conclusions: Trophoblast cells cultured in a novel substrate-free three dimensional system form trophoblast vesicles within 7 days of seeding. These vesicles remain viable after long-term culture and can be repeatedly reformed with repetitive seeding. This new cell culture technique allows us to better study placental cell-cell interactions with the potential of forming microtissues.

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GCM1 Mediates TIMP4 Expression in BeWO and First Trimester Trophoblast Explants. Sascha Drewlo,[1] Dora Baczyk,[1] Craig Pennell,[2] Caroline Dunk,[1] Isabella Caniggia,[1] John Kingdom.[1] *[1]Program in Development and Fetal Health, Deptartment of Obstetrics & Gynaecology Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [2]University of Western Australia, Perth, Australia.*
The transcription factor Glial Cell Missing-1 (GCM1) mediates cell cycle arrest and differentiation of human trophoblast progenitors into villous syncytiotrophoblast and invasive extravillous cytotrophoblast (EVT). Micro-array analysis of total RNA extracted from cultured BEWO cells, in which GCM1 mRNA and protein were repressed using siRNA, identified tissue inhibitor of metalloproteinase-4 (TIMP-4) in the highest (4-fold) upregulated group of genes. Confirmatory rtPCR demonstrated a 7-fold mRNA induction.
In placental villi, GCM1 acts as a transcription factor promoting expression of the fusogenic protein syncytin1 that mediates syncytial fusion into the overlying syncytiotrophoblast. By contrast, syncytial fusion is uncommon in EVT. Rather these cells invade several millimeters into the distal myometrium where they transform spiral arterioles.
To investigate the role of TIMP4 and GCM1 in the trophoblast we assessed its mRNA by qRT-PCR and protein by Western blot in cellular extracts from both BeWO cells grown under standard cultivation conditions (synchronized by prior thymidine exposure) and floating cultured first trimester villous explants cultured in 8% oxygen with prior exposure to either GCM1 siRNA or anti-sense oligo-nucleotides to GCM1.
GCM1 inhibition in the BeWo system was associated with a 50-70% increase in TIMP-4 protein expression and alteration of cell proliferation and differentiation in both models. We are presently utilizing the explant model of EVT invasion (explant tips cultured on Matrigel in 3% oxygen) to test the hypothesis that GCM1 mediates metallo-proteinase expression and EVT invasion via TIMP-4.
Presently we conclude that GCM1-mediated EVT differentiation involves more than an arrest of mitosis and may include promotion of invasion via repression of TIMP-4.
Funding: CIHR.

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Examination of Periattachment Factor (PRR15) in the Human Placenta. Scott H Purcell,[1] Jeremy D Cantlon,[1] Virginia D Winn,[2] Russell V Anthony.[1,2] *[1]Colorado State University, Fort Collins, CO; [2]University of Colorado Health Sciences Center, Aurora, CO.*
Background: Periattachment factor (PF) is a nuclear protein first described in the bovine conceptus. Our research in sheep has shown PF mRNA concentration peaks when the conceptus is undergoing elongation and initial apposition to the endometrium, and that PF is a nuclear protein localized to the trophoblast. *In silico* analysis identified a human homolog, hPRR15. **Objective:** The objective of this experiment was to determine if PF was expressed in the human placenta, and to develop short-hairpin (sh) RNAs for hPF to begin investigating its function. **Materials and Methods:** Immunohistochemistry was performed on paraffin embedded first and second trimester human placental samples. Placental sections were immuno-stained using rabbit polyclonal anti-ovine PF or anti-human cytokeratin-7. Cytotrophoblasts from first trimester pregnancies (n=5) were subjected to an *in vitro* invasion assay and RNA was harvested following 0, 3, and 12 h. Quantitative RT-PCR was performed on these samples with intron-spanning primers for hPF, and normalized on hS15 mRNA concentrations. Based on the human PF sequence, four putative shRNA constructs were generated and cloned into a lentiviral expression vector. BeWo human choriocarcinoma cells were treated with one of four shRNA contructs or an empty vector for 72 h and then RNA was harvested from cells for analysis by quantitative RT-PCR. **Results:** Periattachment factor was present in the nuclei of both first and second trimester cytotrophoblasts. hPF mRNA concentration increased as invasion occurred from 0, 3, to 12 h in all samples; while hypoxia decreased expression at 18 h of invasion compared to 18 h under normoxic conditions. The four lentiviral vectors expressing shRNA against hPF resulted in hPF mRNA concentrations at 2, 18, 83 and 97% of hPF mRNA concentration with the control vector. **Conclusion:** The presence of PF in the human placenta and the increase in PF mRNA during cytotrophoblast invasion may indicate this gene plays a role during implantation. We have developed shRNAs against PF that result in greater than 98% mRNA knockdown and will be using these to begin to elucidate the function of PF in the human placenta, specifically during the invasion process. This project was supported by National Research Initiative Competitive Grant no. 2005-35203-15885 from the USDA Cooperative State Research, Education, and Extension Service.

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Effect of Intermedin (IMD) on the Migration of First Trimester Trophoblast (HTR-8SV/neo) Cells. Madhu Chauhan, Chandra Yallampalli. *Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
BACKGROUND: Intermedin (IMD)/Adrenomedullin (AM) 2 is a novel member of the calcitonin/calcitonin gene-related peptide (CGRP) family. IMD

is emerging as a multifunctional peptide involved in variety of physiological functions such as blood pressure regulation and feto-placental growth. Recently we demonstrated that infusion of IMD antagonist (IMD_{17-47}) in rat caused distorted labyrinth indicative of a deficient vasculature in placenta. We hypothesize that IMD has a role in migration of first trimester trophoblast cell (HTR-8/SVneo) via regulating human leukocyte antigen (HLA-G) and stimulating MEK1/2/ ERK1/2 phosphorylation.

OBJECTIVES: 1) To asses the effect of IMD on migrating capacity of HTR-8sv/neo cells using scratch assay in presence or absence of MEK and RAS/RAF inhibitor, U0126 and manumycin A, respectively ; 2) to assess the effect of IMD peptide on phosphorylation of MEK1/2 and ERK1/2 in first trimester HTR cells 3) to analyze the effects of IMD on the expression of human leukocyte antigen, HLA-G, a critical factor involved in the invasion and vascular remodeling of spiral uterine arteries and subsequent pregnancy in human.

METHODS: HTR-8SV/neo cells were used to assess the effect of IMD (10^{-8} M) on the expression of HLA-G mRNA and phosphorylation of ERK 1/2and MEK1/2 protein by Reverse transcriptase polymeration chain reaction (RT-PCR) and western blot analysis respectively. Scratch wound assay was used to determine the migration capacity of HTR cells. Total RNA was isolated from cells using Trizol reagent and processed for RT-PCR and results are expressed relative to 18S mRNA. Trichloroacetic acid was used for the extraction of total protein for western blot analyses.

RESULTS: Our data demonstrates that, 1) IMD enhances the migrating capacity of HTR cells (compared to the untreated cells) and these effects are inhibited by MEK and RAS/RAF inhibitors, U0126 and manumycin A, respectively; 2) IMD (10^{-8}M) stimulates phosphorylation of ERK1/2 and MEK1/2 proteins in HTR cells, 3) IMD increases the expression of HLA-G mRNA in HTR cells.

CONCLUSION: IMD promotes migration of first trimester HTR cells through MEK/ ERK signaling pathway and modulates the expression of immuno-regulatory molecule, HLA-G in these cells.

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Chymotrypsin-Like Protease Promotes the Placenta Tissue Release of sFlt-1. Yang Gu, Shuang Zhao, David F Lewis, Yuping Wang. *Obstetrics and Gynecology, LSUHSC-Shreveport, Shreveport, LA, USA.*

Objective: The placenta is a major source of soluble VEGF receptor-1 (sFlt-1) in the maternal circulation during pregnancy. Increased placental release of sFlt-1 is believed to play an important role in the pathophysiology and pathogenesis in PE. However, the mechanism of increased placental sFlt-1 release in PE is unknown. We recently reported increased chymotrypsin-like protease (CLP) activity and expression in placental trophoblasts from PE. In this study, we tested if proteolytic effects of chymotrypsin may play a role in promoting sFlt-1 release by placental trophoblasts.

Methods: Placentas delivered by normal pregnant women (n = 5) were used. We tested if chymotrypsin could promote sFlt-1 release by placental tissue, in which villous explants were cultured with DMEM containing chymotrypsin at 1.0, 2.5, and 5.0 μg/ml for 6 hours. The culture medium was then collected for measuring sFlt-1. We then determined the specificity of chymotrypsin induced sFlt-1 release. Villous tissues were cultured with or without chymotrypsin inhibitor (CI) in culture and then the medium was collected and measured for sFlt-1. Soluble Flt-1 was measured by ELISA. All samples were assayed in duplicate. Data are presented as mean ± SE and analyzed by ANOVA. A p level < 0.5 is considered as statistically different.

Results: 1) sFlt-1 concentrations in the medium were increased when chymotrypsin was present in culture and the increased sFlt-1 release induced by chymotrypsin was in a concentration-dependent manner: control: 8.25±1.39; 1.0μg/ml: 10.51±1.89; 2.5 μg/ml: 13.03±2.06; and 5.0μg/ml: 16.12±2.23 ($p<0.01$) pg/mg tissue/hour. 2) CI could attenuate sFlt-1 release. This inhibitory effect was also revealed in a concentration-dependent manner: control: 5.67±0.92; CI 0.5μg/ml: 4.96±1.30; and CI 5.0μg/ml: 3.08±0.47 ($p<0.05$) pg/mg tissue/hour.

Conclusions: Increased placental sFlt-1 release stimulated by chymotrypsin and decreased placental sFlt-1 release inhibited by chymotrypsin inhibitor suggest that the proteolytic effect of CLP may play a role in sFlt-1 generation. Therefore, increased CLP activity in placental trophoblasts may contribute to the increased placental sFlt-1 production in PE. (Supported NIH grants HL65997 and HD36822).

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The Change of Autophagy-Related Proteins, LC3 and Beclin-1, by TNF-a Stimulation in Cultured Primary Trophoblasts. Soo-young Oh, Kyung Hee Kim, Suk-Joo Choi, Jong-Hwa Kim, Cheong-Rae Roh. *Department of Obstetrics and Gynecology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.*

Objective: Our previous work have demonstrated that the expression of LC3, but not beclin-1, was increased in placentas from pregnancies complicated by severe preeclampsia (SGI 2007abstract #209). To understand the regulatory mechanism of these autophagy-related proteins in trophoblast cells, we investigated the changes in these proteins in response to cytokine or hypoxic stimulation in cultured primary trophoblast.

Material and methods: Primary human cytotrophoblasts obtained from normal term placenta were cultured with stimulation of TNF-a or $CoCl_2$ for a given time and the changes of beclin-1 and LC3 were assessed using immunoblot analysis. Paired T test was used for statistic analysis.

Results: TNF-a stimulation induced a significant increase of the expression of LC3-II in cultured primary trophoblasts while decreasing the expression of beclin-1 ($p<0.05$ for each). However, $CoCl_2$ stimulation did not induce a significant change of both LC3-II and beclin-1.

Conclusions: Our data suggests that TNF-a stimulation in cultured primary trophoblasts is associated with increased autophagic activity.

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Monocarboxylate Transporter 8 (MCT8) Represses Proliferation of Fetal Embryonal Neuronal NT2 and Placental/Choriocarcinoma JEG-3 Cells *In-Vitro*. Sally R James,[1,2] Jayne A Franklyn,[1] Vicky E Smith,[1] Elisabet Vasilopoulou,[2] Shiao Y Chan,[2] Margaret C Eggo,[1] Tim G Barrett,[2] Mark D Kilby,[2] Christopher J McCabe.[1] *[1]Division of Medical Sciences, University of Birmingham, Birmingham, West Midlands, United Kingdom; [2]Division of Reproduction & Child Health, University of Birmingham, Birmingham, West Midlands, United Kingdom.*

Background: Thyroid hormones play vital roles in the development of the fetal brain. Mutations in MCT8, recently recognised as a specific thyroid hormone transporter, define a novel syndrome of severe X-linked psychomotor retardation accompanied by elevated serum T3. We previously reported that MCT8 expression in N-TERA-2 (NT2) cells (a human embryonal cell line with characteristics of CNS precursors), as well as MCT8-null JEG-3 choriocarcinoma cells, resulted in markedly reduced cell proliferation. Further, the S448X MCT8 mutation, as reported in males affected by severe psychomotor impairment, resulted in a similar repression of proliferation to wild type, whereas the L471P mutant failed to influence cell turnover compared with control.

Methods: We now examine the effect of "knocking down" MCT8 via siRNA and evaluate the effects of cell proliferation (MTT and [^{3}H]-thymidine assays) and tri-iodothyronine (T_3) uptake.

Results: Repression of endogenous MCT8 expression in NT2 cells by ~90 % caused a significant increase in proliferation compared to matched-dose non-specific siRNA treatment, independent of T_3 concentration (8.6 %, 7.2 % and 8.3 % induction at 0, 10 and 50 nM T3, n=3, $p<0.02$). We also sought to examine the role of MCT8 in T3 uptake. In JEG-3 cells, wild type MCT8 induced a 1.7-fold increase in the uptake of ^{125}I-labelled T_3. By contrast, mutants S448X and L471P failed to significantly augment T_3 uptake, though R271H caused a mild but significant 1.17 fold induction in uptake, hence retaining approximately 25% of WT activity. In parallel experiments, co-transfection of mu-crystallin, a T_3 binding protein, resulted in a similar increase in T3 uptake compared with control (1.9-fold; n=6; $p<0.001$), implying that MCT8 plays only a minor role in thyroid hormone efflux in JEG-3 cells. Mutants S448X, L471P and R271H showed analogous responses to those in the absence of mu-crystallin.

Conclusion: These results further extend the evidence of a potential role for MCT8 in the modulation of cell proliferation, independent of T_3 transport. (Supported by Health Foundation, MRC (UK) & Birminghan Children's Hospital Research Foundation).

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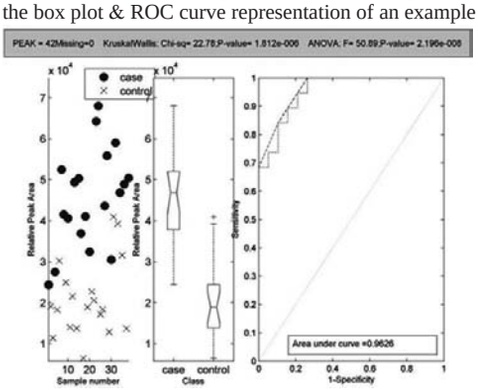
The Application of UPLC/LTQ-Orbitrap Mass Spectrometry to Human Metabolite Biomarker Discovery. A Case Study: Pre-Eclampsia. David I Broadhurst,[1] Marie Brown,[1] Warwick B Dunn,[1] Kathleen B Carroll,[1] Christopher W Redman,[4] Philip N Baker,[3] Douglas B Kell,[1] Louise C Kenny.[2] *[1]The Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, University of Manchester, Manchester, United Kingdom; [2]Department of Obstetrics and Gynaecology, University College Cork, Cork, Ireland; [3]School of Medicine and Manchester Interdisciplinary Biocentre, University of Manchester, Manchester, United Kingdom; [4]Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Oxford, United Kingdom.*

Background: The influence of disease, pharmaceuticals and the environment on human systems is increasingly being studied at the functional level of the metabolome using data-driven approaches. Metabolic profiling is applied to identify biomarkers characterising disease progression, drug intervention/ toxicity, and environmental stress or to investigate the aetiology or biological activity of disease or drug interventions. These studies require powerful analytical technologies to enable the accurate and precise detection of 100-1000s of chemically identified metabolites in the concentration ranges pM to mM.

Pre-eclampsia is a multi-system disorder of pregnancy. Although the aetiology of the disease is unclear there is evidence to suggest that incorrect adaptation of the interface between the maternal vasculature and the developing placenta in early pregnancy leads to the development of a poorly perfused feto-placental unit. This poor adaptation will occur long before any clinical presentation of the disease. Any therapeutic intervention will need to be introduced early in pregnancy, thus inevitably early indicative biomarkers must also be found. A potential source of early biomarkers could be metabolite levels in blood plasma.

Hypothesis: A small case-control study (20 case; 20 control) to determine biomarkers indicative of pre-eclampsia employing UPLC-LTQ/Orbitrap analytical tools is discussed.

Results: Statistical analysis using a modified critical p-value of 0.001 (to account for multiple testing) revealed over 100 significant peaks. Figure shows the box plot & ROC curve representation of an example 'good' metabolite.



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Preeclampsia and Predictors of Labor Induction Failure. Lena H Kim,[1] Anjali J Kaimal,[2] Yvonne W Cheng,[2] Shani Delaney,[2] Angie Child,[2] Aaron B Caughey.[2] *[1]Obstetrics and Gynecology, Kaiser Permanente, Hayward, CA, USA; [2]Obstetrics, Gynecology and Reproductive Sciences, UCSF, San Francisco, CA, USA.*

Objective:

To determine predictors of failure for labor induction in women with preeclampsia.

Study Design:

We conducted a retrospective cohort study to examine cesarean delivery rates in all the preeclamptic women at a single institution undergoing labor induction between 1987-2001 with a singleton pregnancy >=24 weeks gestational age (GA). Bivariate analyses informed the creation of multivariable logistic models to predict the risk of cesarean delivery using multiple predictors (maternal age, race/ethnicity, unfavorable cervix, gestational diabetes, diabetes, and gestational age). Analyses were stratified by parity.

Results:

Our study population included 1,123 preeclamptic women undergoing labor induction. In the bivariate analyses, the risk of cesarean delivery ranged from as low as 10.5% (p=0.01) among multiparous women 24-28 weeks GA to as high as 70.8% (p<0.01) among nulliparous women with diabetes. A total of 1,019 women had adequate data to be included in the multivariable analyses. Odds ratios of the predictors are presented in the table below.

Risk of cesarean delivery (OR, 95% CI)

	Nulliparous (n=725)	Multiparous (n=294)
Maternal age >35	2.03 (1.29 - 3.20)	2.24 (1.18 - 4.28)
Asian	1.28 (0.84-1.93)	1.17 (0.51 - 2.69)
Hispanic	1.11 (0.72 - 1.72)	1.57 (0.69 - 3.55)
African American	1.00 (0.62 - 1.61)	3.23 (1.45 - 7.20)
Unfavorable cervix	1.19 (0.86 - 1.65)	2.36 (1.28 - 4.37)
GDM	1.20 (0.63 - 2.27)	0.88 (0.34 - 2.27)
Diabetes	5.22 (2.08 - 13.07)	2.42 (0.71 - 8.33)
GA 24 - 27	0.85 (0.29 - 2.46)	0.57 (0.12 - 2.81)
GA 28 - 31	2.19 (1.20 - 4.00)	2.97 (1.34 - 6.56)
GA 32 - 37	0.85 (0.59 - 1.24)	1.23 (0.64 - 2.39)

Conclusion:

Preeclamptic women undergoing labor induction have a higher risk of cesarean delivery if maternal age >35 years, diabetic and 28-31 weeks GA if nulliparous; and if maternal age >35, 28-31 weeks GA, African American and unfavorable cervix if multiparous. These factors can be utilized to adjust pre-induction probability of success in a labor induction in the setting of preeclampsia.

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Elevations in Cardiovascular Risk Factors Persist Months Postpartum after Preeclampsia. Robert W Powers,[1,2] Marcia J Gallaher,[1] Dan Plymire,[1] James M Roberts.[1,2,3] *[1]Magee-Womens Research Institute; [2]Obstetrics & Gynecology; [3]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA.*

Objective: Preeclampsia and cardiovascular disease share many risk factors, and women with preeclampsia are at increased risk of cardiovascular mortality later in life. We investigated whether risk factors associated with cardiovascular disease and preeclampsia remain elevated months postpartum.

Methods: We measured plasma sFlt1, endoglin, PlGF, cellular fibronectin (cFN), uric acid, homocysteine, and asymmetric dimethylarginine (ADMA) in 30 women with uncomplicated normotensive pregnancies compared to 20 women with preeclampsia in samples collected at pre-delivery and again several months postpartum (average 10.6±6.2 months). Data are mean±SD or median (interquartile range). Statistical analysis was by Wilcoxin rank-sum or Students unpaired t-tests with statistical significance accepted at p<0.05.

Results: The mean concentration of sFlt1, endoglin, PlGF, homocysteine, ADMA, cFN, and uric acid were all significantly different in samples collected pre-delivery in subjects with preeclampsia compared to controls (table). ADMA, cFN and uric acid remained significantly higher postpartum in subjects with previous preeclampsia compared to postpartum controls (table).

Conclusions: Biological markers associated with altered vascular function or cardiovascular risk are elevated in women with preeclampsia, and some remain significantly higher in postpartum preeclamptic women. These data suggest that vascular dysfunction persists in women with previous preeclampsia, and may contribute to the increased risk of future cardiovascular disease. Funded in part by National Institutes of Health NIH-5MO1-RR00056 and NIH-2PO1-HD30367.

	Pre-delivery Controls	Pre-delivery Preeclampsia	Postpartum Controls	Postpartum Preeclampsia
sFlt1 (pg/ml)	3977.8 (467.4-6215.2)	23802.8 (8634.7-36778.2)*	51.5 (45.1-62.7)	53.9 (48.5-60.2)
Endoglin (ng/ml)	8.7 (5.9-12.2)	66.5 (34.2-96.3)*	5.6 (5.1-6.1)	5.8 (4.9-6.4)
PlGF (pg/ml)	90.7 (3.1-206.8)	31.4 (21.6-66.6)*	6.6 (5.4-8.4)	8.0 (6.6-9.8)
Homocysteine (µmol/L)	4.3±1.7	6.1±2.9*	6.4±1.8	6.5±1.7
ADMA (µmol/L)	0.45±0.10	0.73±0.36*	0.53±0.09	0.67±0.17*
cFN (µg/ml)	41.1±21.6	86.4±44*	30.3±17.1	43.8±26.1*
Uric acid (mg/dl)	4.8±0.9	6.7±1.4*	4.5±1.2	5.3±0.8*

Data are mean±SD or median (IQR), *= statistically different compared to respective gestational age control.

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Resistance to the Angiogenic Effects of Leptin in Blood Vessels from Maternal Food Restricted Offspring Is Secondary to Leptin Signaling Defects. Omid Khorram, Guang Han, Glenda Calvario, Mina Desai, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: We have demonstrated that maternal food restriction (MFR) inhibits angiogenesis in neonatal offspring (AJP, 2007). As leptin is a known potent angiogenic factor we hypothesized that leptin deficiency and/or resistance to leptin-induced VEGF expression might be a mechanism for reduced angiogenesis in MFR offspring.

Methods: Pregnant Sprague-Dawley rats had 50% MFR from day 10 of gestation until delivery. MFR and Control offspring were sacrificed on day 1

of life (p1). Some tissues were used to determine the expression of leptin by Western blot analysis. For culture experiments, thoracic aortas were dissected, cut into 1-2 mm explants and incubated with leptin (25-100ng/ml) In DMEM (10% FBS). After 24 hours of culture, RNA was extracted from the tissues and subjected to real time RT-PCR using specific rat primers for VEGF, VEGFR1 and R2, and Ob-Ra, STAT3 and SOCS3 (18S mRNA as control). Culture media was analyzed for VEGF protein by ELISA.

Results: Expression of leptin mRNA and protein in p1 MFR aortas was significantly reduced. In culture, leptin significantly increased expression of VEGF, VEGFR1 and VEGFR2 mRNA in explants of aortas obtained from the control but not MFR tissues. As expected, control but not MFR aortic explants secreted significantly more VEGF in vitro. To determine the mechanism for resistance to leptin-induced VEGF in MFR offspring, we assessed expression of leptin receptor (Ob-Ra) in explants treated with leptin. Leptin was found to induce the expression of Ob-Ra in aortas from both dietary groups. This upregulation of leptin receptor was accompanied by significant upregulation of STAT3 and SOCS3 mRNA in the control tissues. In contrast, in MFR explants only the 50ng/ml concentration of leptin induced an increase in STAT3 mRNA, and the magnitude of SOCS3 mRNA increase by both concentrations of leptin was significantly less in the MFR explants.

Conclusion: These results indicate that reduced angiogenesis in MFR vessels is in part due to reduced leptin expression and ability of leptin to stimulate VEGF expression. Although in vitro leptin induced the expression of its receptor in both groups, it was only in the MFR group in which leptin up-regulated VEGF and its receptors. Our results suggest that this defect in leptin receptor function in MFR vessels is due, in part, to defects in JAK/STAT signaling.

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Classic Risk Factors Predictive of First Cardiovascular Events in Women with a History of Early-Onset Preeclampsia: Opportunities for Primary Prevention. Bas B van Rijn,[1] Hein W Bruinse,[1] Michiel L Bots,[2] Hieronymus A Voorbij,[3] Mark Roest,[3] Arie Franx.[4] *[1]Perinatology & Gynecology, University Medical Center Utrecht, Utrecht, Netherlands; [2]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, Netherlands; [3]Clinical Chemistry & Hematology, University Medical Center Utrecht, Utrecht, Netherlands; [4]Obstetrics & Gynecology, St Elisabeth Hospital, Tilburg, Netherlands.*

Objective: Women with a history of early-onset preeclampsia are at increased risk of developing major cardiovascular disease (CVD) related events, that have a detrimental effect on their long-term health and life expectancy. In this follow-up study, we measured established risk factors predictive of first CVD events after early-onset preeclampsia.

Study design: Over a 10-year interval, 243 primiparous women with a history of early-onset preeclampsia (delivery <34 weeks gestation) were included and tested for major cardiovascular risk factors at least six months after delivery, in addition to a population-based control group of 374 healthy non-pregnant women. Women with chronic hypertension were excluded.

Results: Mean age was 30.5 years for cases compared to 28.3 years for controls (P<.001). After adjustment for age, we observed significantly increased mean values for weight (P=.002), body-mass index (P<.001), systolic blood pressure (P<.001), diastolic blood pressure (P<.001), total cholesterol (P=.006), LDL cholesterol (P<.001), triglycerides (P=.027), fasting blood glucose (P<.001), and lower HDL cholesterol (P<.001) in women with previous early-onset preeclampsia. No difference was found for height, smoking, diabetes, and ethnicity. Estimated 10-year risk of first CVD events by Framingham Risk Scores remained <10% for all women (low-risk). Nonetheless, at mean (SD) 0.7 (1.0) years after early-onset preeclampsia, 15% of women met the criteria for metabolic syndrome, 89% of women exhibited >=1, 51% of women >=2 and 19% of women >=3 major CVD risk factors.

Conclusion: The majority of women with a history of early-onset preeclampsia exhibit at least one modifiable risk factor for future CVD. Although most of these women are classified as low-risk according to the current AHA guidelines, this is mainly due to their young age masking other, mostly modifiable, major risk factors. Our data thus support life-style intervention programs aimed at primary prevention of CVD in women with a history of early-onset preeclampsia.

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Effect of Antihypertensive Treatment on Activin A and Inhibin A Levels in Women with Hypertensive Disorders in Pregnancy. Asma Khalil,[1] Shanthi Muttukrishna,[2] Nigel Groome,[3] Kevin Harrington,[1] Eric Jauniaux.[2] *[1]Obstetrics, Queen Mary, University of London, London, United Kingdom; [2]Obstetrics, University College London Hospitals, London, United Kingdom; [3]School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, United Kingdom.*

Introduction

It has recently been shown that antihypertensive drugs can stimulate cytokine release in normal and hypertensive pregnancy. There is evidence that these cytokines alter the secretion of inhibin A. Inhibin A and activin A levels are increased in pre-eclampsia (PE), but it is not known if antihypertensive therapy can affect their secretion.

Patients and methods

We recruited 129 women with hypertensive disorders in pregnancy (63 PE and 66 non-proteinuric hypertension [HT]) and 129 matched normotensive controls. Inhibin A and activin A levels, before and 24-48 hours after initiating antihypertensives, were measured in serum and urine, using an ELISA. The same markers were measured using validated assays in 84 placentas delivered at cesarean section at similar gestational age (29 PE, 24 HT and 31 controls).

Analysis

The three study groups were compared using ANOVA multiple comparisons with Bonferroni post hoc testing. The data were normally distributed after logarithmic transformation. Marker levels before and after antihypertensive therapy were compared using paired t-test. We compared placental concentrations between the group which received antihypertensive therapy and the group which did not, using an independent t-test. Data were analysed using SPSS®.

Results

In PE, both serum and urine levels of inibin A and activin A were increased at all gestations (p<0.0001). Activin A (but not inhibin A) level was also increased at all gestations in HT (p<0.0001). After 28 weeks' gestation (but not before), antihypertensive treatment was associated with a significant fall in both inhibin A and activin A serum levels, and urinary inhibin A, in both PE and HT. The placental concentration of inhibin A, but not activin A, was significantly higher in women with PE compared with controls (p<0.0001). There was no significant difference in either marker between controls and women with HT.

Conclusion

The fall in serum levels of inhibin A and activin A following antihypertensive treatment after 28 weeks' gestation may indicate that these drugs have an effect on the pathophysiology of PE other than their known antihypertensive action.

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Angiogenic and Anti-Angiogenic Factors in the Placental Tissue of Normotensive Pregnant Women and Women with Hypertensive Disorders in Pregnancy. Asma Khalil,[1] Shanthi Muttukrishna,[2] Kevin Harrington,[1] Eric Jauniaux.[2] *[1]Obstetrics, Queen Mary, University of London, London, United Kingdom; [2]Obstetrics, University College London Hospitals, London, United Kingdom.*

Introduction

Pre-eclampsia (PE) is a placental disease of unknown etiology. Anti-angiogenic factors, such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng), and pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), are believed to play an important role in its pathophysiology. Maternal plasma concentrations of these markers are altered in PE, even weeks before the clinical manifestations. The aim of this study was to compare the concentration of these markers in placental extracts of normotensive pregnant women, and women with PE and non-proteinuric hypertension (HT).

Patients and methods

Placental samples were collected at cesarean section from women with PE (n = 29), HT (n = 24) and normotensive pregnancies of similar gestational age (n = 32). These samples were stored at – 80ºC. The frozen tissue samples were homogenised and these four markers measured by specific, validated enzyme-linked immunosorbent assays.

Analysis

The three study groups were compared using ANOVA multiple comparisons with Bonferroni post hoc testing. The data were normally distributed after logarithmic transformation. Data were analysed using SPSS®.

Results

The concentrations of both sFlt-1 and sEng were significantly higher in the

placentas of women with PE, but not HT, compared with controls (p=0.0002). There was no significant difference in PlGF concentration between controls and women with PE or HT. Placental VEGF concentrations in both PE and HT were higher than in controls (P<0.0001); there was no significant difference between the levels in PE and HT (P=0.3).

Conclusion

The fact that placental concentrations of sFlt-1 and sEng mirrored the known rise in serum levels in PE suggests that the placenta is the main source of these circulating factors. Although sFlt-1 was significantly raised in PE, PlGF was not reduced. This suggests that the lower levels of free PlGF found in the serum of women with PE are not the result of impaired placental production or secretion, but are due to increased binding by (the increased levels of) sFlt-1 in the serum.

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Biochemical Markers and Uterine Artery Doppler as Predictors of Early-Onset Pre-Eclampsia during the First Trimester of Pregnancy. Mauro Parra-Cordero,[1] Ramon Rodrigo,[2] Pilar Barja,[2] Cleofina Bosco,[2] Virginia Fernandez,[2] Emiliano Soto-Chacon,[1] Susana Quezada.[1] *[1]Fetal Medicine Unit, University of Chile Hospital, Santiago, AM, Chile; [2]ICBM, Faculty of Medicine, Santiago, AM, Chile.*

Objective. The purpose of this study was to evaluate whether systematic screening with uterine artery Doppler (UtAD) and serum biochemical markers of oxidative stress, endothelial dysfunction and vasculogenesis performed during the first trimester predict efficiently pre-eclampsia (PET), specifically early-onset PET, in an unselected Chilean population.

Methods. This nested case-control study involved 2831 asymptomatic pregnancies scanned at 11^{+0}-13^{+6} week of gestation. The subjects for biochemical testing were women who were delivered due to PET (n=43) and normotensive controls (n=129) that were enrolled during the first trimester scan. Mean pulsatility index (PI) of the UtAD was calculated. Blood samples were obtained and stored at –84ºC until biochemical analysis of oxidative stress, endothelial dysfunction and vasculogenesis were performed. Normally distributed data were analysed by the unpaired t test, and non-normally distributed data by the Mann-Whitney Rank Sum test. Chi-square tests were used for the comparison of categorical variables. A probability level of P<0.05 was considered significant. Multiple logistic regressions were used to develop a combined predictive index.

Results. There was 13% and 22% significantly increased of the mean PI UtAD in women who later developed PET or early-onset PET compared to control pregnancies during the first trimester scan. Although oxidative stress and endothelial dysfunction biochemical markers were not different between all PET pregnancies and control groups, plasma levels of sFlt1 (1762.1 ± 397.5 vs. 1169.4 ± 71.1 pg/mL, p<0.05) and placenta growth factor (22.0 ± 3.5 vs. 49.8 ± 5.4 pg/mL, p<0.016) were significantly higher in women who subsequently developed early-onset PET compared to controls. Multivariate logistic regression showed that a combination between abnormal UtAD and both biochemical markers of abnormal vasculogenesis were the best predictor test for early-onset PET, being its detection rate 56% with 10% false positive rate.

Conclusion. This study has shown early and selective changes in markers of impaired placentation and angiogenic state in women who later developed early-onset PET, without alteration in oxidative stress and endothelial dysfunction.

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***In Vivo* Acute-Phase Inflammatory Response to Influenza Vaccination in Women with a History of Early-Onset Preeclampsia.** Bas B van Rijn,[1] Hein W Bruinse,[1] Mark Roest,[2] Hieronymus A Voorbij,[2] Arie Franx.[3] *[1]Perinatology & Gynecology, University Medical Center Utrecht, Utrecht, Netherlands; [2]Clinical Chemistry & Hematology, University Medical Center Utrecht, Utrecht, Netherlands; [3]Obstetrics & Gynecology, St Elisabeth Hospital, Tilburg, Netherlands.*

Objective: Inflammation plays a central role in early-onset preeclampsia. Currently it is unknown whether maternal inflammatory changes are specific to pregnancy or reflect an innate susceptibility to inflammation. C-reactive protein (CRP) and interleukin-6 (IL-6) are markers of the acute-phase inflammatory response and predictive of future cardiovascular events. We compared CRP and IL-6 levels after influenza vaccination, as an *in vivo* model for low-grade inflammation, in non-pregnant women with a history of early-onset preeclampsia and controls with only uneventful pregnancies.

Methods: Forty-four women with a history of early-onset preeclampsia (delivery <34 weeks' gestation) and twenty-nine controls with at least one uneventful pregnancy received an influenza vaccination. We then compared plasma levels of CRP and IL-6 at baseline, 1.3 days and 3.3 days after vaccination.

Results: Median baseline CRP and IL-6 levels of women with a history of early-onset preeclampsia were comparable to controls (1.6 versus 0.8 mg/L; p=0.44 and 5.0 versus 3.4 pg/L; p=0.34, respectively). However, high CRP and IL-6 responses to vaccination were more common in cases (ORs for response >75th, >80th, >85th, >90th and >95th percentile based on the distribution of control values of 2.3, 2.7, 3.1, 4.3 and ∞ for CRP [P for trend 0.11] and of 0.9, 1.4, 1.9, 2.6 and 4.5 for IL-6 [P for trend 0.043], respectively). The relationship between high IL-6 responses and early-onset preeclampsia persisted after adjustment for body-mass index (P for trend 0.048).

Conclusion: Women with a history of early-onset preeclampsia more frequently exhibit an innate pro-inflammatory phenotype not specific to pregnancy.

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TLR4 and NOD2 Gene Variants, Maternal Pro-Inflammatory Phenotype and Susceptibility to Early-Onset Preeclampsia and HELLP Syndrome. Bas B van Rijn,[1] Arie Franx,[2] Eric A Steegers,[3] Christianne J de Groot,[4] Rogier M Bertina,[5] Gerard Pasterkamp,[6] Hieronymus A Voorbij,[7] Hein W Bruinse,[1] Mark Roest.[7] *[1]Perinatology & Gynecology, University Medical Center, Utrecht, Netherlands; [2]Obstetrics & Gynecology, St Elisabeth Hospital, Tilburg, Netherlands; [3]Obstetrics & Prenatal Medicine, Erasmus Medical Center, Rotterdam, Netherlands; [4]Obstetrics & Gynecology, Medisch Centrum Haaglanden, The Hague, Netherlands; [5]Hematology, Leiden University Medical Center, Leiden, Netherlands; [6]Experimental Cardiology, University Medical Center, Utrecht, Netherlands; [7]Clinical Chemistry, University Medical Center, Utrecht, Netherlands.*

Background

Altered maternal inflammatory responses play a role in the development of preeclampsia and the hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. We examined whether allelic variants of the innate immune receptors Toll-like receptor 4 (TLR4) and nucleotide-binding oligomerization domain 2 (NOD2), that impair the inflammatory response to endotoxin, are related to preeclampsia and HELLP syndrome.

Methods and findings

We determined five common mutations in TLR4 (D299G and T399I) and NOD2 (R702W, G908R and L1007fs) in 340 primiparous women with a history of early-onset preeclampsia, of whom 177 women developed HELLP syndrome and in 113 women with a history of only uneventful pregnancies as controls. In addition, we assessed plasma levels of pro-inflammatory biomarkers C-RP, IL-6, sICAM-1, fibrinogen and von Willebrand factor in a subset of 214 women included at least six months after delivery. After adjustment for maternal age and chronic hypertension, attenuating allelic variants of TLR4 were more common in women with a history of early-onset preeclampsia than in controls (OR 2.9 [95% CI 1.2– 6.7]). Highest frequencies for TLR4 variants were observed in women who developed HELLP syndrome (adjusted OR 4.1 [95% CI 1.7– 9.8]). In addition, high levels of IL-6 and fibrinogen were associated with a history of early-onset preeclampsia. Combined positivity for any of the TLR4 and NOD2 allelic variants and high levels of IL-6 was 6.9-fold more common in women with a history of early-onset preeclampsia (95% CI 2.1–23.2) compared to controls.

Conclusions

We observed an association of common TLR4 and NOD2 gene variants, and pro-inflammatory phenotype with a history of early-onset preeclampsia and HELLP syndrome, that suggests involvement of the maternal innate immune system in severe hypertensive disorders of pregnancy.

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Increased Pre-B Cell Colony Enhancing Factor (PBEF/Visfatin) in the Serum of Patients with Preeclampsia. Claire E Wright,[1] Mark Hiraoka,[2] Peter Bryant-Greenwood,[3] Gillian D Bryant-Greenwood.[1] *[1]Pacific Biosciences Research Center; [2]Department of Obstetrics and Gynecology; [3]Department of Pathology, JABSOM, University of Hawaii, Honolulu, HI, USA.*

Objective: Concentrations of PBEF in the serum of patients with mild and severe Preeclampsia (PE) compared to gestational aged-matched normal controls.

Introduction: PBEF is a highly conserved protein with ubiquitous expression and known to be upregulated by NF-KB and AP-1, HIF-1alpha and by stretching. A DNA microarray study has also shown PBEF gene expression upregulated in the placentas of PE patients [Pang et al. J Perinatal Med 2003:115;911-9].

Thus, we sought changes in PBEF in the serum of patients with mild and severe PE, compared with matched controls. Immunodistribution of PBEF in fetal membranes and placentas from similar patients was also studied.
Methods: Serum samples (68) were collected with clinical data including; gestational age, medications, ethnicity and recognized complications. Patients in labor or infection were excluded. The standard BP and proteinuria criteria was utilized to classify cases for PE grouping; no PE (n=45), mild PE (n=8 BP; 140/90-160/110, proteinuria; trace to 1+ or 5-300mg/24hr urine) and severe PE (n=15 BP; >160/110, proteinuria; >2+ or >5g/24hr, or other associated symptoms). PBEF concentration was determined by EIA (Phoenix Pharmaceuticals) in accordance with the manufacturers instructions. Fetal membranes and placentas of additional patients, no PE (n=4) and with PE (n=5) were fixed and embedded in paraffin. Sections (7um) were immunostained with PBEF antibody 1/250 (Pheonix) and treated with ABC reagent (Vector Labs) followed by DAB (0.5% mg/mL), washed, counterstained, mounted and viewed under brightfield microscopy.
Results: The concentrations of PBEF in serum were between 5-150 ng/ml and were significantly higher in those patients with mild PE (p=0.027) and further significantly elevated in those with severe PE (p=0.003) compared with the matched controls. PBEF was detected by immunocytochemistry in the placental syncytiotrophoblast and in the amnion and choriodecidua of the fetal membranes.
Conclusions: PBEF was elevated in the serum of patients according to the degree of PE severity and may be derived from the placenta and/or fetal membranes.
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Platelet Monocyte Aggregation in Pregnancy and Pre-Eclampsia. Fiona C Denison,[1] Amy O Robb,[1] Nicholas L Mills,[2] Jehangir N Din,[2] David E Newby.[2] *[1]Centre for Reproductive Biology; [2]Centre for Cardiovascular Sciences, Edinburgh, United Kingdom.*
BACKGROUND: Platelet-monocyte aggregation (PMA) is a novel sensitive measure of platelet activation and indicates a proinflammatory state (cytokine release). Less sensitive techniques demonstrate platelet activation during pregnancy and pre-eclampsia (PE) but platelet activation has not been assessed by PMA.OBJECTIVE: Longitudinal study of PMA in normal pregnancy and PE. METHODS: 25 healthy, non-smoking primigravida with an uncomplicated pregnancy and 16 primigravida women with PE were studied. PE was defined by standard definitions. Informed consent was obtained and the study had ethical approval. Serial venous blood collected at 16, 24, 32,37 wks in controls, at time of diagnosis in PE cases and 6 wks post-natal (PN) in all. PMA, platelet surface P-selectin (psP-SEL) and monocyte CD40 expression (mCD40) were analysed by flow-cytometry and plasma (p) P-SEL by ELISA. RESULTS: Groups were matched for mean age and BMI. In controls, PMAs, psP-SEL and mCD40 expression and pP-SEL increased with gestation and decreased post-natally (Table 1). For PE analysis, data was divided into pre-term (sampled at mean 30 wks), and term (mean 38 wks). pP-SEL was lower in pre-term PE than control (normal pregnancy 32 wks; p=0.03) There was no significant difference in other measures between PE and control (Table 2). CONCLUSIONS: During normal pregnancy, PMA, psP-SEL and mCD40 expression and pP-SEL increase with gestation. The pattern was similar in PE. Unlike some previous studies we have not demonstrated enhanced platetet activation in PE compared to normal pregnancy.

Table 1. Longitudinal assessment of PMA, psP-SEL and pP-SEL in normal pregnancy

Gestation (weeks)	**16**	**24**	**32**	**37**	**+6**	**ANOVA**
number	n=25	n=25	n=25	n=25	n=25	
%PMA	23.1±1.8	26.9±2.4	26.9±2.3	29.2±2.0	23.9±2.1	p=0.04
%psP-SEL	3.2±0.2	5.7±1.1	6.3±0.7	7.3±0.6	4.1±0.3	p<0.0001
%mCD40	43.0±3.2	51.7±3.4	57.3±3.0	65.7±3.7	47.2±2.8	p<0.0001
pP-SEL	45.5±2.7	48.1±2.6	54.9±3.1*	60.3±3.3	50.5±3.4	p<0.0001

Data as mean±SEM

Table 2. Assessment of PMA, psP-SEL, pP-SEL in PE pregnancy

Mean gestation (weeks)	**30**	**38**	**+6PN**
Number	n=8	n=8	n=16
%PMA	28.9±3.8	27.4±3.0	27.8±2.1
%psP-SEL	4.5±0.5	6.2±1.2	5.1±1.0
%mCD40	56.1±3.0	59.8±4.4	45.1±3.0
pP-SEL	40.7±4.0*	40.7±4.0	39.1±4.0

Data as mean±SEM. *p=0.03

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First Trimester Soluble Endoglin and Soluble fms-Like Tyrosine Kinase-1 Levels for the Prediction of Pregnancies with Subsequent Pre-Eclampsia. Marc U Baumann,[1] Nick A Bersinger,[1] Markus Mohaupt,[2] Henning Schneider,[1] Daniel V Surbek.[1] *[1]Obstetrics and Gynecology, University of Berne, Berne, Switzerland; [2]Nephrology, University of Berne, Berne, Switzerland.*
OBJECTIVE: Much effort has been put into the evaluation of novel markers to identify pregnant women at risk for the development of pre-eclampsia. Soluble endoglin (sEng) and soluble fms-like tyrosine kinase 1 (sFLT1), two antiangiogenic agents, appear to be involved in the pathogenesis of pre-eclampsia. Despite several studies describing higher midtrimester serum concentrations of these markers in women with subsequent pre-eclampsia, information on first trimester serum levels is scarce. The aim of this study was to assess sEng and sFLT1 as first trimester serum markers for the prediction of pre-eclampsia.
METHODS: Sera were obtained between 11+2 and 13+6 weeks of gestation from 46 women who later developed late-onset pre-eclampsia and from controls matched for gestational age, maternal age, maternal pre-pregnancy weight, and storage. Using commercially available microplate enzyme immunoanalytical methods, sEng and sFLT1 were determined and the results analyzed using non-parametric statistical tests.
RESULTS: The serum concentration of sEng was found to be increased in women with subsequent pre-eclampsia when compared to controls (mean ± SD, 5.57 ± 1.18 ng/mL versus 5.02 ± 1.01 ng/mL, P = 0.009, unpaired Mann-Whitney test). Similarly, the serum levels of sFLT1 were higher in women later developing late-onset pre-eclampsia (1764 ± 757 ng/mL) when compared to controls (1537 ± 812 ng/mL, P = 0.036). Sensitivities and specificities for predicting pre-eclampsia were 63% and 57% for sEng and 64% and 56% for sFlt-1, respectively. The combination of the two markers by multiplication yielded a sensitivity of 64% and a specificity of 55%.
CONCLUSION: sEng and sFLT1, showing increased first trimester serum levels in women with subsequent pre-eclampsia, might both fulfill the characteristics of first trimester markers to predict pre-eclampsia. The combination of the two, however, did not improve the sensitivity nor the specificity compared to their individual determinations. Moderate sensitivities and specifities, however, limit the clinical use of these molecules as single markers.

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Pregnancy Is Associated with Increased Monocyte/Platelet Aggregate Formation in Whole Blood. Beth A Bouchard,[1] Adrienne Schonberg,[2] Gary J Badger,[3] Ira M Bernstein.[2] *[1]Biochemistry; [2]Obstetrics and Gynecology; [3]Medical Biostatistics, Univ of VT, Burlington, VT, USA.*
Background Preeclampsia is associated with increased rates of platelet clearance, changes in platelet function and platelet activation. The goal of the current study was to examine basal levels of platelet activation through pregnancy beginning prior to conception, and to examine the association of platelet activation with the development of hypertensive complications during pregnancy. Methods Two indices of platelet activation, platelet CD63 expression (%CD63) and monocyte/platelet aggregate (%MP) formation, were measured in whole blood by flow cytometry using specific, fluorescently-labeled monoclonal antibodies in 16 healthy, nonsmoking women during the follicular phase of their menstrual cycle (PP, cycle day 7.5±0.9). All women subsequently conceived singleton pregnancies and were re-examined in early (EP, 11-16 wks) and late pregnancy (LP, 31-34 wks). Five of these women were diagnosed with hypertensive complications (4 hypertension, 1 preeclampsia) at term although hypertension was not observed at any study time point. Data are expressed as mean±SEM. P<0.05 was accepted for significance. Results Subjects were 29.1±0.9 years old with a BMI of 23.5±0.9 kg/m^2 at the time of prepregnancy studies. A significant increase in the %MP formed over time of pregnancy was observed (p=0.015). There was little change in the %MP formed between PP and EP (PP, 6.0±3.8 % and EP, 10.4±3.8 %, p=0.384). However, the %MP increased significantly in LP (20.9±3.8 %) as compared to PP (p=0.005) and EP (p=0.041). This increase occurred independent of the development of hypertensive complications (p=0.657) and independent of PP platelet activation status. Although statistically significant increases in CD63 expression were not observed, the change in CD63 expression over pregnancy correlated with the change in %MP over pregnancy (r=0.56, p=0.024) and CD63 expression correlated with %MP in LP (r=0.57, p=0.020). Conclusion These combined observations suggest that pregnancy is associated with increases in levels of unstimulated platelet activation and that these increases occur in the presence or absence of subsequent hypertensive complications.

Furthermore, we observed a correlation between the changes in two distinct platelet activation events, %MP and CD63 expression, during pregnancy. Supported by NIH HL 71944.

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A Randomized Double Blind Placebo-Controlled Trial of the Effects of Sildenafil Citrate on Uterine Blood Flow. Ira M Bernstein,[1] Cresta Jones,[1] Adrienne Schonberg,[1] Gary J Badger,[2] George Osol.[1] *[1]Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA; [2]Medical Biostatistics, University of Vermont, Burlington, VT, USA.*

Backgound Sildenafil citrate (SC) has been proposed as a therapy to improve uterine perfusion in pregnancies complicated by IUGR or preeclampsia. We sought to determine the effects of SC on uterine vascular resistance, uterine blood flow and cardiac output in young healthy nulliparous women. Methods Eleven young healthy nulligravid women were studied during the luteal phase (cycle day 22 ± 5) of the menstrual cycle. Women were randomized in a double-blind fashion to receive placebo (PL), or SC at a dose of 25 or 100 mg. Uterine artery vascular resistance, uterine artery volumetric flow, brachial artery volumetric flow and cardiac output were measured at baseline and at 1 and 3 hours post dosing employing color Doppler ultrasound. Comparisons were made by ANOVA between those randomized to PL (n=5) versus SC (n=6). P<0.05 was accepted for significance. Data are expressed as mean ± S.E.M. Results There were no significant differences in subject age, cycle day, body mass index, uterine blood flow, brachial blood flow or cardiac output at baseline comparing the two groups. There was a tendency towards increased uterine blood flow in subjects randomized to receive SC (68% increase) compared to PL (no change), Changes in uterine blood flow, brachial blood flow and cardiac output are outlined in the table below.

Hemodynamic Effects of Sildenafil

		Baseline	1 hour	3 hour	P-value
Uterine (mL/min)	SC	19±7.2	32±7.2	30± 7.2	P=0.20
	PL	21±7.9	21±7.9	20±7.9	
Brachial (mL/min)	SC	44±16	52±16	83±16	P=0.75
	PL	46±18	39±18	80±18	
Cardiac Ouptut (mL/min)	SC	4,599±402	4,669±402	4,567±402	P=0.82
	PL	4,902±440	4,697±440	4,754±440	

Conclusions In these preliminary findings volumetric uterine blood flow demonstrates a tendency towards an increase in response to SC not paralleled by upper extremity brachial blood flow or cardiac output. Additional subjects are currently being recruited for study. This study was supported in part by NIH HL 71944 and NIH HL 73895.

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The Association of Third Trimester Maternal Plasma Volume with Plasma Volume Prior to Pregnancy. Ira M Bernstein,[1] Adrienne Schonberg,[1] Beth Bouchard,[2] Gary J Badger.[3] *[1]Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA; [2]Biochemistry, University of Vermont, Burlington, VT, USA; [3]Medical Biostatistics, University of Vermont, Burlington, VT, USA.*

Objective Reduced maternal plasma volume in the third trimester has been associated with both fetal growth restriction and preeclampisa. We sought to determine the degree to which third trimester plasma volume is dependent on plasma volume prior to pregnancy. Methods Sixteen young (29.1 ± 2.9 years) healthy nulligravid women had their plasma volume measured during the follicular phase (cycle day 7.5 ± 3.6) of the menstrual cycle and subsequently conceived. Subjects were predominantly Caucasian (15/16) with a mean prepregnancy BMI of 23.5 ± 3.5 kg/m². Plasma volume was re-estimated at 32-34 weeks gestation. All patients were placed on sodium and total calorie balanced diets for 3 days prior to each plasma volume determination. Plasma volume was determined employing Evans blue dilution with multiple post injection sampling time points. Data are expressed as mean ± S.D. Results Baseline prepregnant plasma volume was 2,868 ± 446 mL or 123 ± 18 mL/unit BMI. Third trimester plasma volume was 4,306 ± 663 mL representing a 51 % increase (P<0.001). The range of plasma volume expansion was 20-84% dependent upon prepregnant plasma volume. Plasma volume in the third trimester of pregnancy was strongly correlated to prepregnant plasma volume R= 0.65 (P=0.006). Plasma volume expansion was consistent across the range of prepregnancy plasma volume. Conclusions Pre-pregnancy plasma volume contributes approximately 42% of the variance in third trimester plasma volume. The observed increase is plasma volume is independent of prepregnancy volume resulting in a greater percentage increase for those starting at the lower end of the plasma volume range. As third trimester plasma volume is strongly associated with pregnancy outcome the correlation of prepregnancy plasma volume to third trimester plasma volume suggests that prepregnancy status contributes to these adverse reproductive events.This work was supported by NIH HL 71944.

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sFlt-1 Expression Is Increased in Placentae from Molar Pregnancy. Ori Nevo,[1] Isabella Caniggia.[2] *[1]Ob&Gyn, Sunnybrook HSC, MSHRI, University of Toronto, Toronto, ON, Canada; [2]Ob&Gyn, Mount Sinai Hospital, MSHRI, University of Toronto, Toronto, ON, Canada.*

Background: Hydatidiform mole is a rare disorder of pregnancy and may predispose the mother to severe morbidity. Molar pregnancies are known to be associated with high risk for the development of early onset preeclampsia. In recent years, the expression of sFlt-1 (soluble VEGFR-1) was found to be increased in preeclampsia, and contributes to the pathogenesis of the maternal systemic disease. The objective of the present study was to examine the expression of sFlt-1 in placentae from molar pregnancies.

Methods: Placental samples from unique cases of twin pregnancies with complete molar pregnancy in one sac and developing fetus in the other sac were prospectively collected (n=2). The first set delivered at 23 weeks due to excessive bleeding. The second set delivered at 26 weeks due to severe IUGR and elevated blood pressure. mRNA level of sFlt-1 was measured by quantitative real-time PCR using specific TaqMan primers and probe. Protein expression of sFlt-1 in placental tissue lysates were measured by Western blot analysis using a polyclonal antibody against Flt-1. Immunohistochemistry of paraffin embedded samples was performed using specific antibody for sFtl-1.

Results: mRNA level of sFlt-1 was increased by 2.3 fold in the molar placentae compared to matched controls. The placentae of the developing fetuses which were growth restricted exhibited 2.7 fold increase compared to controls. sFlt-1 protein expression in the molar placentae was increased by 7.7 fold compared to controls, while the co-twin placentae exhibited a 1.4 fold increase compared to controls. Immunohistochemistry revealed strong positive immunoreactivity for sFlt-1 in the trophoblast layer of both molar pregnancies and IUGR co-twin relative to controls.

Conclusion: Our data suggest that sFlt-1 expression is increased in placentae from molar pregnancies and thus may explain the increased risk for developing early onset preeclampsia. The expression of sFlt-1 in the growth restricted twin placenta is also increased compared to controls and support our previous observation (Supported by CIHR and OWH/IGH).

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Do Angiogenic Factors Distinguish Severe from Mild Preeclampsia? Sindhu K Srinivas,[1] Jacob Larkin,[1] Jamie Bastek,[1] Mary D Sammel,[2] Ella Ofori,[1] Michal A Elovitz.[1] *[1]Obstetrics and Gynecology CRRWH, University of Pennsylvania, Philadelphia, PA, USA; [2]Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA, USA.*

OBJECTIVE

Recent data suggest that angiogenic factors act as reliable biomarkers for preeclampsia (PRE). Using a large case-control study, we assessed whether angiogenic factors differentiate severe and mild PRE.

STUDY DESIGN

Cases are prospectively identified with mild (including GHTN) or severe PRE based on pre-specified maternal criteria. Controls are women presenting for term delivery. Clinical data and serum were collected for all subjects. ELISAs were used to measure sFlt, Endgolin (ENG) and PlGF. Non-parametric comparisons were made using Kruskal-Wallis and Wilcoxon rank sum tests. MVLR was used to control for confounders. A secondary analysis comparing preterm PRE (cases delivered <34 wks) to cases >=34 wks was performed.

RESULTS

258 patients (GHTN (23), mild (21) and severe PRE cases (64) and 160 controls) were included. For every 10 unit increase in sFlt, there is a 1.7 fold increased odds of being a severe over a mild case (CI[1-3.2], p=0.05) after controlling for significant confounders. ENG and PIGF levels did not differ between mild and severe cases. Given the similarity of GHTN and control sFlt levels, mild cases (excluding GHTN) were compared to severe cases and the association between sFlt and severe cases was no longer present (p=0.22). sFlt and ENG are associated with PRE<34 wks compared to cases >=34 wks.

CONCLUSION

As previously reported, levels of angiogenic factors are significantly different between PRE and controls. However, using ACOG definitions for mild and severe PRE, angiogenic factors do not hold clinical promise in differentiating PRE phenotype. The use of these biomarkers for predicting preterm PRE requires further investigation.

Angiogenic factors by PRE severity

	sFlt mean+/- SD(med)	ENG mean+/- SD(med)	PIGF mean+/- SD(med)
Control	6.8+/-6.0(5.5)	14.0+/-11.8(10.8)	204+/-263.9(130.5)
GHTN	6.9+/-6.7(5.4)	13.9+/-9.6(12.2)	163.4+/-334.2(57.2)
Mild PRE	10.1+/-9.1(6.6)	23.9+/-22.4(15.1)	80.6+/-94(47.6)
Severe PRE	14.7+/-15.9(9.9)	28.3+/-23.5(20.1)	90.3+/-145.1(48.6)
P value	0.004	<0.001	<0.001
PRE <34 wks	25.5+/- 22.5(16.3)	38.5+/-28.9(34.6)	55.8+/-52(34.6)
PRE >=34 wks	9.8+/-9.9(6.4)	21.7+/- 19.4(16.1)	112.9+/-210(52.5)
P value	0.002	0.036	0.29

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No Subsets of Preeclampsia by TNFα:IL10 and HOMA$_{IR}$. Sandra Founds,[1,2] Susan Sereika,[1] Marcia Gallaher,[2] James Roberts.[2,3] *[1]School of Nursing, University of Pittsburgh, Pittsburgh, PA, USA; [2]Magee-Womens Research Institute; [3]Dept Ob/Gyn & Reproductive Sciences and Epidemiology.*

T-helper 2 (Th2) anti-inflammatory cytokines promote maternal immune tolerance in normal pregnancy. T-helper 1 (Th1) pro-inflammatory cytokines are increased and T-helper 2 (Th2) are decreased in preeclampsia. The ratio of Th1:Th2 may better predict pregnancy inflammatory status than either type alone. Insulin resistance is also associated with preeclampsia and in a prior study was not related to inflammatory markers in women with preeclampsia (Kaaja et al., 2004). We hypothesized that there might be two subsets of preeclampsia, primarily inflammatory (high Th1:Th2) or metabolic (high insulin resistance).

Methods

Design was a nested case control study of consented non-laboring fasting primiparous subjects. Preeclamptic cases were hypertensive, hyperuricemic and proteinuric; controls normotensive with normal uric acid and no proteinuria. Women with other medical problems were excluded.

Samples Plasma samples (EDTA) were selected from 45 preeclamptics and 109 controls.

Assays Tumor necrosis factor alpha (TNFα) as Th1 and interleukin 10 (IL10) as a Th2 cytokine were measured by fluorescent bead assays, glucose by colorimetric assay, and insulin by ELISA. Insulin resistance was calculated by homeostasis model assessment (HOMA$_{IR}$=Insulin mU/ml x [Glucose mM/L/22.5]).

Analysis Cases and controls were compared using t-tests for continuous factors and chi-square tests for categorical variables. Hierarchical cluster analysis and logistic regression were conducted. The significance level was p<.05.

Results

Preeclamptic and control groups did not differ by maternal age (p=.46), race (p=.27), or smoking (p=.13). Compared to controls, preeclamptics were earlier in gestation (mean ± SEM in weeks 35 ± .63, 39.5 ± .26; p<.000) and were heavier (prepregnancy BMI ± SEM 27.7 ± 1.0, 25.0 ± .58; p=.02). Although TNFα (p=.01) and IL10 (p=.01) differed between groups, preeclamptics and controls did not differ in TNFα:IL10 (p=.19) or HOMA$_{IR}$ (p=.83). Preeclamptics did not appear to cluster by TNFα:IL10 and HOMA$_{IR}$. TNFα:IL10 and HOMA$_{IR}$ in logistic regression was not related to preeclamptic status (p=.58).

Conclusions

There was no evidence of a dichotomy in preeclampsia by inflammation as measured by Th1:Th2 cytokines and insulin resistance. TNFα and IL-10 were increased in women with preeclampsia. Th1:Th2 cytokines and HOMA$_{IR}$ did not predict preeclampsia versus control group.

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Protein Z Plasma Concentrations in the Umbilical Cord of Patients with Preeclampsia and SGA. Offer Erez,[1] Edi Vaisbuch,[1] Francesca Gotsch,[1] Juan Pedro Kusanovic,[1] Shali Mazaki-Tovi,[2] Chong Jai Kim,[3] Jung-Sun Kim,[3] Tinnakorn Chaiworapongsa,[2] Deborah Hoppensteadt,[4] Jawed Fareed,[4] Nandor G Than,[1] Chia-Ling Nhan-Chang,[2] Moshe Mazor,[5] Pooja Mittal,[2] Sonia S Hassan,[2] Roberto Romero.[1] *[1]Perinatology Research Branch, NICHD, NIH, DHHS, Bethesda, MD, USA; [2]Dept of Ob/Gyn, Wayne State University, Detroit, MI, USA; [3]Dept of Pathology, Wayne State University, Detroit, MI, USA; [4]Dept of Pathology, Loyola University, Maywood, IL, USA; [5]Dept of Ob/Gyn, Soroka University Medical Center, Beer-Sheva, Israel.*

Objective: Preeclampsia (PE) and SGA are associated with placental lesions that represent maternal under-perfusion and fetal vascular thrombo-occlusive disease. A higher rate of protein Z (PZ) deficiency has been reported in patients with PE and SGA neonates. Goals of this study were to determine changes in umbilical cord plasma PZ concentration and rate of PZ deficiency in neonates of patients with PE and SGA neonates.

Methods: A cross-sectional study included: 1) neonates born after a normal pregnancy (n=145); 2) neonates of patients with PE (n=104); and 3) SGA neonates (n=48). Cord blood was collected immediately after delivery and PZ plasma concentrations were measured by ELISA. PZ deficiency was defined as a cord plasma concentration ≤5th percentile of the normal pregnancy group. Non-parametric statistics were used for analysis.

Results: 1) Cord plasma PZ concentration differed significantly among the 3 study groups (Kruskal Wallis, p<0.001); 2) neonates of patients with PE and SGA neonates had a significantly lower median cord plasma PZ concentration than those delivered after normal pregnancy (PE: median 0.45 µg/ml, range 0.05-1.68, p<0.001; SGA: median 0.46µg/ml, range 0.06-3.56, p=0.011; normal pregnancy: median 0.6µg/ml, range 0.04-1.17); 3) there were no differences in the rate of PZ deficiency among the groups; and 4) there was no relationship between placental histologic findings and median cord plasma PZ concentrations between and among the SGA and PE groups.

Conclusions: 1) At the time of delivery, the median cord plasma PZ concentration was lower in SGA neonates and those born to women with PE than in neonates born to normal pregnancies; 2) there was no difference in the rate of PZ deficiency among the study groups, suggesting that the lower median PZ cord blood concentrations in PE and SGA groups may result from activation of the coagulation cascade rather than an inherited PZ deficiency.

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Placental Growth Factor Levels Are Similar between Women with and without Diabetes in the Second Trimester. Mark C Alanis, Christopher J Robinson, Donna D Johnson. *Obstetrics and Gynecology, Medical University of South Carolina, Charleston, South Carolina, USA.*

Introduction:

Diabetes is a significant risk factor for preeclampsia. Women destined to develop preeclampsia exhibit alterations in angiogenic factors such as decreased placental growth factor (PlGF) levels during the second trimester. It is unclear if women with diabetes share this same anti-angiogenic pathogenic pathway compared to other women at risk for preeclampsia. The purpose of this study was to determine if PlGF levels were different in women with diabetes compared to normoglycemic controls.

Methods:

In this IRB approved study, serum samples were collected from women between 15 and 20 weeks' gestation and analyzed for levels of PlGF using ELISA. A validated, research quality database was used to identify women with a singleton gestation and with (cases) or without (controls) diabetes. Exclusion criteria included chronic hypertension or the development of preeclampsia. Levels of PlGF were compared between cases and controls using Student's t-test and multiple logistic regression analysis.

Results:

34 cases and 34 controls met inclusion and exclusion criteria. The mean level of PlGF was similar in both groups (cases = 174.5 pg/mL, SEM 14.5; controls = 165.3 pg/mL, SEM 19.9; p = .7). After adjusting for estimated gestational age and BMI at the time of sample, there was still no statistically significant difference in PlGF levels between cases and controls.

Conclusions:

There appears to be no significant difference in levels of second trimester PlGF in women with and without diabetes. Further study is required to determine if women with diabetes share the same anti-angiogenic pathway that other women at risk for preeclampsia have demonstrated. Alternatively, women with diabetes may possess different pathogenic risk factors than normoglycemic women.

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Periconceptional Multivitamin Use Is Associated with Reduced Risk of Preeclampsia among Normal Weight Women in the Danish National Birth Cohort. Janet M Catov,[1,2] Ellen A Nohr,[3] Lisa M Bodnar,[1,2] Vibeke Knudson,[3] Sjurdur F Olsen,[3] Jorn Olsen.[4] *[1]Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA; [2]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA; [3]Epidemiology, University of Aarhus, Aarhus, Denmark; [4]Epidemiology, UCLA, Los Angeles, CA, USA.*

Objective: Periconceptional multivitamin (MV) use may be related preeclampsia risk. We examined the relation between timing and frequency of periconceptional multivitamin use and the risk of preeclampsia.

Methods: Women in the Danish National Birth Cohort who delivered singleton liveborn infants (n=26,133) reported upon enrollment at 10.8 weeks (SD 3.6) the number of weeks of regular multivitamin use during a 12 week periconceptional period (LMP-4 to LMP+8). Preeclampsia cases were identified using ICD-10 codes (n=605, 2.3%). Logistic regression was used to estimate the effect of

frequency (number of weeks of use) and timing of use (pre-conception [LMP-4 to LMP+2] and post-conception [LMP+3 to LMP+8]). Results were stratified a priori by overweight status.

Results: Overall, 18,551 women (71%) reported MV use in the periconceptional period. After adjustment for BMI, smoking, parity and chronic hypertension, infrequent MV use (<4 weeks of use) had no relation to risk (OR 1.15; 95% CI 0.92,1.44) but regular use (>=8 weeks) was associated with modestly reduced risk (0.84 (0.67,1.06). Similarly, when MV use was modeled as a continuous variable, each additional week of use was related to reduced risk for preeclampsia (OR 0.83, 95% CI 0.67-1.02). This potential dose effect of periconceptional MV use appeared to be limited to normal weight women (BMI <25 kg/m², OR 0.74; 95% CI 0.55-1.01), with no apparent effect among overweight women (BMI ≥25 kg/m², OR 0.93; 95% CI 0.68-1.26). A total of 7,332 women reported regular MV use in both the preconception and post-conception periods, and 5,630 women reported regular use only in the post-conception period. Among normal weight women, regular use in the preconception period had no effect on preeclampsia risk (OR 1.29, 95% CI 0.89-1.85). In contrast, use in the post conception period was associated with reduced risk for preeclampsia (OR 0.56, 95% CI 0.39-0.82).

Conclusions: Regular periconceptional MV use was associated with a modestly reduced risk for preeclampsia among normal weight women. If causal, MV use immediately after conception appeared to be the critical exposure window.

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Effect of Community-Based Group Prenatal Physical Activity on Pre-Eclampsia Rate. Ann F Cowlin,[1] Robyn Brancato,[1] Gil Mor,[2] Daniel Zelterman,[3] Peggy DeZinno.[4] *[1]Research, Women's Health Fitness Institute, Branford, CT, USA; [2]Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT, USA; [3]Biostatistics, Yale University School of Medicine, New Haven, CT, USA; [4]W.E.L.L., Yale-New Haven Hospital, New Haven, CT, USA.*

Background: Pre-eclampsia (PE), a disorder of pregnancy characterized by maternal inflammation, results in immune, cardiovascular and metabolic dysfunction. In non-pregnant persons, inflammatory disorders are treated with and prevented by pharmaceuticals and lifestyle methods such as physical activity (PA). While most pharmaceuticals are contraindicated for pregnant women, PA during pregnancy has been found safe, healthy and beneficial for both mother and baby. Clinical evidence has found PA can beneficially affect pregnancy outcome, decrease excessive inflammation and decrease the risk of PE. Epidemiological studies indicate that PA may be useful in preventing PE.

Unfortunately, previous studies have quantified PA based on recall of postpartum women and have not controlled for differences in women's interpretations of amount, type or intensity of PA. However, investigating PA utilizing a laboratory-based exercise intervention to control these variables inflicts difficulties translating the intervention into a community-based program that attracts and retains pregnant women in order to enhance public health.

Method: A retrospective study was performed to determine the rates of PE among the 9,160 women who gave birth at Yale-New Haven Hospital (YNHH) during 2004 and 2005, and a 90 person subset of this group who performed prenatal PA in a community-based program that is evidence-based and standardized, thereby controlling for type and intensity of PA. Additionally, the program is established in the community, has been offered to the public for 30 years and is internationally known.

Results: During 2004-2005, the PE rate for the general population at YNHH was 7.8%. For the PA group, the rate was 2.2%. Two women in the PA group were diagnosed with PE in the last month of pregnancy and delivered normal infants at term. No PE was observed in this group (PA group) during the second or early third trimesters nor was there any prematurity in this group.

Significance: These findings support the hypothesis that adequate physical activity provided in a standardized community-based group setting may provide a non-pharmacological approach for preventing PE.

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Angiogenic Growth Factors and Their Receptors in First-Trimester Human Decidua of Pregnancies Further Complicated by Pre-Eclampsia or Fetal Growth Restriction. Margreet Plaisier,[1] Esther Streefland,[2] Pieter Koolwijk,[3] Frans M Helmerhorst,[1] Jan Jaap HM Erwich.[2] *[1]Department of Gynecology and Reproductive Medicine, Leiden University Medical Center, Leiden, Netherlands; [2]Department of Obstetrics and Gynecology, University Medical Center Groningen, Groningen, Netherlands; [3]Department of Physiology, VU Univerity Medical Center, Amsterdam, Netherlands.*

Objective: Disturbances in decidual and placental vascular development may play a role in the pathogenesis of pregnancy complications, like pre-eclampsia (PE) or fetal growth restriction (FGR). Whether the regulation of decidual vascular adaptation to implantation is altered in these illnesses, is not elucidated yet. The present study focused on the role of first-trimester angiogenic factors in the pathogenesis of PE and or FGR.

Methods: First-trimester decidua samples were obtained during routine chorionic villous sampling. The expression of vascular endothelial growth factor (VEGF-A), placental growth factor (PlGF), flt-1, KDR, Angiopoietin-1 (Ang-1), Angiopoietin-2 (Ang-2) and TIE-2 mRNA was determined by RT-PCR. The expression of the angiogenic factors was related to the pregnancy outcome, i.e. uncomplicated, PE or FGR.

Results: The first-trimester decidual tissues expressed all angiogenic factors. mRNA levels of VEGF-A, PlGF, KDR, Ang-1, Ang-2 and TIE-2 appeared increased in FGR cases compared to matched controls. In addition, PlGF, Ang-1 and TIE-2 mRNA appeared increased in PE cases compared to matched controls. The differential expression of angiogenic factors was more pronounced in cases with FGR than PE. The large inter-individual variation disallowed a significant outcome.

Conclusions: Various angiogenic factors showed differential mRNA expression in 1st trimester decidua of patients developing PE or FGR in later pregnancy compared to their matched controls. The first-trimester decidual samples provided a unique opportunity to obtain information regarding the onset of PE and FGR. Early 1st trimester changes in angiogenic factor expression may well occur as a compensatory mechanism. In turn, this may set the stage for increased non-branching angiogenesis and altered decidual and placental vascular adaptation, which may be part of the pathogenesis of PE and/or FGR.

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Changes in Levels of Soluble Intracellular Adhesion Molecule-1 (sICAM-1) during Prgnancy Are Related to the Development of Hypertensive Complications. Beth A Bouchard,[1] Adrienne Schonberg,[2] Gary J Badger,[3] Ira M Bernstein.[2] *[1]Biochemistry; [2]Obstetrics and Gynecology; [3]Medical Biostatistics, Univ of VT, Burlington, VT, USA.*

Background Preeclampsia is characterized by endothelial dysfunction. The goal of the current study was to prospectively measure plasma levels of the soluble endothelial cell adhesion molecules, sICAM-1 and sVCAM-1, beginning prior to pregnancy and determine if subjects destined to develop hypertension complicating pregnancy had differences in the concentrations of these molecules. Methods Serum levels of sICAM-1 and sVCAM-1 were measured in 16 healthy, nonsmoking women (cycle day 7.5 ± 0.9, prepregnancy) by ELISA. All women subsequently conceived singleton pregnancies and were re-examined in early (EP, 11-16 weeks) and late pregnancy (LP, 31-34 weeks). Five of these women developed hypertensive complications (4 gestational hypertension, 1 pre-eclampsia) near term. All subjects were normotensive at all study time points. Data are expressed as mean ± SEM. P<0.05 was accepted as significant. Results Subjects were 29.1±0.9 years old with a mean BMI of 23.5±0.9 kg/m² at the time of prepregnancy studies. Significant differences in sICAM-1 levels as a function of pregnancy were observed (p=0.046) and are outlined in Table 1 below.

Changes in sICAM-1 during Pregnancy

	Pre-pregnancy	Early Pregnancy	Late Pregnancy	P-value
Total (n=16)	205±12	198±12	207±12	P=0.046
Normotensive (n=11)	204±14	186±14	200±14	P=0.019
Hypertensive (n=5)	206±20	210±20	214±20	P=0.662

Differences were dependent upon the stage of pregnancy in those women who were not diagnosed with hypertensive complications with a decrease in sICAM-1 levels in EP (p=0.024) followed by an increase in sICAM-1 levels in LP (p=0.044). In women with hypertension in pregnancy, these differences in sICAM-1 levels were not evident (p=0.66). There were no differences in sICAM-1 levels comparing women with or women without hypertensive complications prior to pregnancy (p=0.971). In contrast to sICAM-1, we observed no significant differences in sVCAM-1 levels over pregnancy or

between those with and without hypertension. Conclusions These combined observations suggest that levels of the soluble adhesion molecule sICAM-1 change significantly over time in normal pregnancies. Subjects destined to develop hypertension did not demonstrate the early pregnancy reduction in sICAM-1. Supported by NIH HL 71944.

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Nulliparity and Preeclampsia: Is Angiogenesis a Key Player in the Link? Yuval Bdolah,[1] Uriel Elchalal,[1] Shira Natanson-Yaron,[1] Hadas Caspi,[1] Tali Bdolah-Abram,[1] Angelika Bord,[1] Caryn Greenfield,[1] Debra Goldman-Wohl,[1] Ariel Milwidsky,[1] Franklin H Epstein,[2] S Ananth Karumanchi,[2] Simcha Yagel,[1] Drorith Hochner-Celnikier.[1] *[1]OB/GYN, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [2]OB/GYN & Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA.*

Objectives: Nulliparity is a risk factor for preeclampsia (PE) with a reported incidence of up to 4-5 times higher than multiparous pregnancies. Soluble fms-like tyrosine kinase-1 (sFlt1), a circulating anti-angiogenic molecule of placental origin plays a pivotal role in PE by antagonizing placental growth factor (PlGF). Increased sFlt1 and sFlt1/PlGF have been shown to antedate clinical signs in PE. We therefore hypothesized, that the higher risk of PE in nulliparous pregnancies is associated with high sFlt1 (or sFlt1/PlGF).

Methods: Maternal serum samples from nulliparous (n=68) and multiparous (n=159) term singleton pregnancies without PE, at the time of admission to delivery room, were used. Serum samples were analyzed for levels of sFlt1 and PlGF by ELISA. Statistical analysis was performed applying T-test and the Kruskall-Wallis test and using SPSS software.

Results: For nulliparous and multiparous pregnancies, the mean serum sFlt1 levels were 13,385 ± 960 and 10,584 ± 722, (p=0.021), the mean serum PlGF levels were 215 ± 15 and 249 ± 14 (p=0.117), and the mean ratios of sFlt-1/PlGF were 98 ± 13 and 65 ± 6 (p=0.025), accordingly. In a subgroup of 10 multigravidous nulliparous pregnancies, sFlt1 levels were 14,063 ± 2295. Correcting for maternal age did not alter the results. Moreover, results did not differ between multiparous pregnancies with a 5-10 years interpregnancy interval compared with a 1-5 years interval.

Conclusions: In nulliparous pregnancies, circulating sFlt1 levels and sFlt1/PlGF ratios are significantly higher than in multiparous pregnancies. These findings suggest that the increased risk of PE in nulliparous pregnancies may involve anti-angiogenic imbalance. Nulliparity may be more substantial than primigravity, as a risk factor for PE, suggesting that first semester abortions in primigravidas may not protect from PE in a subsequent term pregnancy. Nevertheless, even 5-10 years intervals from the previous gestation do not increase the risk for PE. Different normograms of angiogenesis should be used, when assessing the risk for PE in multiparous versus nulliparous pregnancies.

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Alterations in Levels of Angiogenic Proteins in First Trimester and Subsequent Risk of Developing Abruption. Sarosh Rana,[1] Alejandro Rauh-Hain,[2] Ananth Karumanchi,[3] Ravi Thadhani.[2] *[1]Obstetrics and Gynecology/ Maternal Fetal Medicine, Women and Infants Hospital, Providence, RI, USA; [2]Department of Medicine, Massachusetts General Hospital, Boston, MA, USA; [3]Departments of Medicine/OB-GYN, Beth Israel Deaconess Medical Center, Boston, MA, USA.*

Objective: We determined whether maternal serum levels of angiogenic proteins namely soluble fms like tyrosine kinase (sFlt-1), soluble endoglin (sEng), and placental growth factor (PLGF) - measured during the first trimester are associated with the subsequent development of placental abruption.

Methods: We performed a prospective, nested case-control study of women enrolled in the Massachusetts General Hospital Obstetric Maternal Study (MOMS). First trimester serum samples from 34 placental abruption cases and 250 normal pregnancies were measured for angiogenic factors. Cases and controls were matched by body mass index and age. Placenta abruption was diagnosed by standard clinical findings and pathological examination of the placenta. Women with confirmed preeclampsia or chronic hypertension were excluded.

Results: Compared to controls, cases had more pregnancies, delivered infants at an earlier gestational age and with lower birth weight. First trimester levels of sEng were significantly increased in cases compared to controls: 7.95 ± 0.48 ng/ml vs. 6.48± 0.15 ng/ml, p <0.05. There were no significant difference in serum levels of PLGF, 40.31± 6.22 ng/ml versus 40.04± 1.79 ng/ml, p=NS, although sflt1 levels were lower in cases: 1.86 ± 1.7 ng/ml vs. 2.7±0.98 ng/ml, p=NS. In logistic regression analysis adjusted for age, race, smoking, number of pregnancies, gestational age at delivery, gestational age of blood sampling, and blood pressure at first prenatal, sEng levels remained independently associated with subsequent risk (Odds Ratio 1.3, 95% CI 1.1-1.6) of placental abruption. Examining this relationship by tertiles of sEng, in the unadjusted model, women in the second (OR 7.4, 95% CI 1.6-33.5) and third (OR 8.8, 95% CI 1.9-39.1) tertiles were at increased risk of developing placental abruption compared with women in the lowest tertile. After adjusting for known risk factors of placental abruption, women in the second (OR 13.1, 95% CI 1.3-129.09) and third (OR 13.3, 95% CI 1.9 95.3) tertiles remained at increased risk for placental abruption.

Conclusion: Increased first trimester maternal serum levels of sEng are associated with increased risk of subsequent placental abruption.

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Levels of Angiogenic Factors (VEGF and sFLT-1) in Plasma and Amniotic Fluid of Pregnancies Complicated by Preterm Birth. Victoria V Snegovskikh,[1] Ramkumar Menon,[2] Stephen J Fortunato,[2] Frederick Schatz,[1] Guoyang Luo,[1] Antonette Dulay,[1] Eric Hodgson,[1] Sarah Lee,[1] Mark Wehrum,[1] Charles J Lockwood,[1] Errol R Norwitz.[1] *[1]Ob/Gyn, Yale University, New Haven, CT; [2]Vanderbilt University, Nashville, TN.*

OBJECTIVE: Although aberrant expression of pro- (VEGF, placental growth factor) and anti-angiogenic factors (sFlt-1, endoglin) has been implicated in pregnancy complications, including preeclampsia and IUGR, little is known about levels in pregnancies complicated by preterm birth (PTB). This study investigates levels of VEGF and sFlt-1 in the circulation and amniotic fluid (AF) of pregnancies complicated by PTB.

METHODS: Plasma and AF samples were collected at the time of term (n=36) and preterm delivery (<36 weeks [n=44]). Pregnancies with preeclampsia, twins, pPROM, IUGR, and medical complications (diabetes, chronic hypertension) were excluded. Of all PTB samples, 23 were from women in unexplained spontaneous preterm labor and 21 had intraamniotic infection (IAI). IAI was confirmed by standard clinical criteria, evidence of histologic chorioamnionitis, and a positive PCR amplification test for microbial DNA. Cases/controls were half African-American and half Caucasian. Total VEGF and sFlt-1 levels were measured in undiluted samples by ELISA. Samples were excluded if levels were below the level of detection of the assay (<9.0 pg/mL for VEGF; <3.5 pg/mL for sFlt-1). Analysis used non-parametric (Mann-Whitney) statistical tests.

RESULTS: Since VEGF and sFlt-1 levels were not significantly different in PTB samples from women in spontaneous labor vs IAI, these samples were analyzed together. Levels of VEGF were similar in women with PTB vs term controls in both plasma (data is shown as median [25%le, 75%le]: 9.84 [3.58, 21.19] vs 5.39 [2.58, 11.18] pg/mL, respectively; p=0.105) and AF (0.95 [0.54, 1.45] vs 0.86 [0.64, 2.89] pg/mL; p=0.546). Similarly, levels of sFlt-1 in women with PTB were not significantly different from term controls in both plasma (3.43 [2.14, 5.83] vs 3.95 [2.08, 7.00] ng/mL; p=0.547) and AF (148.3 [65.0, 247.3] vs 114.8 [63.2, 211.3] ng/mL; p=0.674). No racial differences in VEGF and sFlt-1 levels were noted.

CONCLUSIONS: Levels of angiogenic factors (VEGF, sFlt-1) in both the circulation and AF of pregnancies complicated by PTB are similar to that measured in uncomplicated term controls. Similarly, measurement of VEGF and sFlt-1 cannot distinguish spontaneous preterm labor from that due to IAI.

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Shear Stress and Uterine Blood Flows during the Proliferative and Secretory Phases of the Menstrual Cycle and Pregnancy. Ronald R Magness,[1,2,3] Kreg M Grindle,[1] David J Magness,[1] Andrienne L Schonberg,[4] Ira M Bernstein.[4] *[1]Depts of Ob/Gyn Perinatal Research Labs; [2]Anim Sci; [3]Peds, Univ of WI-Madison; [4]Ob/Gyn, Univ of Vermont.*

Shear stress is the most potent physiologic stimulus for elevating endothelial NO production for flow-mediated vasodilatation. We measured *in vivo* shear stress during the Proliferative and Secretory phases and at 12 and 32 weeks of gestation *hypothesizing* that uterine blood flow (UBF) elevations in turn increase shear stress in early and late gestation. **Methods:** During Proliferative, Secretory phases, and at 12 and 32-34 weeks of pregnancy UA blood velocity and internal radius were measured bilaterally using color Doppler ultrasound. Blood viscosity was measured at shear rates in excess of 60/sec. **Results:** Compared to the Proliferative phase viscosity was decreased at 12 weeks and more so at 32 weeks gestation (P<0.05).

	Menstrual Cycle Phase	Pregnancy

(***P<0.001)	Proliferative; D=1-15 (n=100)	Secretory; D=19-26 (n=55)	Early 12 Weeks (n=15)	Late 32 Weeks (n=13)
UA Internal Radius (cm)	0.096 ± 0.002	0.096 ± 0.002 NSD	0.13 ± 0.004***	0.17 ± 0.008***
UA Velocity (cm/sec)	9.3 ± 0.57	15.3 ± 1.0***	31.4 ± 3.0***	55.7 ± 7.7***
UBF (ml/min)	15.0 ± 0.9	27 ± 2***	101.8± 12.9***	294.2 ± 40.3***
Shear Stress (dynes/cm^2)	20.7 ± 1.5	37.8 ± 2.62***	37.1 ± 3.8***	47.9 ± 6.8***

Internal UA radius was not altered by the menstrual cycle, and was greater at 12 weeks (+35%) and 32 weeks (+77%). Compared to Proliferative, Secretory phase showed significant rises in unilateral UBF and Velocity that rose progressively during gestation. In contrast, shear stress increased in Secretory (80%) and did not rise further in early pregnancy but by 32 weeks shear stresses was further elevated 131%. **Conclusions:** Equivalent rises in shear stress during the Secretory phase and 12 weeks gestation demonstrate increases in radius and profound remodeling of UAs that reflect the physiologic process of "normalization of shear". By late gestation, continued but modest rises in radius illustrate that further increases in shear stress occur almost solely due to rises in UBF via falls in down stream impedance. Continued rises in shear stress into late gestation provide progressive stimuli for NO production by UA endothelium. *NIH HL49210, HD38843, HL63101, HL71944.*

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Endogenous Estrogen Inhibition Using Letrozole in Ovine Pregnancy Decreases Uterine Artery Endothelial Nitric Oxide Production. Ronald R Magness,[1,2,3] FuXian Yi,[1] Steven Vang,[1] Terrance M Phernetton,[1] Jason L Austin.[1] *[1]Depts of Ob/Gyn, Perinatal Research Labs; [2]Peds; [3]Anim Sci, Univ Wisconsin-Madison.*

Estrogen increases NO production in uterine artery endothelial cells (UAEC) via an ER mechanism. ER blockade (ICI 182,780) and NO inhibition similarly lower gravid UBF 35-40%. Limits on the amount of ICI available preclude us from performing chronic ER inhibition studies *in vivo*. This is best tested using aromatase/estrogen synthesis blockers. **Methods/Results:** Aromatase inhibitor (Letrozole) given locally via a UA infusion (0.65ug/ml) in late gestation (d127±8) reduced gravid UBF 30-32% by 2 hrs. C19 estrogen precursor DHEA increased UBF (150ug/kg DHEA IV Bolus) similar to E2β (1ug/kg IV); maximal rises 207±28 ml/min. Letrozole completely blocked DHEA, but not E2β-induced increases in UBF. Chronic studies in late gestation (d120±10) sheep administering IM Letrozole 20mg (day 0) then 125ug/kg/day (day 1-12) vs Control (Vehicle) showed by 2 hrs of Letrozole loading dose a drop in UBF and lower maternal and fetal circulating estrogen but not progesterone. Estrogen replacement therapy (ERT) returned levels of estrogen to Control. Using LC-MS, the C19 steroid levels of Testosterone, DHEA and Androstenedione were not altered by Letrozole ± ERT. We studied the effect of chronic IM injections of Letrozole vs. Vehicle on *ex vivo* ATP (100uM; receptor mediated) and Ionomycin (1uM; nonreceptor mediated)-induced Ca2+ and NO responses in endothelium of intact UAs from late pregnancy (d127±8). Letrozole treatment elevated the initial ATP induced Ca2+ rise (3/6 sheep), but not sustained Ca2+ levels, while substantially reducing stimulated NO production levels. With Ionomycin, equal maximal Ca2+ levels were seen in UAs from in Control and Letrozole sheep, however Letrozole reduced maximum level and rate of NO production *ex vivo*, suggesting lower total eNOS expression. *Ex vivo* measurements of NOx, Ser635 phospho-eNOS, and total eNOS demonstrated significantly reduced UA and placental artery levels with Letrozole, which returned to Control with ERT. **Conclusions:** Letrozole rapidly crosses the placenta in high enough levels to inhibit aromatase activity thereby acutely lowering estrogen and UBF, but did not alter sensitivity of the uterine vascular bed to respond to maximal stimulating doses of E2β. Inhibition of estrogen production in pregnancy decreases eNOS and the ability of UA endothelium to produce NO. *NIH HL49210, HL87144, HD50578, and HD38843.*

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Mechanisms Underlying Maternal Venous Adaptation in Pregnancy. Cresta W Jones, Carolyn J Barron, Ira M Bernstein, George Osol. *Department of Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA.*

Objective: Despite its hemodynamic importance, little is known about the regulation of systemic venous tone in pregnancy. The purpose of this study was to characterize the effects of pregnancy on the mechanical properties, adrenergic reactivity and endothelial function of systemic veins.

Methods: Second-order mesenteric veins from late pregnant (LP, n=21) and age-matched virgin (NP, n = 11) Sprague Dawley rats were mounted in a vasograph for determination of diameter and distensibility as a function of pressure. Reactivity studies were then performed at a physiologic pressure (6 mmHg) using both an adrenergic constrictor (norepinephrine, NE), and an endothelial-mediated vasodilator stimulus (acetylcholine, ACh). Changes in response to ACh were also evaluated in the presence of nitric oxide (NO) synthase inhibition with L-NNA. Data were analyzed using the t-test or Mann-Whitney rank sum test, with p values ≤ 0.05 considered significant.

Results: Data are presented as LP vs. NP for each comparison. Venous baseline diameter was similar in both groups (403 ± 25 vs. 426 ± 30 μm, p = 0.58), and maximal distensibility was unchanged (16 ± 3 vs. 23 ± 8%, p = 0.30). Veins in both groups demonstrated marked constriction in response to NE administration (55 ± 7 vs. 43 ± 4%, p = 0.14). Sensitivity to NE was decreased in pregnancy (EC_{50} = 209 ± 57 vs. 56 ± 12 nM, p = 0.04), while the maximal constrictor response to NE was unchanged (62 ± 7 vs. 73 ± 4%, p=0.19). Sensitivity to ACh was increased in pregnancy (EC_{50} = 194 ± 60 vs. 935 ± 422 nM, p=0.03) as was the maximal dilatory response (56 ± 6 vs. 33 ± 10%, p = 0.05). After preincubation in L-NNA, the ACh EC_{50} (47 ± 19 nM, p= 0.21 vs. LP) and the maximal dilatory response (45 ± 11%, p=0.76) remained unchanged.

Conclusion: There were no significant changes in mesenteric venous diameter or distensibility in pregnancy. However, adrenergic reactivity was significantly decreased. This change was coupled with a concurrent increase in vasodilatory responsiveness which appears to result from non-NO dependent pathways. The combination of decreased adrenergic sensitivity, and increased sensitivity to and maximal effect of ACh would enhance venous capacitance and thereby facilitate the plasma volume expansion of normal pregnancy.

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Effects of Hypertension and Nitric Oxide (NO) Inhibition on Myogenic Reactivity in the Rat Uterine Vasculature in Pregnancy. Carolyn J Barron,[1] Maurizio Mandala,[2] George Osol.[1] *[1]Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA; [2]Cell Biology, University of Calabria, Italy.*

Background: Hypertension during pregnancy is associated with altered uterine vascular reactivity and blood flow, although its effects on arterial myogenic behavior have not been explored. The purpose of this study was to evaluate the effects of hypertension and NO inhibition on myogenic tone in pregnancy, as the ability of a vessel to constrict and dilate in response to pressure plays a key role in regulating blood flow to the uterus.

Methods: Three groups of Sprague Dawley late pregnant (day 20) rats were used: control (n=5), hypertensive (0.5g/L L-NAME in the drinking water, n=9), and treated with L-NAME and hydralazine (also in the drinking water, 0.272 g/L, n=5) to prevent the blood pressure increase, yet maintain NO inhibition. Resistance-sized radial arteries (<150 μm) were mounted in a pressure arteriograph and equilibrated at 60 mmHg (in PSS containing L-NNA and indomethacin) to induce a myogenic response. Vessels were then subjected to pressure steps from 20 to 200 mmHg. Tone (%) was calculated by comparing the vessel diameter at each pressure with the passive diameter at the same pressure (determined by incubation with 0.1mM papaverine and 10μM diltiazem).

Results: Myogenic tone developed in controls (46±5% maximal), and was maintained over a broad range of transmural pressures (40-200 mmHg). Arteries from the L-NAME group did not develop tone at any pressure. Co-treatment with hydralazine reinstated tone (33±4% maximal) over the same range of pressures as in the control group. The reduction in average placental weights in the L-NAME group (401 vs. 425mg, p<0.05) was restored by hydralazine (433mg, p<0.05 vs. L-NAME). Average fetal weights were also reduced in the L-NAME group (1.96 vs. 2.22g, p<0.001), but only partially restored by hydralazine co-treatment (2.11g, p<0.05 vs. control and L-NAME groups).

Conclusions: Surprisingly, radial uterine arteries from the L-NAME group did not develop tone over any range of pressures, despite the fact that matched arteries from late-pregnant controls developed significant myogenic tone. This abolishment of tone was reversed by hydralazine, which also had beneficial effects on fetal and placental growth. These results implicate hypertension rather than NO inhibition as the key factor in the suppression of myogenicitiy and dysregulation of uterine blood flow.

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Inhibition of Uterine Vascular Nitric Oxide Synthase (NOS) Minimally Alters Basal Uteroplacental Blood Flow ($UPBF_b$) in Ovine Pregnancy. Charles R Rosenfeld, Timothy Roy. *Division of Neonatal-Perinatal Medicine, UT Southwestern Medical Center at Dallas, Dallas, TX, USA.*

Background: $UPBF_b$ rises ~30-fold in ovine pregnancy; but the mechanisms responsible for the rise and maintenance are unclear. We (JSGI 2005) reported that uterine vascular smooth muscle BK_{Ca} K^+ channels contribute to uterine vasodilation and $UPBF_b$ maintenance; but up-stream activators are

unclear. Uterine vascular prostacyclin synthesis increases in pregnancy, but cyclooxygenase inhibition does not alter $UPBF_b$ (AJP 1992). Vascular nitric oxide synthase (NOS) also increases; but acute inhibition with L-NAME decreases $UPBF_b$ only ~25% (JCI 1996). It is unclear if L-NAME doses were insufficient or if prolonged NOS inhibition has a greater effect. **Objective:** To determine if local NOS inhibition with L-NAME dose-dependently decreases $UPBF_b$ and if prolonged inhibition exerts a greater effect on $UPBF_b$. **Methods:** 11 pregnant ewes were studied at 100-148d gestation age (GA). 4 had dose-response studies with uterine artery L-NAME infusions to achieve 0.025-1.0 mg/ml over 30min. 7 had 72h arterial L-NAME infusions to achieve and maintain levels of 0.01 mg/ml at 107 (n=6), 122 (n=7) and 135d (n=7) GA, while measuring arterial pressure (MAP), heart rate (HR) and $UPBF_b$ before, during (2,4,6,24,30,48,54 and 72h) and after (72, 96h) infusions. Uterine arterial and venous cGMP were measured. Data were analyzed by ANOVA. **Results:** Acute NOS inhibition decreased $UPBF_b$ 0-20%, but was not dose-related. 72h arterial L-NAME infusions decreased $UPBF_b$ by 2-6h at each GA ($P \leq 0.025$, ANOVA) and values returned to baseline by 96h postinfusion. Sensitivity did not differ between GA ($P=0.1$, ANOVA), $UPBF_b$ falling 20-30% at each GA. Contralateral $UPBF_b$ was unaffected at all GA. MAP rose and HR fell during infusions at 107 and 122d GA, $P \leq 0.03$; but were unaffected at 135d GA. Venous-arterial cGMP concentration differences were seen at 107d and absent at 72h of L-NAME infusion, $P=0.04$. **Conclusions:** Uterine vascular NOS increases in ovine pregnancy, but its inhibition decreases $UPBF_b$ ~30%, suggesting study doses were insufficient to fully inhibit vascular NOS activity. Alternatively, NOS contributes to the maintenance of $UPBF_b$, but other mediators, not yet identified, are more important in activating BK_{Ca} and regulating $UPBF_b$. Notably, L-NAME reached the systemic circulation, and although further diluted, MAP rose 5-10%, suggesting the systemic vasculature may be more sensitive to NOS inhibition than $UPBF_b$.

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Uterine Artery Large Conductance Ca^{+2}-Dependent K^+ Channels (BK_{Ca}) and Signaling Pathways Are Up-Regulated during Ovine Pregnancy. Charles R Rosenfeld, Xiao-tie Liu. *Division of Neonatal-Perinatal Medicine, University of Texas Southwestern Medical School, Dallas, TX, USA.*

Background: Uteroplacental blood flow (UPBF) rises ~40-fold in ovine pregnancy, reflecting increases during pre-implantation, placentation and finally, in the last third of gestation. We reported that BK_{Ca} density in uterine vascular smooth muscle (UVSM) increases in ovine pregnancy (SGI 2007) and inhibition with tetraethylammonium (≤0.3 mM) dose-dependently decreases basal UPBF ~80% in the last third of pregnancy (JSGI 2005). It is unclear how BK_{Ca} subunit expression changes and activity is regulated in pregnancy; since uterine vascular nitric oxide is increased, signaling via cGMP-dependent protein kinase G (PKG) is one potential pathway. **Objective:** To determine simultaneous changes in UVSM BK_{Ca} density and regulatory β1-subunit expression and the cGMP signaling pathway for activation in ovine pregnancy. **Methods:** Endothelium-denuded segments of 2nd generation uterine arteries obtained from nonpregnant (n=3), pregnant (n=14, 56-145d gestation; term 150d) and postpartum (n=8, 2-56d) sheep were used to measure expression of BK_{Ca} α- and regulatory β1-subunits and signaling proteins in UVSM by immunoblot analysis and immunohistochemistry. **Results:** UVSM BK_{Ca} density, reflected as a change in the 100 kDa α-subunit, rose 70% with placentation ($P<0.05$) and was unchanged thereafter. Expression of the 39 kDa regulatory β1-subunit paralleled the rise in BK_{Ca} density during placentation, increasing 50% ($P<0.001$), but increased another 2-fold and exponentially in the last third of pregnancy ($P<0.001$, $R^2=0.99$, n=13). Changes in subunit immunostaining in UVSM paralleled increases in protein. Although UVSM soluble guanylyl cyclase was unchanged in pregnancy ($P=0.8$, R=0.08, n=14), PKG expression rose ~2-fold ($P=0.002$, R=0.86, n=11) and gradually returned to nonpregnant levels by 30d postpartum ($P=0.008$, R=0.83, n=9). UVSM cGMP is being measured. **Conclusions:** These are the 1st data suggesting that increases in ovine UPBF during placentation involve vascular growth and BK_{Ca}-mediated vasodilation. The rise in UPBF in the last third of pregnancy reflects BK_{Ca}-mediated vasodilation due to enhanced channel activation via increases in UVSM PKG, BK_{Ca} phosphorylation and changes in subunit stoichiometry due to an exponential rise in the regulatory β1-subunit. It is unclear what initiates and directs these changes in BK_{Ca} expression and sensitivity.

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Relaxin Regulates Arterial Wall Structure and Composition. Dan O Debrah,[1] Julianna E Debrah,[2] Sanjeev G Shroff,[1] Kirk P Conrad.[3] *[1]Bioengineering, University of Pittsburgh; [2]Magee-Womens Research Institute; [3]Physiology and Functional Genomics, University of Florida.*

Relaxin (Rlx) is a hormone traditionally associated with changes in the female reproductive tract during pregnacy. Recent evidence suggests that Rlx may play a pivotal role in regulating cardiovascular function during gestation. Analogous to pregnancy, administration of recombinant human relaxin (rhRlx) to nonpregnant female rats reduces systemic vascular resistance, as well as increases global arterial compliance. Additionally, we demonstrated that, concurrent with Rlx´s influence on overall cardiovascular function, small renal arteries (SRA) from rhRlx-treated mice and rats are characterized by reduced passive stiffness and increased arterial wall area whereas external iliac arteries (EIA) are not. We hypothesized that Rlx regulates arterial passive mechanical properties by altering the cellular and biochemical composition of the arterial wall.

Nonpregnant female mice were administered rhRlx for 5 days after which SRA and EIA were isolated. We measured arterial collagen, elastin, and total protein using the Sircol collagen assay, the Fastin elastin assay, and the Pierce BCA protein assay, respectively. Additionally, we quantified arterial smooth muscle cell (SMC) density using immunofluorescent techniques.

SRA isolated from rhRlx-treated mice were characterized by a significant reduction in collagen to total protein ratio (0.13±0.01 *vs* 0.19±0.01 µg collagen/µg protein; mean±SEM; $P<0.02$) as well as a significant increase in SMC density (6.1±0.1 *vs* 5.0±0.1 cells/1000 µm²; $P<0.001$) compared to control mice with no significant change in elastin content (104±4 *vs* 97±14 µg elastin/mg dry weight). In contrast, there were no significant changes in collagen to total protein ratio (0.25±0.02 *vs* 0.28±0.04 µg collagen/µg protein), SMC density (3.9±0.2 *vs* 4.0±0.2 cells/1000 µm²) or elastin content (89±12 *vs* 83±5 µg elastin/mg dry weight) in EIA from the rhRlx-treated mice compared to control mice. Of note, comparable results were observed for Rlx knock-out ($Rlx^{-/-}$) and wild-type mice with $Rlx^{-/-}$ mice exhibiting increased arterial collagen and decreased SMC density.

We conclude that the Rlx-induced decrease in passive stiffness of SRA that we previously reported is, at least in part, due to Rlx-induced alterations in arterial wall cellular and biochemical composition. Further, our findings suggest that these vessel wall remodeling effects of Rlx are artery-type specific.

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Changes in Arterial Function by Chronic Relaxin Infusion Are Mediated by the Leucine Rich Repeat G Coupled Lgr7 Receptor. Julianna E Debrah,[1] Alexander Agoulnik,[2] Kirk P Conrad.[3] *[1]Magee Womens Research Institute, Pittsburgh, PA, USA; [2]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA; [3]Department of Physiology and Functional Genomics, University of Florida College of Medicine, Gainesville, FL, USA.*

Relaxin is a peptide hormone that emanates from the corpus luteum of the ovary and circulates during pregnancy. This hormone plays an important role in renal vasodilation and hyperfiltration, two fundamental maternal adaptations in pregnancy. Chronic administration of recombinant human relaxin (rhRLX) to both virgin rats and mice inhibits myogenic reactivity and increases compliance of small renal arteries, thus further mimicking pregnancy. We hypothesize that these arterial responses to rhRLX are mediated by the Lgr7, and not the Lgr8 receptor. Both Lgr7 and Lgr8 receptor-deficient, and wild-type virgin mice were investigated. The mice were chronically infused with rhRLX or vehicle (VEH) for 5 days. Small renal arteries were isolated and mounted in a pressure arteriograph and myogenic reactivity was assessed (% change in diameter over baseline in response to a 20 mmHg step increase in intraluminal pressure). Small renal arteries from rhRLX-infused Lgr7 wild-type mice showed inhibited myogenic reactivity with a 6.1 ± 0.5% increase in diameter whereas the arteries from rhRLX-infused Lgr7 knock-out mice exhibited robust myogenic reactivity with only a 1.2 ± 0.4% change in diameter ($p=0.001$). In contrast, myogenic reactivity of small renal arteries was inhibited in both the rhRLX-infused Lgr8 knock-out and wild-type mice. The VEH-treated Lgr7 and Lgr8 mice, regardless of genotype, exhibited robust myogenic reactivity. Arterial compliance was also assessed for each genotype/treatment group. rhRLX infusion increased arterial compliance of small renal arteries from Lgr7wild-type, but not from Lgr7 knock-out mice ($p=0.01$). In contrast, the arteries from rhRLX-infused Lgr8 wild-type and knock-out mice showed increased compliance relative to VEH-infused animals. **Conclusion:** Relaxin-induced inhibition of myogenic reacitivity and increase in compliance of small renal arteries is mediated by the Lgr7, and not the Lgr8 receptor.

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Cigarette Exposure Induced Fetal Growth Restriction: Role of Peripheral and Uterine Vascular Changes. Arun Jeyabalan,[1,2] Julie Debrah,[1] Stacy McGonigal,[1] Jennifer Rohland,[1] Julie A Deloia,[1,2] Robin E Gandley.[1,3] *[1]Magee-Womens Research Institute; [2]Obstetrics, Gynecology, and Reproductive Sciences; [3]Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, USA.*

Objective: Smoking is associated with adverse pregnancy outcomes including fetal growth restriction. Pathologic effects of smoking on maternal vasculature is a potential mechanism leading to fetal growth restriction. The objective of this study was to determine whether cigarette smoke exposure during pregnancy affects the functional properties of uterine and peripheral arteries using a gravid murine model.

Study Design: Pregnant and virgin C57Bl/CJ mice were exposed to whole body side stream smoke using an inhalational chamber for 4 hours/day. Smoke exposure was increased from day 4 of gestation until late pregnancy (day 16-19) with mean total suspended particle levels of 63 mg/m3, representative of moderate to heavy smoking in humans. Control animals were exposed to ambient room air. Late pregnant and virgin mice were sacrificed and uterine, mesenteric, and renal arteries were isolated and studied in a pressure arteriograph system (n=3-6 in each group). Plasma cotinine was measured by ELISA. Means were compared using t-test or analysis of variance.

Results: Fetal weights were significantly reduced in mice exposed to smoke compared to control fetuses (0.88 ± 0.1g vs 1.0 ± 0.08g, p=0.02), while litter sizes were not different. Cotinine levels in smoking mice were significantly elevated compared to control mice (51.9 ± 9.2 vs 4.2 ± 0.8ng/ml for virgin mice and 33.2 ± 21 vs 2.8 ± 0.4ng/mL for pregnant mice). There was no significant difference in phenylephrine responses between groups. Endothelial mediated relaxation responses to methacholine were significantly impaired in both the uterine and mesenteric vasculature of pregnant mice exposed to cigarette smoke during gestation. No difference in endothelial-mediated relaxation was seen in isolated renal arteries in pregnant mice exposed to cigarette smoke, however relaxation was significantly reduced in renal arteries from smoke-exposed virgin mice.

Conclusions: Passive cigarette smoke exposure is associated with impaired vascular relaxation of uterine and mesenteric arteries in a gravid murine model. Functional vascular perturbations during pregnancy, specifically reduced uterine blood flow and impaired peripheral vasodilation, may be a mechanism by which smoking results in fetal growth restriction.

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The Role of Human Chorionic Gonadotropin in Maternal Vasodilation of Pregnancy. Lee Danielson,[1] Julianna E Debrah,[2] Kirk P Conrad.[3] *[1]University of New Mexico Health Sciences Center, Albuquerque, NM, USA; [2]Magee-Womens Research Institute, Pittsburgh, PA, USA; [3]Physiology and Functional Genomics, and Obstetrics and Gynecology, University of Florida College of Medicine, Gainesville, FL, USA.*

Human chorionic gonadotropin (hCG) is essential during early human gestation for "rescue" of the corpus luteum. However, its potential contribution to the widespread maternal vasodilation of pregnancy that occurs at this stage of pregnancy remains uncertain. Our objective was to use the renal circulation of conscious rats as an experimental model in which to test the vasodilatory potential of hCG. In addition, we investigated both myogenic reactivity and relaxation responses in small renal and mesenteric arteries isolated from rats, as well as in small human subcutaneous arteries using a pressure arteriograph.

We chronically instrumented 10 rats for measurement of renal function. Five were ovariectomized, and 5 sham-ovariectomized. After 7 days of surgical recovery, baseline glomerular filtration (GFR) and effective renal plasma flow (ERPF) were measured on two separate days and the values averaged. Then, an osmotic minipump containing hCG (25 IU/min) was implanted s.c. and renal function was again assessed 2 and 5 days thereafter. GFR and ERPF significantly increased and calculated effective renal vascular resistance decreased from baseline in the intact ($P<0.001$ vs. baseline), but not ovariectomized ($P=NS$) rats on both days 2 and 5 of hCG administration. In the intact, but not ovariectomized rats, plasma osmolality declined and progesterone increased (both $P<0.001$ vs baseline). Plasma hCG concentrations were ~ 2,500 mIU/ml and comparable in both groups of rats. Incubation of small renal arteries from rats with recombinant human relaxin (rhRLX, 1-100 ng/ml), but not hCG (2,500-200,000 mIU/ml) in vitro inhibited myogenic reactivity and relaxed phenylephrine (PE)-constricted arteries. In contrast, both rhRLX and hCG inhibited myogenic reactivity and relaxed PE-constricted small mesenteric arteries from rats and small human subcutaneously arteries.

In conclusion, consistent with our earlier work showing a virtually exclusive role for relaxin in mediating the renal circulatory changes of pregnancy, hCG is likely to play little or no role. In contrast, hCG and relaxin are both likely to contribute to the vasodilation of other organ circulations during pregnancy.

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Mediators of Estradiol Smooth Muscle Relaxation in Rat Uterine Artery. Pierre-Andre Scott,[1,2] Michele Brochu,[2] Jean St-Louis.[1,2] *[1]Research Centre, CHU Ste-Justine, Montréal, QC, Canada; [2]Pharmacology, Université de Montréal, Montréal, QC, Canada.*

Uterine vasculature undergoes major structural and functional changes during pregnancy. Estrogens have been shown to induce increased uterine blood flow in this circulation. We have reported that estradiol (17β-E2) induced direct vasorelaxant response on smooth muscle of the uterine arteries that, for it major part, is not mediated through tissue nitric oxide. To investigate the cellular effectors mediating this vasorelaxant effect of 17β-E2, we set-up uterine arteries of non-pregnant rats in wire myograph systems for microvessels. 17β-E2 and 17α-E2 were equipotent in relaxing PhE (1 µmol/L)-preconstricted uterine arteries, although the later produced significant smaller relaxations. Genistein, a phytoestrogen presumed to inhibit phosphatases, also produced uterine artery relaxation with significant lower potency. To try interfere with the vasorelaxant effect of 17β-E2, tissues were preincubated with different substances. Cycloheximide (protein biosysthesis inhibitor), ICI 182,780 (estrogen receptor modulator), and KT5720 (PKA inhibitor) did not significantly influenced the response to 17β-E2. RP-8-pCPT-cGMPS (PKG inhibitor) slightly displaced the concentration-relaxation curve to 17β-E2 to the right. Inhibitors of potassium channels, penitrem A (BKCa) and glybenclamide (KATP), showed opposite effects for 17β-E2 concentration-response curve; the former producing a right shift and the latter a small not significant left shift. These data indicated that the direct acute effect of 17β-E2 in uterine artery is the result of complex interactions within smooth muscle cells, involving potassium channels, and protein kinases and phosphatases.

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Effects of Aldosterone on Vascular Adaptations to Pregnancy. Mylene Provencher,[1,2] Veronique Houde,[1,3] Michele Brochu,[1,3] Jean St-Louis.[1,2,3] *[1]Research Centre, CHU Ste-Justine, Montréal, QC, Canada; [2]Pharmacology, Université de Montréal, Montréal, QC, Canada; [3]Obstetric-Gynecology, Université de Montréal, Montréal, QC, Canada.*

The renin-angiotensin-aldosterone system is paradoxically activated during pregnancy, since blood pressure decreased. Earlier data showed that the high levels of aldosterone present during pregnancy may be involved in cardiovascular adaptation to pregnancy. In order to delineate this effect of aldosterone on vascular tone, potassium canrenoate, an antagonist of mineralocorticoid receptors (MR), was administered (20 mg/kg/day) to pregnant rats from the day 15 to 22 of gestation. Rats were sacrificed at day 17, 19 and 22 of gestation together with untreated and day 14 pregnant rats. The thoracic aorta was quickly removed and set up in tissue baths as ring (2-3mm) preparation. As observed previously, canrenoate enhanced responsiveness to PhE for all time of treatment tested, but only at day 17 (2 days treatment) for KCl responsiveness. Aortic contractile responses to TEA (tetraethylammonium) gradually decreased during pregnancy to almost disappear at the end of gestation. In the present results, canrenoate treatment made the response statistically different by day 17 of pregnancy. Finally, the activity of Na/K-ATPase was measured by the relaxant effect of KCl added to physiological solution without potassium. The activity of the pump was decreased when approaching parturition compare to day 14 of pregnancy. Canrenoate treatment abolished this effect. The present data show that vascular changes that occurred during pregnancy are markedly modified by treatment of rats with an antagonist of mineralocorticoid receptors, suggesting that aldosterone may be involved in vascular adaptation to pregnancy.

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Effect of Diabetes on Mechanisms of Endothelium-Dependent Vasodilatation in Uterine Arteries of Pregnant Mice. Joanna L Stanley,[1] Nick Ashton,[2] Sandra T Davidge,[3] Michael J Taggart,[4] Philip N Baker.[1] *[1]Maternal & Fetal Health Research Group, University of Manchester, Manchester, United Kingdom; [2]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom; [3]Department of Obstetrics/Gynecology, University of Alberta, Edmonton, AB, Canada; [4]Institute of Cellular Medicine, University of Newcastle, Newcastle, United Kingdom.*

Background Impaired endothelium-dependent vasodilatation has previously been demonstrated in small myometrial arteries from women with gestational

diabetes. This impairment may play a role in mediating the complications observed in diabetic pregnancies. It is not known which mechanisms of endothelium-dependent vasodilatation are affected in myometrial arteries by gestational diabetes.
Aim To investigate mechanisms of endothelium-dependent vasodilatation in uterine arteries using a mouse model of pregnancy complicated by diabetes.
Methods Diabetes was induced in female C57Bl6/J mice (streptozotocin; 200 mg/kg) prior to mating. Mice were culled at day 19 of pregnancy (term) and uterine arteries dissected, mounted on a wire myograph, normalised to ~ 55mmHg and equilibrated (37°C; 5%CO_2/air). Arteries were constricted with phenylephrine (10μM) and a concentration-response curve to the endothelium-dependent vasodilator acetylcholine (ACh; 0.1nM-1μM) constructed in the presence and absence of a nitric oxide synthase inhibitor (L-NNA; 10μM), a cyclooxygenase inhibitor (indomethacin; 10μM) or a combination of the two to determine the contribution of nitric oxide (NO), prostacyclin and endothelium derived hyperpolarising factor (EDHF) to vasodilatation.
Results Sensitivity to ACh was comparable between diabetic and vehicle treated mice (EC_{50} 31.4 ± 8.0nM vs 41.9 ± 15.9nM). The contribution of individual endothelium-dependent vasodilators was significantly altered in arteries from diabetic mice. At 1μM ACh, EDHF-mediated relaxation was significantly reduced (p=0.03, one-way ANOVA) compared with controls (13.17 ± 4.87 vs 67.23 ± 13). In comparison, NO-mediated relaxation was significantly increased (p=0.04, one-way ANOVA) compared with controls (64.88 ± 10.78 vs 17.54 ± 5.77%).
Conclusions Endothelium-dependent relaxation was not reduced in uterine arteries of diabetic mice compared with controls. However, there was a profound change in the contribution of endothelium-dependent vasodilators in arteries of diabetic mice. This may alter compensatory capacity as disease progresses.
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Role of Raf Kinase in Contractile Effects of Phenyleperine on Mesenteric Arteries from Virgin and Pregnant Rats. K Sathishkumar, Uma Yallampalli, Chandra Yallampalli. *Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
Raf-1 serine/threonine protein kinase has been extensively studied as the upstream kinase linking Ras activation to the MEK/ERK module. MEK/ERK has been shown to play a role in the modulation of vascular contraction. However, the role of Raf kinase in vascular contraction and its possible involvement in alteration of maternal vascular function during pregnancy is not known.
Objectives: To determine (1) if Raf kinase contributes to phenylephrine (PE)-induced contractile response, (2) the role of Raf kinase inhibitor (GW5074) in regulating vascular tone during pregnancy, and (3) mechanism by which GW5074 produces vasodilatation in rat mesenteric arteries.
Methods and Results: Conscious non pregnant (NP) and pregnant (P) Sprague Dawley rats received increasing doses of PE in the absence or presence of GW5074. PE induced a dose-dependent increase in MAP in both NP and P rats but responses were substantially depressed in pregnancy. GW5074 shifted the PE-induced dose response to the right in both NP and P rats. GW5074 itself did not affect basal blood pressure. Isometric tension studies in mesenteric arteries showed that GW5074 did not change the KCl-evoked contraction but significantly inhibited the contraction to PE in both NP and P arteries. Interestingly, at a given concentration, GW5074 produced greater inhibition of PE-induced contractile response in P than in NP arteries. Also, in P arteries the inhibitory effects of GW5074 were greater as the pregnancy progressed from day 18 to day 20. Raf kinase expression and activity was significantly decreased in arteries of P compared to NP rats. In mesenteric vascular smooth muscle cells (VSMCs), PE stimulated activation of Raf kinase as indicated by phosphorylation of its immediate target, MEK1/2 in a time- and dose-dependent manner. Measurement of $[Ca^{2+}]_i$ with Fura-2 showed that GW5074-mediated inhibition of PE-induced contraction was not associated with decrease in $[Ca^{2+}]_i$. VSMCs treated with PE exhibited higher levels of the contractile proteins, p-MYPT1 and p-MLC20 which was inhibited by GW5074.
Conclusion: To our knowledge, for the first time we show that Raf kinase plays an important role in the regulation of vascular contractility. Decreased expression and/or activity of Raf kinase during pregnancy may explain in part, for decreased systemic vascular reactivity during late gestation.

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Changes in Expression and Activity of Ras Proteins in Mesenteric Vascular Smooth Muscle during Pregnancy: Influence on Vascular Reactivity. K Sathishkumar, Uma Yallampalli, Rebekah Elkins, Chandra Yallampalli. *Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*
Objectives: The mechanism(s) that contribute to reduced vascular sensitivity to phenyleperine (PE) during pregnancy is not well understood. PE, in addition to activating the classical contractile pathways, also stimulates growth factor pathways that results in activation of Ras/mitogen-activated protein kinases. It is not clear whether these pathways play a role in the modulation of vascular contraction. Hence, the present study was designed to determine 1) if Ras is involved in mediating the pressor response to PE in non pregnant (NP) and pregnant (P) rats, 2) if so, the mechanism by which Ras protein contributes to PE-induced vascular contraction, and 3) any differential expression and/or activity of Ras during pregnancy that might contribute to altered vascular response.
Methods: 1) Mean arterial pressure (MAP) was measured in conscious NP and P rats. 2) Isometric tension was measured in mesenteric arteries of NP and P rats. 3) Expression of contractile proteins, p-MLC20 and p-MYPT1 were studied in cultured vascular smooth muscle cells (VSMCs). 4) Expression and activity of Ras in mesenteric arteries of NP and P rats were measured by Western blot analysis.
Results: (1) Intravenous administration of PE resulted in a dose-dependent increase in MAP but responses were substantially depressed in pregnancy. Inhibiting Ras activation with manumycin, decreased PE-induced increase in MAP in both NP and P rats. Manumycin by itself had no effects on basal MAP. (2) Isometric contraction studies in myograph with mesenteric arteries showed that manumycin shifted PE-induced dose response curve to the right with a decrease in E_{max} in NP and P rats. Higher concentration of manumycin was required to inhibit PE-induced contraction in NP (≥ 10 μM) compared to P (1 – 3 μM) rats. (3) Pretreatment with manumycin inhibited PE-induced increase in the extent of phosphorylation of both MLC20 and MYPT1 in MVSMCs. (4) Compared to P rats, mesenteric arteries of NP rats revealed increased basal and PE-induced expression and activity of Ras. Reduced expression of this contractile Ras pathway might contribute to decreased vascular reactivity during pregnancy.
Conclusion: Ras protein plays a role in regulation of vascular contraction. The decreased amount and activity of vascular Ras may be a novel mechanism that explains the reduced vascular resistance during pregnancy.

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Overweight Affects the Relaxin-Induced Response of Mesenteric Arteries. Joris van Drongelen,[1] Arianne van Koppen,[2] Marc EA Spaanderman,[1] Paul ABM Smits,[2] Frederik K Lotgering.[1] *[1]Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*
OBJECTIVE: Overweight affects pregnancy-induced vascular adaptation and predisposes to gestational hypertensive disease. Relaxin plays a key role in normal gestational vascular adaptation by increasing endothelium-dependent vasodilation and compliance, and reducing myogenic reactivity. We hypothesized that overweight blunts the vascular response to relaxin.
METHODS: The vascular responses to flow and pressure of mesenteric arteries after pre-treatment to human recombinant relaxin (RLX) or placebo (PLAC) were examined in overweight (OW) and normal weight (NW) rats in a pressure-perfusion myograph. Overweight was established by a high-fat diet. The endothelium-dependent vasodilatation was measured in response to flow: E_{50} (flow inducing 50% dilatation) and E_{max} (maximal dilative effect). Active contractile (myogenic reactivity) and passive dilative (compliance) vascular responses to pressure were determined. All vascular responses were calculated as the proportional change in diameter to 40% precontraction with U46619.
RESULTS: In NW rats RLX decreased E_{50} and increased E_{max} to flow and attenuated myogenic reactivity without affecting vascular compliance. In OW PLAC-treated rats, compared to NW rats, an upregulated vasodilative state was present (decreased E_{50} and increased E_{max}, and lower myogenic reactivity). In OW rats RLX increased E_{50} to flow and unaffected E_{max}. RLX increased myogenic reactivity mildly in absence of changes in vascular compliance.
Table 1

		Flow		Myogenic reactivity	Compliance
		E50	Emax	Slope	Slope
		(μl/min)	(%)	(%/10mmHg)	(%/10mmHg)

NW	**PLAC**	50±3	22±1	+1.4±0.2	0.4±0.1
	RLX	30±2#	25±1#	-4.1±0.6#	0.3±0.1
OW	**PLAC**	38±2*	33±1*	-0.8±0.1*	0.3±0.1
	RLX	55±2#	31±1	-2.7±0.6#	0.4±0.1

Values are presented as mean±SEM; * $p<0.05$ between NW/OW, # $p<0.05$ within NW/OW.

CONCLUSIONS: Whereas RLX stimulates endothelium-dependent vasodilation to flow and myogenic reactivity in NW rats, OW overturns the flow-induced response and decreases myogenic reactivity to a lesser extent than present in NW rats. We speculate that these overweight-induced adverse effects of RLX prelude to vascular maladaptation and gestational hypertensive disease.

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Shear Stress Modulation of Endothelial Nitric Oxide Synthase (eNOS) Phosphorylation Responses in Follicular, Luteal and Pregnant Derived Ovine Uterine Artery Endothelial Cells (UAEC). Christopher K Huls,[1] Gladys E Lopez,[1] Dinesh M Shah,[1] Ronald R Magness.[1,2,3] *[1]Depts of Obstetrics & Gynecology; [2]Animal Sciences; [3]Pediatrics, Univ of Wisconsin, Madison, WI, USA.*

Compared to the Luteal (Lut) Phase, uterine blood flow is increased *in vivo* during the Follicular (Fol) Phase and more so during Pregnancy (Preg). Both of these are physiologic states of high estrogen and shear stress. Endothelial cells express eNOS and produce greater amounts of nitric oxide (NO) in response to elevations of shear stress. Phosphorylation of eNOS is a signaling marker of activation. We have recently validated Lut, Fol and Preg UAEC culture models for evaluating eNOS phosphorylation responses to shear stress and vascular mediators.

We **hypothesized** that UAECs derived from Fol and Preg sheep will show greater eNOS phosphorylation than Lut phase UAECs, and with more robust responses in the presence of Estrogen ($E_2\beta$).

Methods: UAECs were cultured until 80% confluence, and then subjected to 0 (static control), 3 or 15 dynes/cm^2 for 48hrs in the absence or presence of $E_2\beta$ (10nM). Western analysis was used to compare optical densities of Ser635-peNOS (peNOS) normalized to total-eNOS (mean $\pm$ SEM).

Results: In Lut UAEC peNOS was equally increased two fold by 3 and 15 dynes/cm^2 (from 1.26 $\pm$ 0.5 to 2.4 $\pm$ 0.008 and 2.56 $\pm$ 0.06, respectively). Compared to Lut UAECs, the static control Fol UAECs appeared to have higher peNOS levels (2.2 $\pm$ 1.5) and this was further increased 1.5-1.8 fold with 3 dynes/cm^2 (3.2 $\pm$ 0.8) and 15 dynes/cm^2 (3.9 $\pm$ 0.38). As seen with Fol UAECs, Preg UAEC static peNOS (1.8 $\pm$ 0.4) appeared higher than Lut UAEC, but unexpectedly, neither 3 nor 15 dynes/cm^2 significantly raised these levels of peNOS (1.76 $\pm$ 0.05 and 1.6 $\pm$ 0.04). Regardless of shear stress level, $E_2\beta$ replacement in the culture media only increased the peNOS levels in the nonpregnant, but not the pregnant derived UAECs.

Conclusions: Increasing amounts of shear stress have a corresponding increase in the ratio of eNOS that is phosphorylated at Serine635 in Lut and Fol UAECs. Pregnant UAECs do not appear to increase the constitutive ratio of Serine635 phosphorylation of eNOS at either 3 or 15 dynes/cm^2. In contrast to our hypothesis, chronic treatment with $E_2\beta$ for 48hrs did not augment the ratio of eNOS phosphorylation in pregnancy. *NIH HL49210, HD38843, HL87144.*

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In Situ Detection of S-Nitrosylated Proteins in Estrogen Treated Endothelial Cells. Honghai Zhang, Wu Xiang Liao, Dong-bao Chen. *Reproductive Medicine, University of California San Diego, La Jolla, CA, USA.*

S-nitrosylation (SNO) is a rapid, reversible, and nitric oxide (NO) dependent post-translational protein modification critical for signal transduction. Estrogen stimulates endothelial cell (EC) NO production but yet to be determined is if this leads to increased formation of SNO-proteins in EC. **Hypothesis**: We hypothesize that estrogen stimulates the formation of SNO via a receptor and endogenous NO dependent pathway in HUVEC or ovine uterine artery EC. **Methods**: Cells on glass coverslips were treated with 1 mM of NO donor GSNO or DEA NONOate, estradiol-17β (10 nM) in the presence of L-NAME (1 mM) for 30 min. The cells were fixed with methanol. In situ SNO-proteins were detected by a rapid biotin derivatization method by blocking thiols by methylmethanethiosulfonate (MMTS), followed by ascorbate reduction and labeling with Texas Red-labeled ethylmethanethiosulfonate (MTSEA) and examined by fluorescence microscopy. Cells (~3x10^6/group) were lysed in HEN buffer, and then similarly prepared but labeled with biotin-MTSEA. A portion of the samples were separated on SDS-PAGE and blotted with anti-biotin antibody for total SNO-protein patterns. The biotin-labeled SNO- proteins were captured by avidin, followed by immunoblotting with specific antibodies. **Results**: Basal SNO-protein labeling was apparent in both EC types. Exogenous NO donated by GSNO or DEA NONOate significantly increased red fluorescence labeling of SNO-proteins in the cytosol of both EC types. Treatment with estradiol-17β also increased total SNO-protein labeling in both EC types. Pretreatment with L-NAME significantly reduced SNO-protein labeling. SDS-PAGE and immunoblotting with anti-biotin antibody analyses identified multiple bands of SNO-proteins. In avidin captured biotinylated samples, multiple proteins were indentified. **Conclusion**: Exogenous NO donated by GSNO or DEA NONOate and endogenous NO upon estrogen stimulation increased S-nitrosylated proteins in EC (Supported by NIH RO1 HL70562 and 74947).

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Ginger Root Juice Down-Regulates Renin Secretion by Term Human Decidual Cells *In Vitro*. Ziming Yu, Dinesh M Shah, Xiu-Yu Yi. *Department of Obstetrics and Gynecology, University of Wisconsin, Madison, WI, USA.*

Background and Objective: The renin-angiotensin system (RAS) functions both systemically and locally as a primary regulator of blood pressure and fluid volume and is therefore involved in the etiopathogenesis of essential hypertension as well as preeclampsia, a pregnancy-induced hypertensive disorder in pregnant women. While much progress has been made in the development of strategies to block the systemic RAS and thus treat essential hypertension, relatively less advance has been achieved in the development of effective local RAS inhibitors to treat preeclampsia, primarily due to the fact the conventional systemic RAS inhibitors generate potential teratogenic risks to pregnant women during critical stages of their pregnancies. Ginger has been widely and effectively used in pregnant women to treat pregnancy-induced nausea and vomiting with minimal adverse effects. The present study was designed to investigate whether ginger could down-regulate renin secretion by human decidual cells (the major source of renin in the tissue-based uteroplacental RAS), as an initial step toward a long-term goal to develop potential safe and effective RAS inhibitors for use in obstetric patients. **Methods**: Full-term normal human placentas were obtained within one hour of vaginal deliveries or Cesarean sections. Decidual cells were isolated from the decidua parietalis. After an initial culture for 2~3 days in a serum-containing medium, the decidual cells were treated with ginger juice at various doses in a serum-free medium for 24 hours. The culture supernatants were then harvested and subject to Western blot analyses of renin protein contents. The ginger juice used was freshly prepared from the ginger roots purchased from a local grocery store and different batches of juice preparations were standardized based on their optical density values at a wavelength of 400 μM on a spectrophotometer. **Results**: A dominant band of renin at approximately 47 kD was detected in all samples. When compared with the control (i.e. 0%), ginger juice decreased the renin protein contents in the culture supernatants in a dose-dependent manner. No significant difference in the cell morphology was observed between the control and treatment groups. **Conclusion**: Ginger root juice down-regulates renin secretion in human decidua, showing a great potential of a novel RAS inhibitor, particularly, in obstetric patients.

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Noninvasive Quantification of the Autonomic Nervous System throughout Pregnancy. Dietmar Schlembach,[1] Karoline Pickel,[1] Daniela Ulrich,[1] Philipp Klaritsch,[1] Isa Alkan,[2] Uwe Lang,[1] Manfred G Moertl.[1] *[1]Obstetrics and Gynecology, Medical University of Graz, Graz, Styria, Austria; [2]CNSystems Medizintechnik AG, Graz, Styria, Austria.*

Introduction:

The analysis of heart rate variability (HRV), blood pressure variability (BPV), and baroreflex sensitivity (BRS) has become a powerful tool for the assessment of autonomic control. Although HRV analysis was initially developed for risk stratification in cardiology, the field of clinical application has broadened in recent years. The aim of our study was to investigate the adaptation of autonomic control during pregnancy based on analysis of heart rate variability and baroreceptor sensitivity.

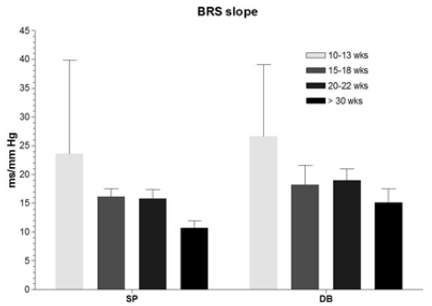
Methods:

20 patients with uncomplicated pregnancy were measured with the TASKFORCE® Monitor 2040i made by CNSystems. All measurements were performed in supine position under standardized resting conditions. Autonomic parameters were recorded at rest and within the “deep breathing method” as a “cardiovascular challenge test”.

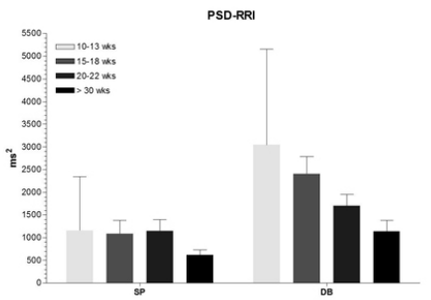
Results:

Throughout pregnancy a shift to higher sympathetic activity respectively a

lower parasympathetic activity was observed. Baroreflexsensitivity decreased throughout pregnancy (23.55±16.27 [10-13 wks], 16.15±5.54 [15-18 wks], 15.76±7.28 [20-22 wks], and 16.15±5.54 [>30 wks]) (figure 1).



During the deep breathing maneuver we could show an increase of the sympathetic / parasympathetic ratio (figure 2).



Discussion:
The noninvasive determination of autonomic parameters throughout pregnancy is possible. These results can be used as basic parameters for classifying and assessing autonomic changes in pathological conditions in pregnancy such as hypertensive disorders. How far the characterization and challenge of these autonomic functions can be utilized for diagnosis and prediction of preeclampsia has to be shown in future studies.

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Hemodynamic Parameters Measured Noninvasively throughout Pregnancy. Daniela Ulrich,[1] Manfred G Moertl,[1] Karoline Pickel,[1] Philipp Klaritsch,[1] Isa Alkan,[2] Uwe Lang,[1] Dietmar Schlembach.[1] *[1]Obstetrics and Gynecology, Medical University of Graz, Graz, Styria, Austria; [2]CNSystems Medizintechnik AG, Graz, Styria, Austria.*

Background:
Hemodynamic changes throughout pregnancy have been measured predominantly by invasive techniques. The discussion on the valence and the low acceptance of these invasive procedures by pregnant women demands a noninvasive method for evaluating and distinguishing cardiovascular adaptative mechanisms throughout normal and especially complicated pregnancy.

Method:
20 healthy patients with uncomplicated pregnancy were measured with the TaskForce® Monitor 2040i (CNSystems, Austria) at different time points throughout pregnancy. Cardiovascular parameters were recorded in supine and left lateral position under standardized conditions.

Results:
Throughout pregnancy an increase of global parameters such as heart rate (HR), blood pressure (BP), total peripheral resistance (TPR) and total peripheral resistance index (TPRI) were observed. Stroke volume (SV), stroke index (SI), cardiac output (CO), contractility index (CI), acceleration index (ACI), and left ventricular ejection time (LVET) decreased throughout pregnancy (table).

Discussion:
The noninvasive determination of cardiovascular parameters throughout pregnancy is possible and the results of this pilot study can serve as basic parameters for classifying and assessing cardiovascular changes in pathological conditions in pregnancy such as hypertensive disorders. Assigning pregnant hypertensive women into hyper- and hypodynamic groups may aid in planning individual therapeutic strategies.

Cardiovascular parameters throughout pregnancy

	10-13 wks	15-18 wks	20-22 wks	> 30 wks
HR [bpm]	74±9	79±16	80±11	88±16
sBP [mm Hg]	108±12	109±12	109±11	115±10
dBP [mm Hg]	67±8	66±7	67±7	74±6
MAP [mm Hg]	78±9	79±9	79±8	86±6
SV [ml]	92.53±15.04	91.34±16.25	86.67±16.39	65.31±11.42
SI [ml/m2]	54±8	52±10	49±10	36±6
CO [l/min]	6.86±1.34	7.06±1.20	6.82±1.20	5.59±0.93
CI [1000/s]	3.93±0.69	4.02±0.72	3.85±0.73	3.04±0.52
TPR [dyne*s/cm5]	918.40± 171.58	899.45± 207.71	925.34± 170.17	1244.00 ±219.80
TPRI [dyne*s*m2/ cm5]	1597.67± 274.01	1586.11± 381.98	1644.79± 327.36	2288.23± 410.25
ACI [100/s2]	92.27±23.91	95.91±28.09	89.85±25.06	62.41±16.10
LVET [ms]	315±13	303±22	295±18	274±27

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Increased Circulating GnRH II in Pregnancy and in Spontaneous Abortions. Theresa Siler-Khodr,[1] Kimberlee Sorem,[2] Collin Smikle.[3] *[1]Center for Investigation Cell Regulation & Replication, San Antonio, TX, USA; [2]Physicians Foundation, Califormia Pacific Medical Center, San Francisco, CA, USA; [3]Laural Fertility Care, San Francisco, CA, USA.*

Objective: Both GnRH I and GnRH II are expressed in humans. GnRH I is produced by the hypothalamus under the regulation of gonadal steroids and stimulates pituitary gonadotropins. During pregnancy GnRH I is also produced by the placenta and affects hCG. GnRH II, the ancient isoform, is produced by numerous human tissues including immune and reproductive tissues and circulates in blood in quantifiable levels. We have demonstrated that GnRH II analogs directly affect fertilization and uterine function, and propose that it acts via immune regulation. During pregnancy it is known to regulate numerous hormone productions, yet the levels of GnRH II has not been reported. In these studies we have determined the circulating levels of GnRH II throughout pregnancy and in early pregnancy loss. **Study Design:** Thirty-three women having normal pregnancies were followed prospectively. Plasma samples were drawn at 8, 10, 12, 14, 16, 28, 36 weeks gestation and during labor. Plasma was also collected from patients have spontaneous abortions (n=8). Circulating GnRH II and CRH and GnRH I were measured by specific radioimmunoassays. **Results:** Circulating GnRH II increased from non-pregnant levels (65+/-2 pg/ml, mean+/-sem) to 83+/-2 pg/ml by 10-week LMP. GnRH II concentrations continued to increase through 16 weeks gestation over the 10-week levels, although a significant increase was only attained by 28 weeks. GnRH II continued to increase in late term, attaining levels of 99+/-1 pg/ml which was significantly higher than that of early gestation. During labor and delivery GnRH II in maternal plasma was further increased to 106+/-2 pg/ml, i.e., 1.5 times that of 8-week gestation (73+/-4 pg/ml). Circulating GnRH II did not parallel GnRH I in early pregnancy but did in late gestation. GnRH II did correlate with CRH in early pregnancy but not in late gestation. Patients having spontaneous abortion had increased circulating GnRH II at 8-weeks LMP (85+/-3 pg/ml) as compared to normal pregnancies (73+/-4 pg/ml). **Conclusion:** GnRH II increased throughout pregnancy attaining highest concentrations during labor and delivery. Patient with early pregnancy loss had increased GnRH II expression. The function of GnRH II or factors affecting this increased GnRH II in normal or abnormal human pregnancy should be investigated.

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Plasma Levels of Anandamide Are Elevated in Early Pregnancy Failure. Mark Finney, Anthony H Taylor, Patricia MW Lam, Stephen C Bell, David J Taylor, Justin C Konje. *Endocannabinoid Research Group, Reproductive Sciences, Cancer Studies & Molecular Medicine, University of Leicester, Leicester, United Kingdom.*

Introduction: Plasma levels of the endocannabinoid, anandamide (AEA) decrease during the luteal phase of the menstrual cycle and early pregnancy and increase during parturition.[1] High plasma AEA levels at 6 weeks in women undergoing IVF-ET was associated with a failure to achieve an on-going pregnancy[2]. What is uncertain is what happens to AEA levels when the pregnancy has already failed. We aimed to quantify AEA levels in women presenting in early pregnancy with a diagnosed non-viable pregnancy and to compare them to those of a viable pregnancy.

Methods: Plasma AEA was measured by a sensitive isotope dilution HPLC-MS/MS method from 45 women in early pregnancy (5-12 weeks) of whom 25 had a viable pregnancy and 20 had a non-viable pregnancy. Serum hCG and progesterone were measured in blood samples by standard ELISA methods.

Results: The ages and BMI of both groups were similar (29 ± 4.9 versus 28 ± 4.7 years; mean ± SD; P=0.45; Student's unpaired t-test and 24 ± 2.7 versus 25 ± 2.9 Kg/m^2; P=0.91) respectively. Plasma AEA levels in women with non-viable pregnancies were significantly higher than those in women with viable pregnancies (1.68 ± 0.77 versus 1.15 ± 0.30nM; P=0.007). There was no correlation found between AEA levels and hCG or progesterone levels

(Pearson correlation r=-0.203; P=0.272 and r=0.274; P=0.075, respectively), despite progesterone being significantly lower in the non-viable group (40.0 ± 35.1 versus 61.0 ± 26.3ng/mL; P=0.027).

Conclusion: Higher plasma AEA levels were associated with early pregnancy failure, and this association appeared to be independent of serum hCG or progesterone concentrations. These data suggest that plasma AEA levels are linked with pregnancy failure through a mechanism that does not involve hCG or progesterone production. The precise involvement of anandamide in early pregnancy needs to be investigated further.

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Restricted Protein Diet in Pregnancy Decreases Delta 5 and 6 Desaturases and Elongases 2 and 5 Expression and Docosahexaenoic (DHA) in Maternal Rat Liver. Claudia J Bautista,[1] Nimbe Torres,[1] Guillermo Ordaz-Nava,[1] Maricela Rodriguez-Cruz,[1] Armando R Tovar,[1] Fernando Larrea,[1] Peter W Nathanielsz,[2] Elena Zambrano.[1] *[1]Biologia de la Reproduccion y Fisologia de la Nutricion, Instituto Nacional de Ciencias Medicas y Nutricion SZ, Mexico City, Mexico; [2]Department of Obstetrics, UTHSCSA, San Antonio, TX, USA.*

Introduction. DHA (22:6n-3) and arachidonic (AA, 20:4n-6) acids, the major brain long-chain polyunsaturated fatty acids (LC-PUFA) are generated by elongation-desaturation of dietary essential fatty acids (EFA). The maternal liver is principally responsible for EFA elongation-desaturation.

Objetive. We determined effects of protein restriction in pregnancy on expression of Δ5D, Δ6D and elongases 2 and 5 (ELOV 2 AND 5) in the maternal liver rat.

Methods. Pregnant rats were fed control (20 % casein; C) or restricted (10 % casein; R) isocaloric diets. At day 19 gestation maternal blood and livers were collected. Serum triglycerides, cholesterol and glucose were determinate with the Synchron CX autoanalyser and leptin and insulin by RIA. Liver fat determination was performed by the Soxhlet method. Liver ARA and DHA were calculated by gas chromatography based upon retention times from methyl ester standards. Liver Δ5D, Δ6D, ELOV 2 and 5 mRNA were measured by RT-PCR and northern blot. Data are M ± SEM; analysis by t-test.

Results. Liver weights did not differ in C and R. Total liver fat (760 ± 39 vs 543 ± 22 mg) serum leptin (5 ± 0.1 vs 7 ± 0.7ng/ml) and insulin (0.2 ± 0.04 vs 0.9 ± 0.09 ng/ml) differed in C and R respectively ($p<0.03$). Serum triglycerides, cholesterol, and glucose did not differ. Desaturase and elongase mRNA and % of maternal liver AA and DHA were lower in R (Fig 1).

Conclusion. Low liver fat content and desaturase and elongase mRNA in R indicate impaired LC-PUFA synthesis which may adversely impact fetal development, especially the brain.

Fig 1. C and R desaturase and elongase mRNA and % maternal liver FA at day 19 gestation. M ± SEM, n = 6. * $p < 0.05$ vs C.

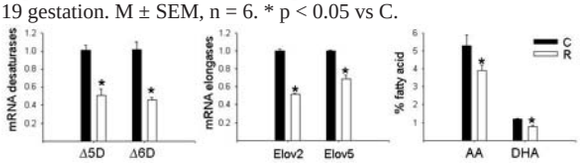

Figure 1

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Association of Maternal Intravascular Fetal Material and Obstetrical DIC. Michael D Benson,[1] Navneet Cheema,[2] Michael K Kaufman,[3] Robert A Goldschmidt,[3] Jennifer L Beaumont.[4] *[1]Obstetrics and Gynecology, Evanston Northwestern Healthcare and Northwestern University, Evanston, IL, USA; [2]Medical Student, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [3]Pathology, Northwestern Healthcare and Northwestern University, Evanston, IL, USA; [4]Center On Outcomes, Research and Education, Northwestern Healthcare and Northwestern University, Evanston, IL, USA.*

Objective: To test the hypothesis that obstetrical DIC results from an excessive leak of fetal material into the maternal circulation. A secondary objective was to assess maternal morbidity.

Methods: All Cesarean hysterectomy cases for hemorrhage at our hospital from 1993 to 2002 were included. Intravascular presence of fetal material was determined by two pathologists, blinded to each other and any clinical information. The percentage of those with any fetal material in the maternal circulation was calculated for each diagnosis for hemorrhage. For a given diagnosis, the percentage of intravascular fetal material in those with the given diagnosis was compared to those without that diagnosis using Fisher's Exact Test. Most patients had multiple hemorrhage diagnoses. A two sample t-test was used to evaluate the difference in mean blood loss between those with and without intravascular fetal material.

Results:

Primary Outcome*

Hemorrhage Diagnosis	N (with diagnosis)	ANY intravascular fetal material N (%); P > 0.5 for all
Uterine rupture	5	1 (20.0)
Abruption	3	1 (33.3)
Atony	31	9 (29.0)
Previa	19	5 (26.3)
Acreta, etc.	24	8 (33.3)
Coagulopathy (DIC)	7	2 (28.6)
Retained placenta	7	2 (28.6)

Mean EBL (cc):
No fetal material: 2759
+ Fetal material: 2389
P = 0.4971
*82% power to detect significant difference if 80% of DIC group had fetal material and all others had 20%

Secondary Outcomes:
89% of patients received blood products and 40% received clotting agents. The average blood loss was 2650 cc. There were no maternal deaths and 4 injuries to adjacent organs, all to the bladder.

Conclusions: There was no association between the presence of intravascular fetal material and any specific hemorrhage diagnosis or amount of blood loss. Although the power to detect a relationship was low, the excessive leakage of fetal material as a specific and exclusive mechanism of obstetrical DIC, as postulated, seems unlikely. While 90% of the population was transfused, there were few intraoperative complications and no maternal deaths.

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Gestational Prehypertension – A New Predictor of Pregnancy Complications? Stacy L Jeronis,[1] Lauren Averbuch,[1] Alexandra L Hanlon,[2] Eliezer J Holtzman,[3] Enrique Hernandez,[1] Ossie Geifman-Holtzman.[1] *[1]Obstetrics & Gynecology, Temple University School of Medicine, Philadelphia, PA, USA; [2]School of Public Health, Temple University, Philadelphia, PA, USA; [3]Nephrology & Hypertension, Tel-Aviv University, Sheba Medical Center, Ramat-Gan, Israel.*

Objective: The joint national committee on high blood pressure (JNC7) determined blood pressure of 120-139/80-89 in adults to be Prehypertension that requires health promoting life style modifications to prevent cardiovascular disease. Similarly, we hypothesize that gestational prehypertension in pregnancy is associated with increased risk of pregnancy complications and its management would improve pregnancy outcome.

Methods: Prospective and retrospective recruitment resulted in a study group consisting of 40 patients who were diagnosed in the first and early second trimester (<15 weeks) with blood pressure of 120-130/80, and a control group consisting of 87 patients with blood pressure < 120/80 at similar gestational age. Comparisons between the two groups were accomplished for demographic characteristics and outcome measures using the chi-square test statistic and two-sample t-tests for categorical and continuous variables, respectively.

Results: All outcome measures analyzed resulted in poorer results being associated with the study group compared with control as follows: 10% versus 2% of the study and control group pregnancies, respectively, experienced preeclampsia (p=0.07); and 14% versus 0% of the study and control group, respectively, were associated with gestational hypertension (p=0.0005). 10% of the control group deliveries were associated with admission to the NICU, compared to only 3% in the control group (p=0.16); 17% versus 10% of the study and control group deliveries, respectively, were preterm (p=0.36); Twenty-nine percent of the study group were cesarean deliveries, compared to 38% in the control group (p=0.05). On admission mean SBP and DBP was 132 and 76 mmHg, respectively, in the study group, compared to 122 and 70 mmHg in the control group (p=0.0003 for SBP and p=0.03 for DBP).

Conclusions: "Gestational Pre-hypertension" may be a real pregnancy condition that when is recognized, physician attention, patient close monitoring during prenatal care and delivery and early intervention in patients at risk, may all promote improved pregnancy outcome. Larger scale study is in progress to evaluate and confirm these findings in the larger pregnant population.

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Are Contractions at 24-32 weeks Gestation Less Painful Than Those at Full Term? Kristy A Ruis, Karin Blakemore, Abimbola Aina-Mumuney, Valerie Jones. *Gynecology and Obstetrics, Johns Hopkins Hospital, Baltimore, MD, USA.*

Objective

To determine if gestational age plays a role in severity of pain associated with uterine contractions.

Study Design

Self-reported pain from women in labor, on a scale of 1-10, is assessed and recorded in an obstetrical database by nurses at regular intervals at two hospitals within our medical system and was retrospectively reviewed from 2002-2007. Pain at various dilatations (3-10cm) of women who were laboring and then delivered at 24-32 weeks' gestation was compared to women in labor at term. Maternal demographics of age, race, education level, BMI, presence of support person, request for analgesia at any point in labor, and epidural placement were abstracted from maternal records. Categorical data were analyzed using chi square or Fisher exact test; continuous variables using Student t-test.

Results

99 laboring patients between 24-32 weeks gestation and 409 at term were identified. Term and preterm patients did not differ with respect to maternal demographics. Pain reported by preterm patients was significantly less at 3-4 cm dilatation (4.8 vs. 6.3, p=0.01) and significantly greater at 9-10 cm (4.2 vs. 2.3, p=0.03). Pain reported at 5-6 cm and 7-8 cm was not statistically different between the groups. Of note, fewer of the preterm patients received an epidural despite the request for analgesia.

Conclusion

Preterm laboring patients between 24-32 weeks gestation may perceive less pain at 3-4 cm dilatation compared to term laboring patients; however, their pain perception at advanced dilatation was comparable to those at term despite the fact that they were less likely to have an epidural in place at the time of pain evaluation. In light of these findings, the widely accepted etiology of labor pain as the result of cervical dilatation may need to be re-examined. Also, factoring in the patient's discomfort level into the evaluation for onset of early active labor may not be valid in the preterm patient.

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Uterine Electromyographic Activity in Pregnant Women with Asthma. Egle Bytautiene,[1] William L Maner,[1] Roberto Romero,[2] George R Saade,[1] Robert E Garfield.[1] *[1]Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA; [2]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI, USA.*

Background and Objective: Asthma is a risk factor for adverse pregnancy outcome. This has been attributed to the effect of allergy in pregnancy. Mast cell mediators can induce uterine contractility. The objective of the study was to test the hypothesis that pregnant women with asthma have different uterine electrical signals from those generated by normal pregnant women.

Materials and Methods: Uterine electromyography (EMG) recordings from gestational age matched pregnant women at term were analyzed. The cases consisted of patients with asthma and the controls, those women without asthma. EMG was recorded for approximately 30 minutes from surface electrodes placed upon the maternal abdomen, with the electrical signals filtered in the uterine-specific range of 0.34 to 1.00 Hz to remove noise components. The recordings were analyzed in their entirety first by power spectrum analysis and then by Lyapunov analysis. The power-spectrum-largest-peak frequency and the largest Lyapunov exponent were calculated for each patient, and then the mean and standard deviation of these parameters was compared using Student's t-test.

Results: Patients with asthma had significant lower power spectrum frequency and higher Lyapunov exponent than women without asthma. See table.

Patients	With Asthma	Without Asthma	P value
Power Spectrum Frequency	0.39 ± 0.01	0.46 ± 0.01	0.040
Lyapunov Exponent	0.13 ± 0.02	0.08 ± 0.01	0.048

Conclusions: 1) The power spectrum frequency and the Lyapunov exponent used to analyze uterine EMG signals were different in women with and without asthma; 2) The results suggest that uterine electrical activity is altered in women affected by asthma; 3) These findings may explain the observed increased frequency of preterm birth in women with asthma. Further studies are required to dissect the mechanisms.

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Fetal Microchimerism and Tissue Repair in the Appendix of Pregnant Women. Margarida AR Santos,[1] Keelin O'Donoghue,[1,2] Josephine Wyatt-Ashmead,[3] Nicholas M Fisk.[1,2] *[1]Institute of Reproductive and Developmental Biology, Imperial College London, London, United Kingdom; [2]Centre for Fetal Care, Queen Charlotte's and Chelsea Hospital, London, United Kingdom; [3]Department of Pathology, Hammersmith Hospitals NHS Trust, London, United Kingdom.*

Background:

Fetal microchimeric cells trafficked into the maternal circulation persist in blood and tissues for years after pregnancy. Increasing data suggest that microchimerism occurs after every pregnancy, but the biological role of fetal microchimerism or the cell types involved is unclear. While persistent fetal cells were initially implicated in autoimmune disease, animal studies suggest these fetal cells play a broader role in response to tissue injury.

Methods:

Appendix specimens were acquired from 9 women undergoing appendicectomy during pregnancy. Detailed reproductive histories were obtained. Fluorescence in situ hybridisation (FISH) with two different probes allowed investigation of the presence of male presumed-fetal cells and nested PCR amplification of SRY gene confirmed male DNA in the appendix. Immunostaining was used to determine the fetal cell phenotype.

Results:

Male cells were identified in appendix tissues from women with known male pregnancies (n=7) and also from a woman with no sons and a previous miscarriage of undetermined gender (n=1). No male cells were observed in the control (n=1), a woman with 3 daughters. Male cells of presumed fetal origin were evenly distributed in the muscle, mucosa and submucosa layers of the appendix. Morphology and co-localisation analysis suggested the identified male cells had differentiated primarily into muscle cells or lymphocytes. Combined immunostaining and Y-FISH demonstrated male desmin+ muscle cells and CD3+ and CD19+ lymphocytes. Finally, the presence of male DNA in the appendix specimens was confirmed by nested PCR.

Conclusions:

Male cells of presumed fetal origin were identified in the appendix of pregnant women. Microchimerism frequency varied according to the reproductive history and the degree of inflammation. Microchimerism rates were higher in the appendix from women with current male pregnancies than in those with previous male pregnancies and were higher in those with histological acute inflammation, when compared to milder cases. Male microchimeric cells identified were of haematopoietic and mesenchymal origin. This study suggests that fetal cells are present in sites of tissue injury and may participate in tissue repair during pregnancy.

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Collagen Iα2 and Collagen-Binding Integrins mRNA Expression in Rat Cervix during Gestation. Huiling Ji, Tanya L Dailey, Vit Long, Edward KS Chien. *Obstetrics and Gynecology, Maternal Fetal Medicine, Women and Infants' Hospital of Rhode Island, Providence, RI, USA.*

Objective

Cervical remodeling is associated with cell proliferation and extracellular matrix (ECM) remodeling. Integrins are a family of multifunctional cell adhesion receptors which mediate ECM-cell interactions including binding collagen. Of the 24 integrin (I) heterodimers, α1β1,α2β1, α10β1 and α11β1 are primary receptors for collagen. The predominant cervical collagen is Collagen type 1. The purpose of this study is to investigate collagen and integrin expression in the pregnant rat cervix.

Study design

The cervix was harvested from timed pregnant Sprague-Dawley rats. Non pregnant (NP)and timed pregnant day 12, 16, 18, 20, 21 and 22 animals were euthanized using a protocol approved by the IACUC. Four animals were sacrificed on each day of gestation. Quantitative RT-PCR was used to evaluate mRNA expression and normalized to β-actin. A standard curve was generated from a single sample. Data was analyzed using ANOVA and multiple comparisons testing**.**

Results

Collagen Iα2 mRNA expression increased through mid-gestation but decreased to NP levels on day 22. A similar pattern was seen with the Integrin beta 1 subunit. The pattern of integrin alpha subunit expression was different for each subunit during pregnancy. The changes in Collagen, Integrin alpha 1, 10, 11 and Integrin beta 1 were found to be statistically significant. (Figure).

Conclusion

The pattern of collagen binding integrin alpha subunit expression during the

rat gestation appears to vary independently of each other. The expression of the β1 subunit paralleled Col 1α2 expression. Collagen binding integrins may play independent roles in signaling and biomechanics during gestation.

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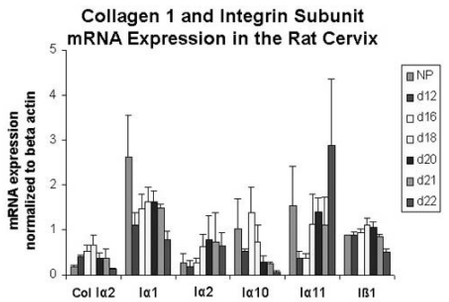

Figure1: Collagen and collagen binding integrin mRNA expression in pregnant rat cervix. Data reported as Mean ± Standard Deviation (error bars). Col Iα2 Collagen I α2; Iα1 Integrin α1; Iα2 Integrin α2; Iα10 Integrin α10; Iα11 Integrin α11; Iβ1 Integrin β1

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The Pregnant Baboon as a Model for Studies on the Effects of Chemotherapy during Pregnancy. Kristel Van Calsteren,[1] Roland Devlieger,[1] Luc De Catte,[1] Thomas D'Hooghe,[1,2] Daniel Chai,[2] Jason Mwenda,[2] Frederic Amant.[1] *[1]Obstetrics & Gynecology, UZ Gasthuisberg, Katholieke Universiteit Leuven, Leuven, Belgium; [2]Reproductive Biology, Institute of Primate Research, Nairobi, Kenya.*

Background. The effects of chemotherapy on human fetal development are poorly studied, mainly due to the lack of adequate animal models in which placentation and embryological development resembles humans and pharmacokinetic studies, prenatal sonography as well as histological studies can be performed.

Aim of the study. To test the baboon as model for studying pharmacokinetics and fetal effects of chemotherapy administered during pregnancy.

Methods. Experiments were performed in 11 pregnant baboons at a mean gestational age of 134 (+/-10) days. Detailed ultrasound examination (biometry, Doppler, screening) was performed 3 days before, at and 1 day after the drug administration. The administration of different schemes of chemotherapy and the fetal samplings occurred under endotracheal anaesthesia. Maternal blood samplings, as well as percutaneous ultrasound guided fetal blood (2ml) and amniotic fluid (4ml) samplings were performed at least once immediately after drug administration. In case of fetal demise, the fetus underwent detailed macro- and microscopic examination. In the other animals mother and fetus were euthanized 24h after the experiments, with collection of blood, amniotic fluid and tissues for further analysis.

Results. All 11 mothers survived the experiments, one mother developed paralitic ileus. None of the fetuses died during the acute phase of the experiment but 3/11 (27%) fetuses died within 24h following the experiment. None of the animals showed significant clinical or histological signs of infection or anaemia. A total of 50 ultrasound examinations were performed in 11 fetuses, allowing the creation of sonographic growth charts in this model. At the time of drug administration the mean maternal weight was 16.8kg (range 14.0-20.2) and the estimated fetal weight was 405gr (range 317-484). Sixteen out of 18 cordocenteses (89%) and all 18 amniocenteses (100%) were successful in obtaining the required samples for analysis.

Conclusion. The pregnant baboon can be used as a model for studies on pharmacokinetics and fetal effects of chemotherapy administered during gestation. Prenatal ultrasound is comparable to the human situation, and amniocentesis and cordocentesis can be performed with a high success rate and short term survival.

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Reference Values for Progesterone, 17 Hydroxy Progesterone, Cortisol, Estradiol, and Inhibin throughout the Baboon Gestation and Early Postpartum Life. Juan DeLeon-Luis,[2] Isabel Galan,[2] Mehmet Genc,[1] Julian Robinson,[1] Joaquin Santolaya-Forgas.[1] *[1]Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA, USA; [2]Amarillo Women's Health Reaserach Institute, Texas Tech University Health Science, Amarillo, TX, USA.*

OBJECTIVE: A 2006 Cochrane Review as well as a review by Marc Keirse concluded, "Information regarding the potential harms of progesterone (P) therapy to prevent preterm birth is limited". Moreover, 17-hydroxyprogesterone (17OHP) caproate is a category D drug according to the FDA (evidence of fetal harm).

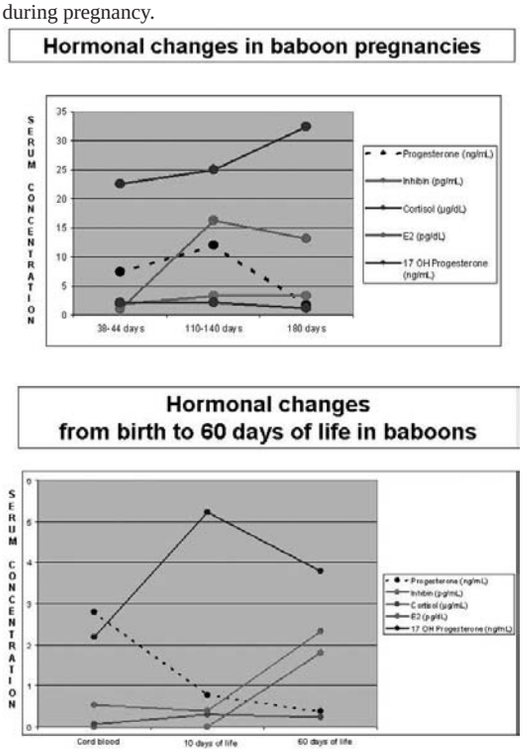
P is produced in the adrenal glands, gonads and brain. After 8 weeks gestation P is secreted from the placenta independently to the mother and the fetus. P is the precursor of aldosterone and after conversion to 17OHP, of cortisol and androstenedione.

Baboons have similar reproductive system and development to humans and are used to study mechanisms of labor and prevention of preterm labor. Currently, there are no normative data on the concentration of P, 17OHP, Cortisol ©, Estradiol (E2) or Inhibin (I) throughout their pregnancy. Therefore, a dose-response or the teratogenic effects of P or 17OHP caproate cannot be studied in a controlled manner in this animal model. The aim of this study was to generate reference values for these hormones during baboon gestation and early postpartum life.

MATERIAL AND METHODS: 43 hormonal quantitative measurements were performed (IMMULITE Analyzer)

RESULTS: The mean concentrations of these 5 hormones are depicted in **Figure 1** (maternal serum, from conception to term ~180 days) and **Figure 2** (newborn serum)

CONCLUSION: This study provides reference values for 5 hormones quantified during the baboon gestation and early postpartum life. This may be useful for future research concerning the effects of P and 17OHP administration during pregnancy.



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Heat-Shock Proteins and the Stress Response Following the Events of September 11, 2001 in Pregnant and Non-Pregnant Women of New York City. Men-Jean Lee,[1] Leah Ahoya,[2] Caroline Tang,[2] Se-Te J Huang,[2] Mortimer Levitz,[2] Edward Kuczynski,[2] Charles J Lockwood,[2] Seth Guller.[2] *[1]Obstetrics and Gynecology, Mount Sinai School of Medicine, New York, NY, USA; [2]Obstetrics, Gynecology, and Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA.*

OBJECTIVE: To evaluate serum adrenocorticotropin hormone(ACTH), cortisol, and heat shock proteins-70 and 90(HSP70, HSP90) as markers for stress in non-pregnant and pregnant women receiving health care in NYC before and after 9/11/01.

STUDY DESIGN: A nested cohort study of banked serum samples from two groups of reproductive-age women in NYC from 2000 to 2001 was performed: (1)non-pregnant women eligible for IVF treatment and (2)pregnant women receiving prenatal care. The pre-9/11 samples were drawn in the year prior to 9/11/01; the post-9/11 samples were drawn within 6 months after. The pre-9/11 and post-9/11 normal pregnant samples were drawn at 20±2 weeks' gestation. The non-pregnant samples were from IVF candidates with serum E2 levels<75

pg/mL and FSH serum levels<12.5 mIU/mL. The samples were assayed for ACTH and cortisol by a commercially available Immulite™ system. HSP70 and HSP90 were measured by commercially available ELISA. These results were compared in the patient populations before and after 9/11. T-tests were used for analysis. Statistical significance was set at $p<0.05$.

RESULTS: In the pregnant subjects, there were no statistical differences in the mean levels of ACTH, cortisol, and HSP70 pre- and post-9/11(Table 1). Only serum HSP90 levels were significantly increased in the pregnant women post-9/11.* Mean serum ACTH, cortisol, HSP70, and HSP90 levels were all significantly different in non-pregnant subjects pre- and post-9/11.

CONCLUSIONS: Except for HSP90, serum markers for stress were unchanged in pregnant subjects after the stress of 9/11/01 in comparison to non-pregnant women whose serum HSP90, HSP70, cortisol, and ACTH levels were significantly different. The lack of change in serum markers of stress in pregnant women suggests that the hormonal milieu of pregnancy may buffer against acute environmental stressors.

Table 1

		ACTH (pg/ml)	Cortisol (ug/ml)	HSP70 (ng/ml)	HSP90 (ng/ml)
Non-Pregnant	Pre-9/11	78.3+8.8	61.2+16.7	290.4+170.4	18.3+8.6
Non-Pregnant	Post-9/11	84.9+10.5	49.2+16.0	581.6+384.5	12.7+8.5
Pregnant	Pre-911	9.1+8.2	14.0+4.3	140.2+155.6	3.6+3.2*
Pregnant	Post-911	7.3+6.7	15.7+6.5	113.3+115.1	17.5+12.8*

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Relationships between Maternal Body Composition Prior to Conception and Duration of Gestation. Jim F Johnstone,[1] Hazel Inskip,[2] Sarah Crozier,[2] Rohan M Lewis,[3] Mark Hanson,[3] John RG Challis,[1] Keith M Godfrey.[2,3] *[1]Department of Physiology, University of Toronto, Toronto, Canada; [2]MRC Epidemiology Resource Centre, University of Southampton, United Kingdom; [3]Centre for Developmental Origins of Health and Disease, University of Southampton, United Kingdom.*

Objective: In human pregnancy, maternal body composition provides an indicator of maternal nutritional status and metabolic capacity. Previously, we reported that 11ß HSD-2 activity was significantly reduced in term placentas from thin women and women with a smaller mid-upper arm circumference before conception. This suggests that these fetuses may have been exposed to inappropriate levels of maternal cortisol, which is a mediator of gestation length. In this study, we hypothesize that the duration of gestation will be shorter in women who tend to be thin and have a lower mid-upper arm circumference prior to conception.

Methods: Within the longitudinal, population-based Southampton Women's Survey (SWS), analyses were performed on 1301 women whose estimated date of conception was set using an algorithm that combined menstrual and early ultrasound scan data and who had a spontaneous onset of labour and delivered after 37 weeks of gestation. Linear regression was used to examine the relationships between maternal body composition and the duration of gestation.

Results: Within the 1301 women surveyed, lower maternal body mass index, mid-upper arm circumference and arm muscle area before conception were all associated with shorter duration of gestation at delivery (r=0.12, p=0.00001; r=0.10, p=0.0002; and r=0.09, p=0.0009, respectively). A lower subscapular skinfold thickness, sum of skinfolds and height were also associated with shorter duration of gestation (r=0.08, p=0.006; r=0.07, p=0.009 and r=0.06, p=0.03, respectively). Mother's age, own birthweight and ratio of subscapular/triceps skinfold thickness were not related to the duration of gestation.

Conclusions: In this study, we found that thinner women with a lower mid-upper arm circumference and arm muscle area tended to have a shorter duration of gestation. Our findings of shorter gestation length in thinner women are in keeping with our previous observation of lower 11ß HSD-2 activity in term placentas from thinner women. We conclude that metabolic capacity prior to conception could influence duration of gestation through mechanisms that include alteration in placental metabolism and fetal cortisol exposure.

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FIPP Research Initiative: Impact of Fecal Incontinence Postpartum on Quality of Life. Jamie O Lo,[1] Patricia Osterweil,[2] Hong Li,[2] Motomi Mori,[3] Karen B Eden,[4] Jeanne-Marie Guise.[2] *[1]School of Medicine, Oregon Health & Science University (OHSU), Portland, OR, USA; [2]Obstetrics & Gynecology, OHSU, Portland, OR, USA; [3]Public Health & Preventive Medicine, OHSU, Portland, OR, USA; [4]Medical Informatics & Clinical Epidemiology, OHSU, Portland, OR, USA.*

Objective

Fecal incontinence (FI) is a debilitating condition affecting 2-10% of the US. Our prior studies found that 29% of women report new onset FI after childbirth. The goal of our study was to examine the impact of FI on postpartum quality of life (QOL).

Methods

Women reporting FI on a statewide survey who agreed to participate in a 2-year study of QOL were included in the analysis. Women were considered to have FI based upon the NIH definition of FI. The quality of life survey was based upon the Uebersax incontinence impact questionnaire and was administered every 6 months for 2 years. QOL in women with FI was examined using Chi square, and impact of severe FI (stool incontinence) was determined by multivariate logistic regression.

Results

2907 women with FI were surveyed and of those, 1247 (43%) returned at least 1 survey during the study period, completed all survey questions, and were included in the final analysis. Among women with FI, 51% felt frustrated due to FI, 26% reported FI impacted their emotional health, 18.5% reported FI impacted child-caring abilities, and 16.7% reported a negative impact on social activities. QOL was similar across survey periods. One out of three women with FI reported severe symptoms (incontinence of stool). Women with severe symptoms were 4-7 times more likely to report negative impacts on QOL compared to milder (e.g. flatus) FI after adjusting for age, parity and urinary incontinence.

33.9% felt their stool was stored elsewhere before bowel movements, 13.9% reported using digital defecation and more than half (52%) reported symptoms of urinary leakage.

Despite the substantial impact on postpartum quality of life, few women sought medical help with only 10% of women at 6 months, 13.5% at 1 year, and 16.7% at 2 years ever reporting their symptoms to a medical provider.

Conclusion

Postpartum women report that fecal incontinence has a substantial negative impact on their QOL after delivery including their emotional health and ability to care for their newborn. Despite this profound impact, few women will discuss FI with medical providers. These data suggests there may be a benefit for providers to inquire about FI at postpartum visits.

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How Do Predictors of Breastfeeding Differ among Racial/Ethnic Groups? Maria Carolina Court, Robyn Lamar, Aaron Caughey. *Obstetrics and Gynecology, School of Medicine, University of California, San Francisco, San Francisco, CA, USA.*

Objective: The objective of this study was to determine what characteristics contribute to racial/ethnic differences in breastfeeding rates.

Study Design: A retrospective cohort study of all women who delivered a viable infant (N=26,781) was conducted. The primary outcome was breastfeeding upon discharge of the hospital. We first examined the association between race/ethnicity and breastfeeding. Next, we conducted stratified analyses examining a variety of predictors of breastfeeding within the racial/ethnic groups including maternal demographics, obstetric interventions, and perinatal complications.

Results: We found that both Asians (OR 0.53, 95% CI 0.47- 0.61) and Blacks (0.32, 95% CI 0.27-0.37) had statistically significant lower rates of breastfeeding, while Latinas (OR 1.18, 95% CI 0.99-1.41) showed a trend towards higher rates of breastfeeding. In Latinas, we found that 3rd and 4th degree lacerations were significantly associated with lower rates of breastfeeding, but were not predictive of breastfeeding in the other racial/ethnic groups. Epidural use was predictive of lower rates of breastfeeding in Caucasians and Blacks, but was not predictive in Latinos and Asians. Some of the other predictors which differed between the racial/ethnic groups were obesity and induction of labor (Table 1).

Conclusion: Race/ethnicity is significantly associated with breastfeeding, with Blacks and Asians having the lowest rates of breastfeeding at discharge. A variety of other factors are associated with breastfeeding and interestingly, their effect appears to differ between the racial/ethnic groups. Future studies might elucidate the sociocultural and biomedical reasons that explain the differences. These results help focus our efforts during the peripartum period to advocate for mothers who have factors that might impede breast feeding.

Table 1

	Obesity	Perineal Lacerations	Induction of Labor	Epidural
White	0.52* (0.38-0.71)**	1.04 (0.69-1.54)	0.57 (0.46-0.72)	0.71 (0.57-0.87)
Black	0.89 (0.69-1.14)	1.09 (0.70-1.71)	0.82 (0.62-1.06)	0.74 (0.62-0.92)
Latino	0.90 (0.59-1.38)	0.45 (0.24-0.82)	0.75 (0.51-1.11)	0.84 (0.59-1.17)
Asian	0.92 (0.61-1.39)	0.94 (0.71-1.24)	0.94 (0.73-1.22)	1.1 (0.92-1.34)

* Odds Ratio; **95% CI

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Epidural or Not? Women Perceptions and Beliefs Regarding Epidural Influencing Decision Making. Sarah Azad,[1] Anita S Sit,[1] Tim Park,[2] Aaron B Caughey,[3] Neena Duggal.[1] *[1]Obstetrics & Gynecology, Santa Clara Valley Medical Center, San Jose, CA, USA; [2]Anesthesia, Ohio State University Medical Center, Columbus, OH, USA; [3]Obstetrics, Gynecology & Reproductive Science, University of California San Francisco Medical Center, San Francisco, CA, USA.*

OBJECTIVE: Racial and ethnic disparities exist in the proportion of patients receiving epidural analgesia during labor and delivery. The purpose of the study is to determine how women perceptions and beliefs about epidural influencing their decisions in accepting intrapartum epidural analgesia.

METHODS: We conducted a 12-question survey-study of term, laboring patients admitted for labor regarding their perceptions of epidural analgesia. Demographic information, obstetrics, and labor and delivery outcomes were collected. A multivariable logistic regression model was used to evaluate the predictors of desire for and acceptance of epidural analgesia.

RESULTS: A total of 564 surveys were collected. 388 questionnaires were completed and analyzed. 39% of patients reported intention to receive epidural analgesia in labor. After controlling for potential factors such as age, education, parity, and pain score, significant predictors of decreased acceptance of epidural use included beliefs that epidural 1) causes back pain; 2) can harm the baby; and 3)decreases one' s self perception of womanhood (Table). Spanish-speaking (adjusted odds ratio 0.53, 95% CI 0.30-0.94) and Hispanic ethnicity (adjusted odds ratio 0.39, 95% CI 0.15-0.99) are also independent risk factors for decreased acceptance of epidural analgesia.

CONCLUSION: Perceived risks and cultural beliefs are significant predictors of women's acceptance of epidural analgesia. This finding highlights the importance of educational and counseling efforts in targeting these concerns in the decision making of women seeking pain-control during labor. Such interventions may help to alleviate the racial and ethnic disparities in the receipt of epidural analgesia.

Predictors of decreased acceptance of intrapartum epidural

Perceptions	adj OR	95%CI	p- value
Believe epidural causes back pain	0.24	0.15-0.69	<.005
Feel less of a woman if had an epidural	0.19	0.04-0.97	0.046
Believe getting epidural may hurt the baby	0.11	0.03-0.44	0.002

adj OR: adjusted Odds Ratio; CI: confidence interval

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Determinants of Folic Acid Use in Early Pregnancy in a Multi-Ethnic Urban Population. Sarah Timmermans,[1,2] Vincent WV Jaddoe,[2,3,4] Johan P Mackenbach,[5] Albert Hofman,[3] Regine PM Steegers-Theunissen,[1,3,4,6] Eric AP Steegers.[1] *[1]Department of Obstetrics and Gynecology/Division of Obstetrics & Prenatal Medicine, Erasmus Medical Centre, Rotterdam, Netherlands; [2]The Generation R Study Group, Erasmus Medical Centre, Rotterdam, Netherlands; [3]Epidemiology, Erasmus Medical Centre, Rotterdam, Netherlands; [4]Pediatrics, Erasmus Medical Centre, Rotterdam, Netherlands; [5]Public Health, Erasmus Medical Centre, Rotterdam, Netherlands; [6]Clinical Genetics, Erasmus Medical Centre, Rotterdam, Netherlands.*

Background Recommendations on folic acid use to prevent neural tube defects are launched in several countries. However, the adequate use of folic acid supplements during the periconception period seems to be low.

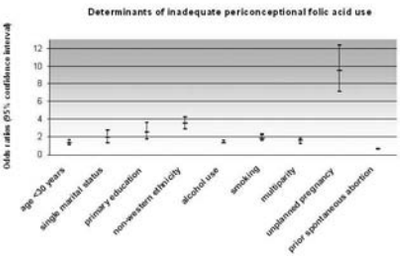
Objective To assess the prevalence of adequate folic acid use, defined as the preconception start of supplements, and to identify its determinants in a multi-ethnic population.

Design The study was embedded in the Generation R Study in Rotterdam, the Netherlands, a population-based prospective cohort study from early pregnancy onwards.

Methods From all women in the cohort who delivered between April 2002 and January 2006 information on folic acid use and potential determinants was obtained by questionnaires and physical examination. Logistic regression models were used to identify determinants of periconception folic acid use.

Results. Data from 6,940 pregnant women were available. Of all women 37% adequately used folic acid supplements. The most important risk factors for inadequate use were unplanned pregnancy (OR 9.5, CI 7.2-12.4, p<0.001), non-western ethnicity (OR 3.5, CI 2.9-4.3, p <0.001) and a low educational level (OR 2.5, CI 1.8-3.6, p<0.001). Other risk factors were single marital status, smoking, multiparity (all p<0.001) and alcohol use (p<0.05). Prior spontaneous abortion was associated with increased adequate folic acid use (p<0.001).

Conclusion Adequate periconceptional folic acid use is low. Improved preconception care and public health education programs are necessary to improve the uptake of folic acid.



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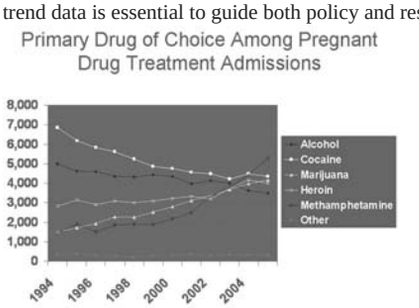
Methamphetamine Use and Pregnancy: Trends in Prevalence and Risk Factors in Drug Treatment Admissions 1994-2005. Mishka Terplan,[1] Michael J Koslowski,[1] Harold A Pollack.[2] *[1]Obstetrics and Gynecology, University of Chicago, Chicago, IL, USA; [2]School of Social Service Administration, University of Chicago, Chicago, IL, USA.*

Although methamphetamine (MA) use has been front and center of the United States drug control policy since 2005, little attention has been given to MA use in pregnancy, despite the fact that pregnant admissions to drug treatment for MA have been rising sharply. To determine trends in the prevalence of MA treatment admissions during pregnancy, we undertook a secondary analysis of the Treatment Episode Data Set (TEDS), an administrative data set that captures at least 70% of all known treatment admissions in the US. In particular, we investigated risk factors for MA use and how these characteristics have changed over time. Demographic, geographic and substance use data were collected for the 219,335 pregnant admissions captured between 1994 and 2005. Logistic regression models were constructed by year. Confounding was assessed via backwards elimination with a change-in-estimate criteria of 0.1 considered substantial. Trend results were reported as adjusted proportions and represented graphically. Overall MA prevalence, reported as the primary drug of use upon admission, rose from 8.1% in 1994 to 24.5% in 2005. Although white women had 3 times the odds of using MA in 1994 (adjusted OR (95%CI) 3.2 [2.8, 3.8]), this had dropped to 1.9 [1.7, 2.1] by 2005, mostly due to an increase in Latina MA use in pregnancy. Pregnant women admitted for MA had fewer prior treatment admissions, were more likely to be unemployed, and less likely to use alcohol or marijuana. We found great geographic variability in use. MA was more common in the Pacific region and least common in New England. There was little change in regional variation over the study time period. Less is known about the perinatal effects of MA compared with other substances, yet it is the primary drug of choice for pregnant women admitted into treatment. As pregnant women occupy a unique place in drug treatment, analysis of national trend data is essential to guide both policy and research.



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An Examination of the Epidemiology and Antecedents of Extremely Preterm Delivery before the 28th Week of Gestation. Thomas F McElrath,[1] Jonathan L Hecht,[2] Elizabeth N Allred,[3] Alan Leviton,[3] Andy Onderdonk.[4] *[1]Obstetrics and Gynecology, Brigham & Women's Hospital, Boston, MA, USA; [2]Pathology, Beth Israel Hospital, Boston, MA, USA; [3]Neuro Epidemiology, Children's Hospital, Boston, MA, USA; [4]Channing Laboratory, Brigham & Women's Hospital, Boston, MA, USA.*

Background: Epidemiologists have grouped the multiple disorders that lead to extremely preterm delivery in a variety of ways. We sought to identify characteristics that would support the combining or dividing of the disorders that lead to preterm delivery.

Methods: We enrolled 1,006 women who delivered a live born singleton infant between 23 and 27 completed weeks gestation at 14 tertiary centers in the United States. Each delivery was classified according to the complication that prompted presentation: preterm labor, preterm premature rupture of

fetal membranes (pPROM), preeclampsia, placental abruption, cervical incompetence, and fetal indication/intrauterine growth restriction (IUGR). We compared these entities on the frequency of characteristics identified by standardized interview, chart review, histological examination of the placenta, and culture of placenta parenchyma.

Results: The percents of women who presented with each antenatal complication were: Preterm labor (40), pPROM (23), preeclampsia (18), placental abruption (11), cervical insufficiency (5), and fetal indication/IUGR (3). After considering antecedents and correlates of the processes leading to preterm delivery, we observed two overarching epidemiologic patterns. The first pattern, characterized by recovery of organisms from the placenta and by histologic chorioamnionitis, tended to be associated with preterm labor, pPROM, placental abruption, and cervical insufficiency. The second pattern, characterized by a paucity of organisms and inflammation and the presence of histologic features of dysfunctional placentation, tended to be associated with preeclampsia and fetal indications/IUGR.

Conclusions: Disorders leading to preterm delivery can be categorized broadly into two groups: those associated with intrauterine inflammation and those with aberrations of placentation.

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Predictors of Compliance with Tuberculosis Screening in Pregnancy. Nadav Schwartz,[1] Sarah Wagner,[1] Sean Keeler,[1] May Tam,[1] Julian Mierlak,[1,3] Aaron Caughey.[2] *[1]Obstetrics and Gynecology, NYU School of Medicine, New York, NY; [2]Obstetrics and Gynecology, UCSF, San Francisco, CA; [3]Obstetrics and Gynecology, Gouverneur Health Care Services, New York, NY.*

Objective: Poor compliance with tuberculosis screening and treatment is a major obstacle to the containment of this disease. We sought to identify predictors of PPD and CXR compliance during pregnancy.

Methods: A retrospective cohort study at a single institution which serves a largely immigrant population in NYC, from November 1, 2001 through June 30, 2006. Data on maternal age, ethnicity, country of origin, level of education, PPD and CXR status were collected.

Results: Of the 4,049 pregnancies, Asian and Hispanic race/ethnicities accounted for 50.4% and 44.0% of the population, respectively, with 11.2% being US-born. The mean age was 27.02 years and the overall PPD+ rate was 50.3%. There was 5% non-compliance with PPD testing, and 5% of PPD+ patients were non-compliant with their chest x-rays. Asian women were more likely to be PPD-compliant than Hispanic or Caucasian women. PPD+ Asian women were also more likely to be compliant with CXR. US-born women were significantly less likely to be compliant with their PPD or with their CXR. Women >40 years old were less likely to be compliant with their CXR, while women with an elementary school education or less were more likely to be compliant. (See Table for Odds Ratios and 95%CI)

Conclusions: Age, education, immigrant status and other cultural and ethnic factors appear to play a role in compliance with tuberculosis screening. Further elucidation of these effects may help clinicians target at-risk sub-populations and improve overall compliance, working towards better control of this disease.

	PPD compliance	**PPD +**	**CXR compliance**
Age category	NS	<20 y/o: 0.55 (0.42-0.73)	>40 y/o: 0.25 (0.09-0.68)
Highest level of Education	NS	College graduate: 0.66 (0.46-0.95)	Elementary school: 2.11 (1.20-3.73)
Asian vs Caucasian	4.94 (2.34-10.45)	3.15 (1.62-6.14)	12.7 (3.44-46.7)
Asian vs Hispanic	2.64 (1.81-3.85)	1.55 (1.35-1.80)	2.7 (1.65-4.42)
Hispanic vs Caucasian	NS	2.06 (1.06-4.01)	4.93 (1.37-17.8)
US Born	0.44 (0.30-0.64)	0.08 (0.05-0.13)	0.22 (0.07-0.67)

NS: not significant (i.e. p>0.05)

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Preterm Delivery: What Role Do Age and Body Mass Index Play? Chuka B Jenkins,[1] John P Pezzullo,[2] Sina Heari,[3] Helain Landy,[2] Mauro V Leo,[4] Celeste Sheppard,[5] Menacheam Miodovnik.[3] *[1]OB/GYN, Harbor Hospital, Baltimore, MD, USA; [2]Ob/GYN, Georgetown University, Washington, DC, USA; [3]Ob/GYN, Washington Hospital Center, Washington, DC, USA; [4]OB/GYN, Franklin Square Hospital, Baltimore, MD, USA; [5]MFM, Hill Country MFM, Austin, TX, USA.*

OBJECTIVE

To determine the association between maternal age, body mass index and preterm delivery.

STUDY DESIGN

We performed a retrospective review of all singleton pregnancies (n=26,691) delivered at four regional medical centers from October 2004 to May 2007. Pregnancies with multiple gestations were excluded. Our primary outcome was the incidence of preterm delivery (PTD), defined as delivery at less than 37 weeks gestation. The cohort was divided by age into adolescent (< 20 years), adult (20-34 years), advanced maternal age (> 35 years); and by body mass index (BMI) into normal (< 25 kg/m2), overweight (25-29 kg/m2), obese (30-35 kg/m2), and morbidly obese (> 35 kg/m2). Association of maternal age and BMI with preterm delivery was assessed using multiple logistic regression analysis. This study was approved by our Institutional Review Board.

RESULTS

Data were available for 26,691singleton deliveries. The odds ratio (OR) for PTD for teenage women (age <20) was 1.1 (CI 0.8-1.3). OR for women 35 years or older (AMA) was 1.5 (CI1.3-1.8). The OR of PTD was 0.41 (CI 0.34-0.50) for overweight women (BMI at delivery 25-30), 0.34 (CI 0.28-0.41) for obese women (BMI 30-35) and 0.37 (CI 0.30-0.45) for morbidly obese women (BMI>35), when compared to normal weight women (BMI <25).

CONCLUSION

Our findings confirm that advanced maternal age confers a higher risk for PTD. A higher BMI is associated with a decreased incidence of PTD. Teenage women are not at increased risk for PTD.

Incidence of PTD by Age and BMI

BMI	<20 years	20-34 years	35+ years	All Ages
<25 (Normal)	52/372 (14.0%)	135/1255 (10.8%)	34/269 (12.6%)	221/1896 (11.7%)
25-29.9 (Overweight)	30/808 (3.7%)	201/4056 (5.0%)	68/986 (6.9%)	299/5850 (5.1%)
30-34.9 (Obese)	25/551 (4.5%)	140/3606 (3.9%)	54/770 (7.0%)	219/4927 (4.4%)
35+ (Morbid Obese)	11/426 (2.6%)	11/426 (2.6%)	61/671 (9.1%)	242/4805 (5.0%)
All BMI's	118/2157 (5.5%)	646/12625 (5.1%)	217/2696 (8.0%)	981/17478 (5.6%)

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Preterm Delivery: How Significant Is the Impact of Maternal Race and Obstetrical History? Chuka B Jenkins,[1] John P Pezzullo,[2] Sina Heari,[3] Helain Landy,[2] Mauro V Leo,[4] Celeste J Sheppard,[5] Menachem Miodovnik.[3] *[1]OB/GYN, Harbor Hospital, Baltimore, MD, USA; [2]Ob/GYN, Georgetown University, Washington, DC, USA; [3]Ob/GYN, Washington Hospital Center, Washington, DC, USA; [4]OB/GYN, Franklin Square Hospital, Baltimore, MD, USA; [5]MFM, Hill Country MFM, Austin, TX, USA.*

OBJECTIVE

The objective of this study was to determine the effect of maternal ethnicity and obstetric history on the incidence of preterm delivery.

STUDY DESIGN

We performed a retrospective review of all singleton pregnancies (n=26,691) delivered at four regional medical centers from October 2004 to May 2007. Pregnancies with multiple gestations were excluded. Our primary outcome was the incidence of preterm delivery (PTD), defined as delivery at less than 37 weeks gestation. Association of race and obstetric history with preterm delivery was assessed using multiple logistic regression analysis. This study was approved by our Institutional Review Board.

RESULTS

Of the 26, 691 deliveries reviewed, 39.3 % were Caucasian (C), 42.1% African American (AA), 2.2% Hispanic (H) and 16.4% Other (O). 6.6% (n=1659) of the total population had preterm deliveries (C 4.2%, AA 8.3%, H 4.2%, O 6.0%). After controlling for body mass index (BMI), age, and delivery site, the odds ratios (95% confidence interval) for preterm delivery, compared to Caucasian women were: AA 2.0 (1.6, 2.3), O 1.4 (1.1, 1.8), H 0.4 (CI 0.16, 1.2). In women without a previous PTD, the rates of PTD were: C 3.6%, AA 6.9%, H 3.9%. History of one previous PTD yielded rates of: C 9.8%, AA 17.2%, H 8.3%; and 2 previous preterm deliveries was associated with the following rates: C 20.8%, AA 23.3%, H 33.3%.

CONCLUSION

Our findings confirm a strong association between maternal race, obstetrical history and preterm delivery. Close observation and more intensive intervention is warranted in women with one previous PTD as the incidence of subsequent PTD nearly triples in this population.

Incidence of PTD by Ethnicity and OB History

Prior PPD	White	Black	Hispanic	Other
None	352/9705 (3.6%)	683/9902 (6.9%)	22/570 (3.9%)	224/4139 (5.4%)
1	65/666 (9.8%)	177/1029 (17.2%)	2/24 (8.3%)	31/195 (15.9%)
2	21/101 (20.8%)	52/223 (23.3%)	1/3 (33.3%)	4/25 (16.0%)
3+	3/17 (17.6%)	20/86 (23.3%)	0/0	2/6 (33.3%)
All	441/10489 (4.2%)	932/11240 (8.3%)	25/597 (4.2%)	261/4365 (6.0%)

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Social Epidemiology of Cervical Cancer: The Case of Literacy and Communication. Melissa A Simon,[1] Veenu Randhawa,[2] Priya John,[3] Ludmila Cofta-Woerpel,[4] Bonnie Spring.[2] *[1]Obstetrics & Gynecology, Northwestern University, Chicago, IL, USA; [2]Preventive Medicine, Northwestern University, Chicago, IL, USA; [3]Medicine, University of Chicago, Chicago, IL, USA; [4]Behavioral Science, University of Texas, MD Anderson, Houston, TX, USA.*

Background: In the US, differential outcomes in cervical cancer among medically underserved women are linked to multiple barriers impacting loss to follow-up and failure or delay in diagnostic resolution. Prior studies have found risk factors for acquisition of HPV and for inability to obtain a pap smear. No study has explored health literacy and physician communication as a key factor.

Methods: This research represents the formative phase of a randomized controlled trial of African American (AA) and Hispanic women aimed to improve communication and abnormal pap smear follow-up in Chicago. Semi-structured interviews and focus groups were conducted face to face in Spanish or English. Each interview lasted 45 minutes, and each focus group lasted 60-90 minutes. We recruited 20 patients (10 Hispanic, 10 AA) and 20 providers from a purposive sample representative of two large clinics that serve low-income women.

Results: All interviews were transcribed by two investigators. Each interview was coded by two investigators separately and then a third investigator reviewed the transcripts and coding to achieve triangulation. Codes for these themes were developed and the responses were tabulated using the coding scheme. Atlas ti was utilized to analyze all qualitative data. From these data, we uncovered Provider-Patient challenges in cancer communication including: the providers' trade off between medical accuracy and/or literacy when communicating with their patients. For the patients, the word cancer was important to hear since they wanted the truth and needed to hear this word in order to encourage them to respond more quickly. However, many providers believed that the word cancer was too "scary" and to "extreme" of a word that may communicate too much exaggerated information. Both the Hispanic and AA patients did not seem to differ in their responses.

Conclusion: Results exemplify not only the importance of health literacy and patient provider communication, but demonstrate the wealth of information gained from qualitative research- a method of data collection seldom utilized in OB/GYN investigations.

Funding: Women's Reproductive Health Research Award: HD050121-02; R21 CA126450 (Spring).

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Construction of Weight Gain Charts in a Low Risk Obstetric Belgian Population. Kristine Beckers,[1] Isabelle Guelinckx,[2] Greet Vansant,[2] Roland Devlieger.[1] *[1]Obstetrics and Gynaecology, University Hospital Gasthuisberg, Leuven, Belgium; [2]Clinical Nutrition, Katholic University Leuven, Leuven, Belgium.*

Objective: To generate reference charts for weight gain during pregnancy for the different BMI-categories (underweight, normal weight, overweight, obesity), based on recent data in a homogeneous Caucasian population.

Methods: In a retrospective study at the department of Obstetrics of the Leuven University Hospital (Belgium), weight gain and pre-pregnancy BMI were determined in 605 Belgian pregnant women with accurately dateable, uncomplicated singleton pregnancies. Centile curves for the different BMI-categories were constructed using of the Linear Mixed model, one set of charts based on the absolute weight gain, another set based on the relative (expressed as percentage) weight gain. The effect of parity on weight gain was examined.

Results: Overall mean weight gain was 14.8 ± 4.7 kg (32.63 ± 10.36 lbs). Mean weight gain was 15.4 ± 4.1 kg (33.95 ± 9.04 lbs) in the underweight population, 15.1 ± 4.5 kg (33.29 ± 9.92 lbs) in the population with normal weight, 13.7 ± 5.3 kg (30.20 ± 11.68 lbs) in the overweight population and 12.0 ± 5.9 kg (26.46 ± 13.01 lbs) in the obese population. Weight gain (pattern and amount) of the underweight and normal weight patients differed significantly of the overweight and obese patients. Parity had a statistical, but no clinical significant influence on amount and evolution of weight gain.

Conclusion: By using strict inclusion criteria, BMI-category-specific reference charts were generated representing the optimal gestational weight gain, rather than the mean weight gain. This enables the weight charts to be used as a clinical tool during the counselling of pregnant women. Further studies are required to assess the effectiveness of this clinical tool.

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Intravenously Injected Bone Marrow Cells Restore Ovarian Folliculogenesis and Steroid Hormones Production in Female FSHR (-/-) Mice. Mohsen Ghadami,[1,2] Ebtehal El-Demerdash,[1] Dong Zhang,[1,2] Memmy H Hassan,[1] Manubai Nagamani,[1] Xinlei Chen,[4] Jorge Chedress,[3] M Ram Sairam,[4] Ayman Al-Hendy.[1,2] *[1]Dep of Ob/Gyn, UTMB, Galveston, TX, USA; [2]Dep of Ob/Gyn, Meharry Medical College, Nashville, TN, USA; [3]Dep of Ob/Gyn, Uni of Saskatchewan, Saskatoon, SK, Canada; [4]Mol Rep Res Lab, Univerisité de Montréal, Montreal, QC, Canada.*

Introduction: Recent studies showed that BMT rescues fertility and fecundity in chemotherapy-treated young adult female mice (Selesniemi et al. SGI 2007). Female FSHR(-/-) mice are sterile, because of a block in folliculogenesis at the primary follicle stage, show decrease in E2 and elevated FSH. This animal model is an appropriate model for studying hypergonadotropic ovarian dysgenesis and Infertility, caused by C566T mutation in FSHR gene. **Objective:** To investigate the effects of BMT on serum hormonal levels, follicular maturation and fertility of FSHR(-/-) mice. **Methods:** FSHR (-/-) mice at 6-10 weeks of age were randomized into treated versus control groups.BM from 1 syngenic female donor was injected into the tails of 2 recipients. Control group received vehicle alone. Vaginal smears were collected, body weight was measured daily. Sample animals were sacrificed at 0, 1, 2, 3, and 4 weeks post BMT. All organs were weighted and examined by H&E. FSH, E2, and P4 were measured before and after treatment. For donor cell tracking, DNA was extracted from various organs. Specific primer sets were designed for normal and mutant hFSHR gene. PCR amplification was done and PCR products were analyzed in 1% agarose gel. **Results:** Total body weight significantly increased in treated animals($P< 0.02$). Significant increase in both the total number of follicles, and the collective diameter of the follicles in treated animals observed($P<0.03$ and $P< 0.002$). Six out of 8 treated animals showed estrogenic changes in daily vaginal smear. E2 level increased 2.5-7.5 times and FSH level dropped to 50% in treated animals. Normal (donor allele) FSHR gene was amplifiable in 6 out of 8 recipient mice, and was detected only in the ovaries and uterus but not in any other tested organs.Control group did not show any changes in vaginal smear, hormonal level, and normal FSHR allele. **Conclusion:** Intravenously injected syngeneic bone marrow cells were able to home to the ovaries of female FSHR (-/-) mice and restore folliculogenesis and resume steroid hormone production. Potential mechanisms for these observations will be discussed.

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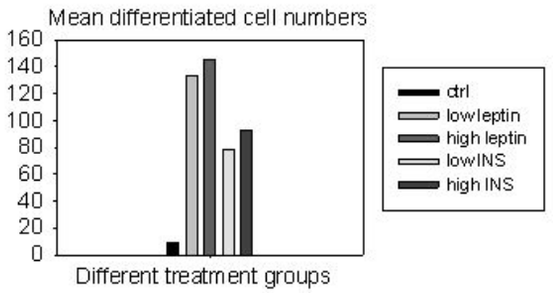
Leptin/Insulin-Induced Neural Progenitor Differentiation in the Rat Hypothalamus. Radmila Runic, Tri Nguyen, Stanislav Karsten, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Fetal hypothalamic development includes formation of orexigenic/anorexigenic neuronal pathways. IUGR offspring demonstrate impaired formation of anorexigenic pathways, contributing to offspring obesity. As IUGR newborns have low leptin and insulin (INS) levels, and both hormones serve as potential CNS growth factors, we speculate that reduction in leptin and INS contributes to reduced neural pathway development. Using multipotent CNS stem cells, we examined the effect of leptin/INS on newborn hypothalamic neural progenitor differentiation.

Methods: Neural progenitor cultures(NPC): tissue around the third ventricle of newborn hypothalami (P1) is digested and cells washed in neurobasal medium (NB). Cells are plated in 10-ml flasks for free floating neurospheres, all in NB containing 2μM L-glutamine, 25 ng/ml recombinant human fibroblast growth factor-2 (FGF-2), and heparin (1:25,000 dilution). Cells are grown in this medium, with FGF-2 replaced every 72 hrs. At day 7, cells are replated in NB medium alone or containing 0.1 (low) or 1 μg/ml (high) leptin, 25 μg/ml (low) or 1 mg/ml (high) INS in 12-well plates. Differentiation of NPCs: cells undergo differentiation in coated dishes until 72 hr. Immunohistochemistry confirmed pure neuronal cultures. Mouse monoclonal neurofilament antibody, SMI-311 is used as neuronal marker. 10 x magnification pictures are used to count the neuronal differentiated cells in all the fields (n=2).

Results: Leptin and INS increase neuronal stem cell differentiation in hypothalamic neuronal cell cultures (Fig). Low dose leptin increases the differentiated cell number 14-fold, high dose leptin 15.5-fold, low dose INS 8.3-fold and high dose INS 9.9-fold.



Conclusion: Neural stem cells (NSCs) give rise to progenitors, which expand by rapid proliferation until cell cycle arrest followed by differentiation along one of 3 CNS cell lineages: neurons, astrocytes and oligodendrocytes. Increased differentiation of neuronal cells with INS and even more with leptin suggests that IUGR-associated reduction of these growth factors may contribute to impaired hypothalamic pathway development.

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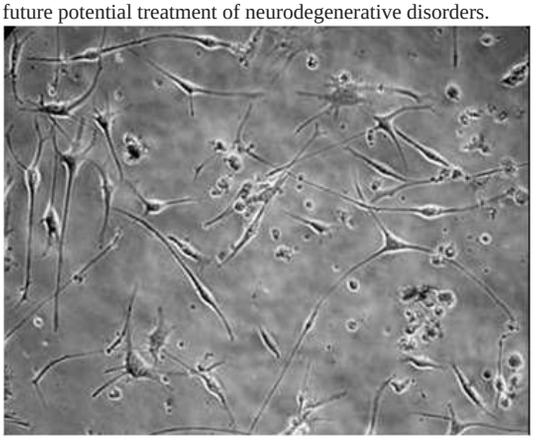
Three-Dimensional (3D) Collagen Fibrous Scaffolds for Neural Engineering of Human Umbilical Cord Blood (HUCB)-Derived Progenitors. Uriel Elchalal,[1] Marian Bercu,[1] Hadar Arien-Zakay,[2] Anat Perets,[3] Peter L Lelkes,[3] Yosef Ezra,[1] Reuven Or,[4] Philip Lazarovici.[2] *[1]Obstetrics and Gynecology, Hadassah Hebrew University Medical Center, Jerusalem, Israel; [2]Pharmacology and Experimental Therapeutics, School of Farmacy, Faculty of Medicine, The Hebrew University, Jerusalem, Israel; [3]School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, USA; [4]Bone Marrow Transplantation Unit, Hadassah Hebrew University Medical Center, Jerusalem, Israel.*

Objective: To develop a technology of modulating HUCB in order to achieve neuronal cells for future potential replacement of damaged neuronal tissue.

Design: We developed a two-dimensional (2D) tissue culture technology for isolation and differentiation of collagen-adherent HUCB neural progenitors (HUCBNPs). We further used the extracellular matrix protein collagen, organized in a three-dimensional (3D) gel, supplemented with neuronal conditioning medium and nerve growth factor (NGF), to facilitate *ex-vivo* long term neuronal differentiation of HUCBNPs. We developed a stable green fluorescence protein (GFP)-PC12 cell model, to be used as a positive control for monitoring neuronal outgrowth for proper evaluation of the HUCBNPs growth in the 3D scaffold, mimicking a neural tissue organization.

Results: Our experimental data indicate that 3D collagen environment is neuronal biocompatible, supporting attachment, long-term survival, proliferation and differentiation of both HUCBNPs and GFP-PC12 cells.

Conclusions: Improvement of the 3D technology with cultures of HUCBNPs that is in progress in our laboratory might be the first step in validating the concept for the feasibility of generating a neuronal 3D tissue construct for future potential treatment of neurodegenerative disorders.



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Mouse Trophoblastic Stem (TS) Cells from *In Vitro* (IVF) and *In Vivo* Fertilized Embryos Show a Similar Differentiation Potential. Luisa Delle Piane, Justin Tsai, Gnanaratnam Giritharan, Emin Maltepe, Paolo Rinaudo. *Obstetrics, Gynecology & Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA.*

Objective: IVF embryos are often used to generate stem cells. However, it is unknown if stem cell lines originated from *in vitro* generated embryos are different from stem cell originated from *in vivo* embryos. Trophoblastic cells, due to their external position within the embryo, are most susceptible to environmental factors encountered *in vitro.* Furthermore, our previous data showed that trophoblast transport functions may be impaired after culture *in vitro* and that the number of trophoblastic cells is reduced in the embryo after IVF. In this study, we assess the differentiation characteristics of TS cell lines obtained from *in vivo* and *in vitro* fertilized embryos.

Methods: Oocytes were isolated from superovulated C57BL/6J female mice and in *vitro* fertilized with sperm from male C57BL/6J mice. The resulting late-cavitating blastocysts were harvested. *In vivo* controls were obtained by flushing the blastocysts from the uteri of superovulated pregnant mice 4 days post hCG. TS cells from the two groups were allowed to develop and maintained in vitro in the presence of FGF4 and heparin, using a feeder layer of human placental fibroblasts. TS cells were then allowed to differentiate without FGF4 and heparin, fixed on day 8, stained with α-tubulin and ZO-1 antibodies and then observed under fluorescence microscopy. Three TS cell lines per group were analyzed and their differentiative capacity was evaluated using morphological criteria.

Results: *In vivo* and IVF derived TS exhibit a similar differentiation pattern. In particular, the number and timing of trophoblast giant cells and spongiotrophoblasts derivation is similar in the two groups. There are no obvious abnormalities in the immunologic staining morphology of the different cell lines at different time points.

Conclusion: There are no apparent morphological alterations in TS cells lines derived from IVF embryos as compared to *in vivo* embryos. This finding in an animal model increases our confidence in the reliability of human stem cells derived from IVF.

As a further investigation, markers of trophoblast giant cells, spongiotrophoblast, syncytiotrophoblast and chorionic trophoblast cells will be examined and compared in the two different groups by Northern Blot hybridization.

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Genomewide High Density SNP-CGH Reveals Several New Deletion Copy Number Variants on the X Chromosome in POF Patients. Erik AH Knauff,[1] Cisca Wijmenga,[2] Ruben van 't Slot,[2] Lude Franke,[2] Bart CJM Fauser.[1] *[1]Reproduction & Gynecology, University Medical Centre, Utrecht, Netherlands; [2]Complex Genetics Group, University Medical Centre, Utrecht, Netherlands.*

Introduction:

Around 1% of women have a post-menopausal hormonal profile before age 40, referred to as premature ovarian failure (POF). POF could act as a genetic model for accelerated follicle loss and may render useful information about the polygenetic background of major individual differences in menopausal age. Macrodeletions on the X chromosome are associated with POF but karyotyping has a maximal resolution of ~10Mb. When using genotyping whole genome arrays it is now possible to detect submicroscopic deletions and duplications (copy number variants (CNV)) up to 50 kb effective resolution. We have screened for microdeletions on the X chromosome in a well-phenotyped cohort of POF patients.

Methods:

Our study included 108 Caucasian, 46,XX patients with spontaneous secondary amenorrhea before age 40, FSH > 40 IU/L and absence of (low) 45X/46 XX mosaicism and FMR1 premutations. DNA analysis was performed using Illumina 370k CNV arrays containing 370,404 probes. 12,556 probes were located on the X chromosome (~1 probe for every 12 kb), of which 1,348 probes were specifically designed to detect known CNVs. Ten samples with call rates <99% were removed from the analysis and one sample gave inconsistent X probe intensities. We screened for deletions (~60 kb) using an algorithm detecting at least five consecutive probes with intensities (logR > -0.20) below the mean probe intensity.

Results:

We identified a total of 183 X chromosomal microdeletions, divided in 84 loci and varying between 60-200 kb in size. 46 of the 97 samples showed at least one deletion on the X chromosome. 27% of the identified deletions had already been recorded in the Database of Genomic Variants. In the newly identified

loci, we found 26 coding regions containing 38 genes. Eight of these are established or potential POF candidate genes, including five that are clustered on the terminal Xq critical POF region.
Conclusions:
We observed abundant variation in the CNV regions, a proof-of-principle for this specially designed CNV-chip. SNP comparative genomic hybridization revealed possible new deletions specific to POF on the X chromosome. Data on duplications, validation and whole genome CNV analysis, compared to a control cohort, will become available soon and will be presented at the SGI Annual Scientific Meeting.

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Unexplained Intrauterine Fetal Death (IUFD) Is Associated with Long QT Syndrome. Irene Cetin,[1] Patrizio Antonazzo,[1] Sabrina Cozzolino,[1] Stefania Calabrese,[1] Lia Crotti,[2] Francesca Ferrari,[3] Roberto Insolia,[2] Fabio Facchinetti,[3] Peter J Schwartz.[2] *[1]Dept. of Obst/Gynecol, Univ. of Milano, Milano, Italy; [2]Molec Cardiol Lab, Policlinico San Matteo, Pavia, Italy; [3]Mother-Infant Dept., Univ. of Modena/Reggio Emilia, Modena, Italy.*
Introduction: In developed countries, nearly 1 in every 200 pregnancies ends in late fetal loss. Many IUFDs can be attributed to maternal disorders, fetal pathology, placental pathology and fewer to complications of labor and delivery. However, 25-50% of cases remain unexplained. We recently demonstrated that 10% of Sudden Infant Death Syndrome (SIDS) cases carry functionally relevant genetic variants in Long QT Syndrome (LQTS) genes. Aim of the study was to analyze whether LQTS genes are associated with IUFD.
Materials and Methods: 60 patients with IUFD were enrolled in two years, as part of an Italian multicentre study. IUFD was defined as fetal death at 22 weeks or more of gestation according to the definition of late fetal death of the WHO. 32 out of 60 cases were classified as "unexplained stillbirths" according to the Wigglesworth and Aberdeen classifications. At birth placental and/cord samples were collected and DNA extracted. The main LQTS genes KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 were screened through DHPLC and sequence analysis. Any amino-acid substitution identified in the samples was checked for in a control population of 122 Caucasian women with uneventful pregnancies. Preliminary data on the first 17 cases (gestational age of death: 23-38 weeks) are reported.
Results: A total of 3 missense mutations were identified in 3 of 17 stillbirths (18%), two on SCN5A and one on KCNH2. The two mutations on SCN5A (V1951L; P2006A) were observed in two IUFD occurred at term; they had been previously associated to SIDS and shown to increase the late sodium current. The mutation on KCNH2 is a novel genetic variant absent in 244 reference alleles, never described in any control populations; this mutations was present in a case of IUFD diagnosed at 33 weeks of gestation. We are currently performing the electrophysiological cellular studies to define its functional effect.
Conclusions: These preliminary data indicate that a potentially significant number of currently unexplained IUFD might be caused by ion channel diseases such as LQTS. The potential prevention of SIDS or IUFD recurrence and the identification of other affected family members could have important implications for the affected families.

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Alternative Splicing of ePAB Is Regulated by Exonic Splicing Enhancers. E Seli, A Yaba, O Guzeloglu-Kayisli, MD Lalioti. *Ob & Gyn, Yale U., New Haven, CT, USA.*
Introduction: Alternative splicing is an important mechanism by which the genome gives rise to the observed diversity of proteins. Embryonic poly(A) binding protein (ePAB), expressed exclusively in oocytes and early embryos, mediates translation of maternal mRNAs. We identified an alternatively spliced form of ePAB lacking exon 10 (cEx10del), and investigated its regulation as a model for alternative splicing in early development. Specifically, we evaluated: imprinting (expression from maternal or paternal allele only); RNA editing (post-transcriptional single nucleotide substitution); and exonic splicing enhancers (ESEs, exonic sites that bind splicing proteins).
Methods: A single nucleotide polymorphism (SNP) detected in exon 9 (c1290A/G) served as a marker for the parental origin of the spliced form. SNP genotyping was performed by PCR amplification of exon 9 followed by restriction enzyme digestion. To evaluate imprinting, we characterized heterozygous mice (A/G) that inherited the SNP from either the mother or the father. To test for RNA-editing and exonic enhancer contribution we tested mice homozygous for the exon 9 SNP (A/A or G/G). Efficiency of alternative splicing in different genetic backgrounds was tested using real-time PCR normalized to actin expression.
Results: In mice heterozygous (A/G) for the exon 9 SNP, cEx10del was ONLY expressed from the (A) allele. However, this was independent of the parental origin of the allele, ruling out imprinting. In mice homozygous (G/G) for the exon 9 SNP, the cEx10del variant also contained (G). Therefore, RNA editing did not occur. Further sequence analysis led to the identification of an additional SNP in exon 10 (c1383G/C) that co-segregated with the exon 9 SNP. Presence of c1383G led to the formation of an ESE that binds splicing regulatory protein SRp40, leading to efficient exclusion of exon 10. Real time PCR revealed a five-fold increase in the expression of the cEx10del alternative splicing variant in animals carrying the enhancer (homozygous G/G) for the exon 10 SNP compared to those that did not (homozygous C/C) at the same locus ($p < 0.001$).
Conclusions: In this study, we found that ESEs mediate the alternative splicing of oocyte-specific transcripts. Our findings suggest that single nucleotide polymorphisms may alter the ratio between alternative splicing variants of oocyte-specific proteins. The role that these subtle differences play in determining individual reproductive outcome remains to be identified.

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Epigenetics and Chromosomal Abnormalities in Human Oocytes. Ilse van den Berg,[1,2] Joop SE Laven,[1] Robert Jan Galjaard,[2] J Hikke van Doorninck.[1,2] *[1]Obstetrics & Gynaecology; [2]Clinical Genetics, Erasmus Medical Center, Rotterdam, Netherlands.*
Humans have a low fertility rate compared to other mammalian species. Moreover their fertility declines with increasing age. Both phenomena are largely due to an increasing number of chromosomal abnormalities in human oocytes during life. Identifying factors that cause aneuploidy in oocytes may offer possibilities to diminish these abnormalities *in vitro* or *in vivo*. Chromosomal segregation errors can result from aberrant recombination but little is known about epigenetic factors that may cause aneuploidy. Epigenetic modifications such as histone acetylation and subsequent de-acetylation in oocytes are necessary for a correct progress through meiosis. If disturbed it may lead to aberrant segregation of chromosomes or chromatids due to decreased kinetochore function and imperfect spindle figures resulting in aneuploidy. Mice oocyte data have shown a correlation of abnormal histone de-acetylation and aneuploidy and a correlation between age, remaining histone acetylation and aneuploidy (Akiyama et al., 2006).
Our research focused on human oocytes and investigated whether a similar relationship between histone acetylation, age and aneuploidy is present. Human oocytes were surplus from standard IVF/ICSI treatments (IC+). Human oocytes showed immunostaining for histone 4, lysine 12 acetylation (H4K12) at the germinal vesicle stage and complete deacetylation at the MII stage in 45% of the oocytes, while 55% keep high levels of H4K12 acetylation. Treatment of germinal vesicle oocytes with a histon deacetylase inhibitor (TSA) during *in vitro* maturation until MII stage resulted in high levels of acetylation. Remaining acetylation in TSA treated oocytes was correlated significantly with abnormal spindle figures (tubulin staining), a hallmark of developing aneuploidy. Similarly, 80% of oocytes with naturally remaining H4K12 acetylation showed abnormal spindle figures. In contrast 80% of the normal de-acetylated oocytes showed normal spindles ($p=0.002$). This suggests that defective de-acetylation of H4K12 in human oocytes leads to abnormal spindle figures and subsequent aneuploidy. Advanced maternal age is associated with a reduction in de-acetylation during *in vitro* maturation and an increase in spindle abnormalities. These results may stimulate the development of assays for histone modifications as biomarkers to follow oocyte quality in *in vitro* maturation studies or in optimizing general IVF treatments.

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High Throughput Genetic Analyses Reveal Significant Racial Disparity in Preterm Birth. Ramkumar Menon,[1] Digna R Velez,[2] Errol R Norwitz,[3] Charles J Lockwood,[3] Scott M Williams,[2] Stephen J Fortunato.[1,3] *[1]The Perinatal Research Center, Centennial Women's Hospital, Nashville, TN, USA; [2]Vanderbilt University, Nashville, TN, USA; [3]Obstetrics, Gynecology and Reproductive Science, Yale University School of Medicine, CT, USA.*
OBJECTIVE: Racial disparity in preterm birth (PTB) is unexplained and genetic risk factors are suspected as a major cause. This large scale candidate gene study examines differences association of single nucleotide polymorphisms [SNPs] in Caucasians (C) and African Americans (AA) to help elucidate racial disparity in preterm birth (PTB)
METHODS: In this case (preterm birth <36 weeks) control study (term birth > 37 weeks) maternal and fetal DNA from 370 (172 cases and 198 controls) C and 279 (82 PTB and 197 term) AA were collected. A high-throughput candidate

gene association study was performed examining 1442 SNPs in 130 genes of selected from hypothesized PTB pathways. Single locus association analyses were performed separately on maternal and fetal samples.
RESULTS: SNPs in 23 genes associated with PTB ($p<0.01$) were common between races with both maternal and fetal DNA analyses. However, SNPs in 24 genes in C and 32 in AA in both maternal and fetal DNA differed in its association with PTB. In C maternal DNA, the single strongest association between PTB and SNP was in plasminogen activator tissue (PLAT) gene (C-4443T; rs879293) at both allelic ($p = 2.00x10^{-3}$) and genotypic ($p=2.0x10^{-6}$) level with an odds ratio (OR) of 2.80 [CI 1.77-4.44]. The single strongest effect in C fetal DNA was observed in a SNP in the interleukin-10 receptor antagonist gene (A18792G; rs17121510) for both allele ($p=0.01$) and genotype ($p=3.34x10^{-4}$), OR 1.92 [CI 1.15-3.19]). In AA, the strongest associations were in Interluekin-15 (C13929T-rs10833, allele $p=2.91x10^{-4}$, genotype $p=2.0x10^{-3}$) in maternal DNA with an OR=0.54 [CI 0.37-0.78] and in fetal DNA Interleukin-2 receptor B (A14481G; rs84460, allele $p=1.4x10^{-4}$, genotype $p=6.3x10^{-4}$) with an OR 2.36[CI 1.49-3.73].
CONCLUSION: Large scale high throughput analyses of SNPs in candidate genes of PTB pathway reveals significant differences between races at both maternal and fetal levels. We found that the strongest single locus associations differed in the two races in both maternal and fetal DNA samples. These findings support the hypothesis that underlying genetic predispositions may differ between these populations, perhaps partly explaining racial disparity in preterm birth.

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Candidate Gene Association Study Indicates Differential Etiologies in GDM and in T2DM. Johann Urschitz, Tarik Sultan, Kenneth Ward. *OBGYN, JABSOM, University of Hawaii, Honolulu, HI, USA.*
OBJECTIVE: Recently several genome-wide association studies have associated multiple single nucleotide polymorphisms (SNPs) in multiple genes with increased risk of type 2 diabetes. As risk factors are similar between T2DM and gestational diabetes (GDM), we investigated a possible association of those 18 SNPs with GDM.
STUDY DESIGN: Blood was collected from Caucasian (100 cases, 364 controls) women who met Coustan-Carpenter criteria for GDM and non diabetic controls. DNA was extracted and a candidate gene association study was performed (TaqMan, ABI).
RESULTS: Chi^2 contingency tests were used to analyze genotype and allele frequencies in controls and GDM affected pregnancies (see table below). None of the SNPs showed a significant association after Bonferroni correction.
CONCLUSION: Several polymorphisms which displayed highly significant associations with type 2 diabetes are not associated with gestational diabetes. These results suggest that GDM is not a simple unmasking of a T2DM predisposition by the metabolic demands of pregnancy; rather it appears that different biological mechanisms are responsible for the respective diseases.

MAF and p-values

	CDKAL1	**CDKN2B**	**EXT2**	**EXT2**	**EXT2**	**FTO**
	rs7754840	rs10811661	rs1113132	rs3740878	rs11037909	rs8050136
GDM	0.363 (G)	0.163 (C)	0.330 (G)	0.354 (G)	0.347 (T)	0.370 (C)
CONTROL	0.344 (G)	0.191 (C)	0.307 (G)	0.308 (G)	0.325 (G)	0.376 (C)
p-value*	0.630	0.385	0.291	0.224	0.318	0.334
	HHEX	**IGF2BP2**	**IGF2BP2**	**PPARG**	**PKN2**	**SLC30A8**
	rs1111875	rs1470579	rs4402960	rs1801282	rs6698181	rs13266634
GDM	0.351 (G)	0.328 (A)	0.314 (G)	0.129 (C)	0.399 (C)	0.274 (C)
CONTROL	0.442 (A)	0.351 (A)	0.348 (G)	0.113 (C)	0.377 (C)	0.346 (C)
p-value*	0.026	0.570	0.373	0.544	0.585	0.065
	WSF1	**WSF1**	**WSF1**	**Chr.10-94471897**	**Chr.11-41871942**	**Chr.11-42203294**
	rs734312	rs6446482	rs10010131	rs7923837	rs9300039	rs7480010
GDM	0.447 (G)	0.360 (C)	0.345 (A)	0.344 (G)	0.097 (C)	0.242 (A)
CONTROL	0.434 (G)	0.360 (C)	0.359 (A)	0.393 (G)	0.072 (C)	0.278 (A)
p-value*	0.744	0.999	0.714	0.213	0.273	0.329

*before Bonferroni correction

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Acetylation and Phosphorylation Status of HDACS and the Epigenetics of Myometrial Quiescent and Pro-Contractile Genes. Kelly A Harper, Stephen C Robson, GN Europe-Finner, Alison J Tyson-Capper. *Surgical & Reproductive Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom.*
Introduction: Histone acetylation/deacetylation plays an important role in the regulation of gene expression. Histone deacetylase inhibitors (HDACi), in general, maintain gene expression, although in some cases they cause repression of specific genes. In this context we have previously shown that the HDACi TSA suppresses activation of the pro-contractile gene COX-2 in human myometrial and amnion-derived cells [*Reproductive Sciences*, Vol 14, No 1 (supplement, #9)]. We have also shown that expression profiles for HDACS (1-6, 8) differ significantly within upper and lower regions of the myometrium during pregnancy and in labour.
Objectives: Since changes in both acetylation and phosphoryation status can influence the activity of individual HDACs we aimed to define whether there are any changes in the levels of acetylation and phosphoryation of myometrial HDAC 1, 2 and 8 during pregnancy and labour.
Methods: Protein homogenates prepared from non-pregnant, term and labouring myometrium were treated +/- shrimp alkaline phosphatase and subjected to SDS-PAGE and Western immunoblotting (WB) using antibodies to HDAC1, 2 and 8. The acetylation status of the HDACs was assessed by a co-immunoprecipitation assay using anti-HDAC and anti-acetylated lysine antibodies.
Results: Distinct patterns of acetylation were observed for the individual HDACs; HDAC 1 and 2 appeared to be more acetylated in non-pregnant and labouring lower tissues when compared to pregnant myometrium. In contrast, HDAC8 appeared to be slightly more acetylated in lower uterine samples from pregnant and labouring myometrium. In experiments to evaluate changes in phosphorylation status we observed that 1) myometrial HDAC1 appeared to less phosphorylated in both upper and lower labouring samples when compared to non-pregnant and pregnant samples; 2) HDAC2 appeared to be more phosphorylated in labouring samples than non-pregnant and pregnant myometrium; 3) No changes in phosphorylation levels were observed for HDAC8 using this test system.
Conclusions: The difference in HDAC acetylation and phosphorylation levels in the human myometrium may indicate differential regulation of the activity of the HDACs within the distinct myometrial regions, perhaps leading to the alteration of their epigenetic effect on genes related to myometrial quiescence and contraction and the subsequent onset of both term and preterm labour.

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A Genetic Polymorphism Related to Cardiac Health Evaluated in a Pregnant Hawaiian Population. Janet M Burlingame, Johann Urschitz, Kenn Ward. *Pacific Center for Early Human Development, John A. Burns School of Medicine, Honolulu, HI, USA.*
Objective: Native Hawaiians and Pacific Islanders experience a higher perinatal morbidity secondary to the increased incidence and earlier onset of heart failure. This increased incidence is likely multi-factorial and includes co-morbidities and genetic factors. Mutations in the human adrenergic receptor gene may play a role in the determination of heart function. Mutations in the b1-adrenergic receptor (ADRB1) located on chromosome 10 have been identified as a cause progressive cardiomyocyte loss leading to a dilated cardiomyopathy. The minor allele frequencies for most of these polymorphisms have been determined for Caucasian, Japanese, African-American and Chinese as part of the HapMap project. The frequencies have not been determined in the Pacific Islander groups. This study helped determine the Hawaiian allele frequency and determine the allele frequency in the presence of cardiac or other vascular co-morbidities such as hypertension, preeclampsia, and gestational diabetes.
Study Design: Real time PCR technology (*TaqMan Genotyping Assays, Applied Biosystems)* was used to screen maternal DNA (n= 1315) from the Phenotyping Sample Core of the Pacific Center for Early Human Development (PRCEHD) for the rs1801253 single nucleotide polymorphisms (SNPs). Genotype frequencies were also determined in 100% complete ethnic controls as determined by a four grandparent descent.
Results: Chi square was used to analyze allele and genotype frequencies differences between controls and affected pregnancies. The results are summarized in the following tables.
Conclusion: The rs1801253 SNP was not significantly associated with hypertension, preeclampsia or GDM in our overall Hawaiian population. The genotype frequency in the 100% Pacific Islander group was significantly different from the Caucasians ($p=0.04$) and Asians ($P=0.008$) in our overall Hawaiian population.

rs1801253 Allele and Genotype Frequencies

Allele	Overall	Chronic Hypertension	Preeclampsia	GDM	Pacific Islander
G	21.4%	25.6%	21.4%	21.2%	10.7%*
C	78.6%	74.4%	78.6%	78.8%	89.3%*
Genotype					
GG	4.7%	8.1%	7.7%	2.2%	0
CC	62.2%	56.8%	65.0%	59.8%	78.6%
CG	33.1%	35.1%	27.3%	38.0%	21.4%

* p=0.04 vs. Caucasian; 0.008 vs. Asian

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Angiotensin-Converting Enzyme and α-Adducin Polymorphisms in Preeclamptic Mothers and Fetuses. C Mando,[1] P Antonazzo,[1] S Tabano,[2] F Colleoni,[1] A Martinelli,[1] S Calabrese,[1] C Benedetto,[3] L Marozio,[3] F Facchinetti,[4] M Miozzo,[2] I Cetin.[1] *[1]Inst.Obstetrics and Gynecology, Fondaz. IRCCS Policlinico Mangiagalli Regina Elena, Univ. Milan, Milan, Italy; [2]Dept. Biology and Genetics, Univ. Milan, Italy; [3]Dept.Obstetrics and Gynecology, Univ. Torino, Italy; [4]Mother-Infant Dept., Univ. Modena and Reggio Emilia, Modena, Italy.*

Background

The genes encoding angiotensin-converting enzyme (ACE) and α-adducin (ADD1) share the potential of influencing blood pressure. Previous studies demonstrated in humans the association of hypertension with the combined effect of both ACE insertion/deletion (I/D) polymorphism, which leads to a different activity of the enzyme, and ADD1 G460W non-sense single nucleotide polymorphism (SNP). ACE I-I genotype has been associated with low serum ACE activity.

Controversial studies concerning the association of ACE polymorphism with preeclampsia (PE) were reported and the possible combined effect of both ACE I/D and ADD1 G460W polymorphisms yet remains to be investigated.

Population association study

We genotyped 2 polymorphisms (ACE I/D and ADD1 G460W) in 497 women: 197 with PE (119/197 with severe PE) and 300 controls. Moreover, we investigated a subset of their fetuses: 24 from severe PE, 17 from mild PE and 300 controls, in order to identify specific maternal and/or fetal genotypes conferring a higher risk to develop PE.

We both evaluated the single and the combined effects of ACE and ADD1 genotypes on mother-fetus couples and singularly on mothers and fetuses.

ACE I/D genotype was analyzed using a PCR method; an Allelic Discrimination approach was performed to detect the ADD1 SNP.

Results

In mother-fetus genotype couples, neither ACE nor ADD1 polymorphisms are associated with PE, nor are the combination of their genotypes separately in mothers and in fetuses. Nevertheless in mothers with mild PE ACE I-I genotype is significantly less frequent (8% vs 13%; $p<0.05$) and ACE I-D is significantly more frequent (59% vs 42%; $p<0.02$) compared to controls. ACE I allele frequency is not significantly different in mild PE compared to controls (37% vs 38%).

Conclusions

In women with mild PE the I allele seems to move from I-I to I-D genotype. It leads to the increase in mild PE women of those genotypes with a higher ACE activity conferring a higher susceptibility to develop PE. This association with mild PE and not with severe PE could be due to the contribution in the latter of many other genetic and environmental factors.

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CPEB Is Expressed in Mouse and Human Oocytes and Early-Preimplatation Embryos Prior to Zygotic Genome Activation. IE Sasson, O Guzeloglu-Kayisli, S Uckac, E Seli. *Ob & Gyn, Yale U., New Haven, CT, USA.*

Introduction: Maternal mRNAs stored in the oocyte are critical for early development as transcription ceases upon oocyte maturation, and gene expression until zygotic genome activation (ZGA) is mediated by translation of maternal transcripts. Cytoplasmic polyadenylation element binding protein (CPEB) plays a central role in this process by stabilizing maternal mRNAs and regulating their timely activation. This function has been well characterized in Xenpous, and a similar role in mammals is suspected as the CPEB knockout mouse displays infertility as a result of arrested oogenesis. In order to investigate if translational regulation of maternal transcripts by CPEB is maintained in mammals, we characterized the spatial and temporal expression of CPEB-1 in the mouse and human.

Methods: Ten different somatic tissues, testes, and ovaries were tested by RT-PCR for the expression of CPEB mRNA in mouse and human. CPEB mRNA expression in mouse was also tested in prophase I (PI) and metaphase II (MII) oocytes, 1-cell, 2-cell, 4-cell, 8-cell embryos and blastocysts. In human, PI and MII oocytes, 8-cell embryos and blastocysts were evaluated. Sequencing of the PCR products was performed to confirm specific amplification of CPEB. Amplification with actin primers provided a positive control and allowed semi-quantitative analysis.

Results and Discussion: Highest level of CPEB mRNA expression was detected in ovaries and testis of both mouse and human. In addition, differential expression of CPEB in somatic tissues was also observed. Among these, prominent expression was present in brain, where a role for CPEB in facilitating gene expression through cytoplasmic polyadenylation has been proposed. These data would suggest that translational control of mRNA by CPEB may be a mechanism utilized by multiple somatic tissues to regulate gene expression. In the mouse, CPEB was expressed in PI and MII oocytes and 1-cell and 2-cell embryos, and became undetectable in 4-cell or more advanced embryos. Human CPEB was expressed in PI and MII oocytes, but not in 8-cell embryos or blastocysts. ZGA occurs at late 2-cell stage and 4-to-8-cell stage, in mouse and human, respectively. The tightly controlled temporal expression of CPEB prior to ZGA in both mouse and human is consistent with a conserved role for CPEB in the regulation of maternal mRNA expression during early development in mammals.

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Stem Cell Markers of Embryonic and Trophoblast Lineages in Rhesus Macaque Blastocysts. AJ Harvey,[1] DR Armant,[2,3] CA Brenner.[1,2] *[1]Physiology, Wayne State University, Detroit, MI, USA; [2]OB/GYN, Wayne State University, Detroit, MI, USA; [3]Reproductive Biology and Medicine Branch, NICHD, NIH, Bethesda, MD, USA.*

Background Embryonic stem cells (ESC) express specific transcription factors that are indicative of their ability to maintain pluripotency. These factors are activated during preimplantation embryo development. The interplay between the transcription factors POU5F1 (Oct3/4) and caudal-homeobox domain 2 (Cdx2) is thought to regulate inner cell mass (ICM) and trophectoderm (TE) differentiation; however, the mechanism of cell fate determination in mammalian embryos is poorly understood. Genetic ablation of Oct3/4 in mice prevents ICM development, while Cdx2 knockout embryos fail to implant. ESC deficient in Nanog, a second key regulator of pluripotency, fail to maintain pluripotency and undergo differentiation. Expression of Nanog in primate embryos has not been investigated, therefore the localization of Oct3/4, Nanog and Cdx2 was determined in rhesus macaque blastocysts.

Methods Oocytes were collected from rhesus macaque females, fertilized and cultured in vitro to the blastocyst stage. Embryos were collected 144 hours post insemination, then fixed prior to incubation with primary antibodies directed against Oct3/4, Nanog and Cdx2. Following incubation with Cy3 conjugated secondary antibodies, nuclei were counterstained with DAPI and embryos visualized using an Olympus BX41 fluorescence microscope.

Results Nanog protein was restricted to the ICM of monkey blastocysts. Unlike the mouse embryo, Oct3/4 protein was detected in both the ICM and TE, based on two different antibodies. The expression of Cdx2 was localized specifically to the TE.

Conclusions The ubiquitous pattern of Oct3/4 expression is consistent with observations in human, cow and pig embryos. Significantly, lack of restricted Oct3/4 protein, and ICM localization of Nanog in primate blastocysts, suggests that Nanog more specifically determines cell fate in primate embryos. These results contrast markedly with current mechanistic hypotheses, although other factors may lie upstream of Nanog. Importantly, this difference may underlie observations that regulatory mechanisms in ESC differ between mice and primates. Further investigations will focus on determining the onset of marker expression, and the upstream regulators of Nanog activation. *Supported by NIH grants 1R01HD045966 & 1R21RR021881, and the intramural research program of NICHD.*

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***In Vitro*-Conceived Mice Tend To Be Smaller at Birth and throughout Their Life.** Luisa Delle Piane, Annemarie Donjacour, Francesca Di Sebastiano, Gnanaratnam Giritharan, Paolo Rinaudo. *Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA.*

Objective: Epidemiological evidence indicates that IVF is associated with an increased incidence of low birth weight. This phenomenon has not been

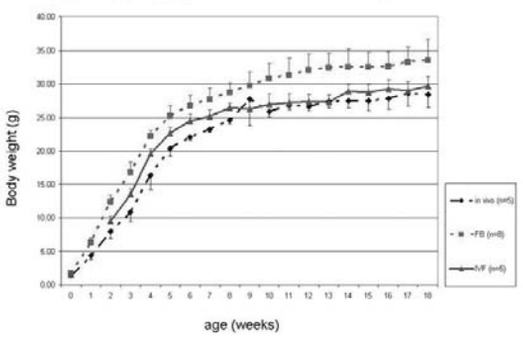
studied in a mouse model; in addition, it is unknown if the resulting offspring show different growth pattern later in life. We therefore created an IVF mouse model to follow growth patterns till adulthood.

Methods: We generated 3 experimental groups of B6 mice: one cohort of *in vivo* generated mice (*in vivo* group), one cohort of *in vitro* generated animals (IVF group) transferred to CD1 foster mothers and one cohort of animals fertilized *in vivo* and transferred to CD1 foster mothers (flushed blastocysts group, FB). The FB group was generated because our preliminary results showed that embryo transfer to B6 foster mothers was not successful. All pups were delivered at term, measured and weighed at birth and then weekly up to 18 weeks. Parametric tests (Anova with Bonferroni correction) were used as appropriate. A mixed model was used to compare growth curves.

Results: Average litter size was 6.3 (*in vivo*), 3.7 (FB) and 3.5 (IVF). Birth weight of male mice both IVF (1.57 mg) and FB (1.73 mg) was larger than male *in vivo* mice (1.30 mg) ($p<0.05$). IVF mice tended to be smaller than FB mice in both sexes ($p= 0.0533$). The BMI (body mass index) was not different among all the groups. Male FB growth curves were different from *in vivo* mice ($p<0.0001$) and more importantly from IVF growth curves ($p=0.0516$) (Fig. 1).

Conclusion: The method of conception and the maternal environment play a significant role in determining birth weight in this mouse model, emphasizing that the FB group is the best control for the effects of IVF in this model. These preliminary results confirmed for the first time an IVF effect on growth and interestingly the IVF males did not show a catch-up growth. The finding of smaller IVF mice when compared to FB is both noteworthy, because it confirms human data, and worrisome, because lower birth weight is associated with long term sequelae according to the Barker hypothesis.

Fig.1 Average weight gain of male animals from birth up to 18 weeks.



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The Effect of Betamethasone Exposure in Mid-Gestation on Renal Sodium Excretion in Male Sheep. Lijun Tang,[1] Luke Carrey,[1] Nancy Valego,[1] Philip Deibel,[1] James Perrott,[1] Jorge Figueroa,[1] Mark Chappell,[2] James C Rose.[1] *[1]Obestetrics and Gynecology, Wake Forest University, Medical School, Wiston-Salem, NC, USA; [2]Hypertension Center, Wake Forest University, Medical School, Wiston-Salem, NC, USA.*

Objective: Whereas prenatal exposure of ovine fetuses to clinically relevant doses of glucocorticoids during the time of peak nephrogenesis results in a reduction in nephron number in adulthood, there is little information about its effect on sodium excretion. In the present study, we evaluated the effect of exposure to betamethasone on renal sodium excretion in adult male sheep.

Methods: We studied nineteen conscious adult rams at 1.5 years of age which were exposed to either vehicle or betamethasone at 80-81 days gestation. We implanted vascular and bladder catheters and then allowed the animals a 5-7 days recovery period prior to study. Inulin and para-aminohippuric acid (PAH) clearances were performed for estimating glomerular filtration rate (GFR) and renal plasma flow (RPF) respectively. Following determination under basal conditions, an acute hypertonic sodium load was administrated intravenously by a continuous infusion of NaCl (0.0275 mEq/kg/min at 0.55 ml/min) for 60 minutes. Urine was continuously collected for determination of Na^+ excretion.

Results: Basal GFR was decreased in steroid exposed adults (2.267 ± 0.10 ml/min/kg) compared with the vehicle animals (1.935 ± 0.08 ml/min/kg) ($P=0.028$). RPF was similar in the vehicle and steroid exposed group (15.483 ± 0.40 ml/min/kg *vs* 13.561 ± 0.80 ml/min/kg). At basal conditions, Na^+ excretion ($U_{Na}V$) was similar in vehicle and steroid exposed group (16.60 ± 5.37 μmol/h *vs* 14.37 ± 2.46 μmol/h). This similarity was also present after normalization by body weight (0.278 ± 0.09 μmol/h/kg *vs* 0.234 ± 0.04 μmol/h/kg). The vehicle group excreted 53.65 ± 9.71% of the dose of Na during the experiment while the betamethasone exposed group excreted only 37.07 ± 4.42% ($P<0.05$). GFR and PRPF did not change during the experiments.

Conclusion: These results suggest that prenatal exposure to glucocorticoids affects renal function in adult male sheep which results in a decreased basal GFR and an attenuated ability to excrete on acute sodium load. The elevated blood pressure previously observed associated with this prenatal steroid treatment may be related to the alteration in renal function.

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Characterisation of Human Fetal Cardiac Stem Cells. Marah Alfakir, Nicholas Dawe, Annette Meeson, Stephen C Robson. *School of Surgical & Reproductive Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Objectives: For many patients with severe cardiac disease treatment is limited to surgical intervention and/or heart transplantation. The heart has a limited regenerative capacity but it is insufficient to repair extensive damage. Cellular therapies might provide an alternative treatment. We have identified stem cells (SP cells) in the adult mouse and human heart and have shown that the mouse cardiac SP cells can differentiate along a cardiac lineage. SP cells can be identified using FACS combined with Hoechst 33342 dye efflux. This ability to efflux Hoechst dye is due to expression of Abcg2 (an ABC transporter). We have hypothesized that the fetal heart would contain more cardiac stem cells, relative to the adult, and the aim of this project was to determine the spatial and temporal expression of known stem cells markers in the embryonic/fetal heart.

Methods: 15 human fetal hearts were collected aged between 6-16 wk. We used RT-PCR, using primers for several stem cell (Abcg2, CD34, CD45) and cardiac specific (Mlc-2v, αMHC) markers, and IHC on frozen and paraffin sections, using a panel of antibodies (Abcg2, CD34, CD45, islet1). cDNA was generated from the following regions of the heart at different developmental stages: right and left atrium, right and left ventricle, and outflow tract.

Results: Abcg2 mRNA was highly expressed in all regions of the fetal heart between 7-10 wk. Expression was down regulated at 11-15 wk especially in the LA and LV. A similar pattern of expression was observed for CD34. CD45 showed low/moderate expression in all heart regions examined; however, this expression was absent in the LV at 13-15 wk. mRNA and protein analysis showed similar results. Whilst protein expression of Abcg2 was robust at 7 wk (approximately 3-6%), it declined with increasing gestational age. CD34 was also strongly expressed at 7 weeks of age. There was no co-localisation between Abcg2 and islet1.

Conclusion: Abcg2 and CD34 are both expressed between 7-10 wk of age. Based on this analysis, further studies are underway to isolate and characterise both the Abcg2 and CD34 expressing fetal cardiac cells which might be a potential source of cardiac stem/progenitor cells.

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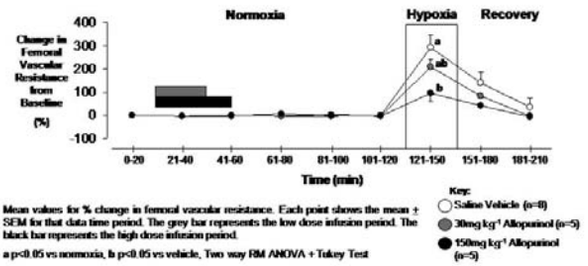
Xanthine Oxidase Plays a Significant Role in the Fetal Cardiovascular Defence to Hypoxia. JA Hansell, A Kane, E Herrera, DA Giussani. *Physiology, Cambridge University, United Kingdom.*

Prenatal hypoxia remains a major concern in obstetrics. The fetal defence to hypoxia includes redistribution of the cardiac output, away from peripheral and towards essential ciculations, such as those perfusing the brain (Cohn et al. *AJOG* **120**:817,1974). The physiology underlying this response is well characterised and involves chemoreflex and endocrine responses (Giussani et al. *Fet Mat Med Rev* **6**:17, 1994). More recently, local factors such as nitric oxide (NO) and reactive oxygen species (ROS), and the interaction between them, have been shown to play a role. Antioxidants, such as vitamin C, scavenge hypoxia-induced ROS, maintain NO high and thereby depress peripheral constriction (Thakor *et al.*, SGI 2006). In this study, we address the source of hypoxia-induced ROS production and investigated the effects on the *in vivo* fetal femoral constrictor response to hypoxia of maternal treatment with the xanthine oxidase inhibitor allopurinol in sheep.

Methods: Under anaesthesia, 8 sheep at 0.8 gestation, were instrumented with maternal and fetal catheters and a fetal femoral Transonic probe. Five days later, all animals were subjected to 2 h normoxia, 0.5 h hypoxia (mat FiO_2 to reduce fetal PaO_2 to *ca.* 10 mmHg) and 1 h recovery, either following maternal i.v. treatment with vehicle or allopurinol in low (30 $mg.kg^{-1}$ over 20 min) or high (150 $mg.kg^{-1}$ over 30 min) doses. Treatments finished 100 min prior to hypoxia. The low allopurinol dose was adopted from human studies (Benders et al. *Arch Dis Child* **91**:163, 2006). The timing of the hypoxic challenge coincided with peak concentrations in fetal sheep plasma of oxypurinol (active metabolite).

Results: During hypoxaemia, a similar fall in fetal P_aO_2 occurred in all fetuses. Maternal treatment with allopurinol lead to a dose-dependent suppression of the fetal femoral constrictor response to acute hypoxaemia (Fig. 1).



Conclusion: Generation of reactive oxygen species, secondary to activation of the xanthine oxidase pathway, plays a significant role in the fetal cardiovascular defence to prenatal hypoxia. Treatment with allopurinol during pregnancy should be viewed with caution.
The Bristish Heart Foundation.

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Increased Term Fetal, as Compared to Newborn, Corticotrophin Releasing Factor Receptor Subtype 1 (CRF-R1) Expression Pattern in the Distal Small Intestine and Colon: Role in the Timing of Meconium Passage. John D Richard, Michael G Ross, Sharon K Sugano, Guo L Liu, Reuben Lakshmanan, Brian H Im, Jayaraman Lakshmanan. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*
Objective: Studies in adult rats indicate that CRF-R1 and CRF-R2 receptors regulate gastrointestinal (GI) motility during times of stress. Whereas CRF-R1 activation stimulates, CRF-R2 activation inhibits GI motility. We hypothesize that stress-induced fetal meconium passage is analogous to stress-induced defecation in adult rats, and is likely mediated by the CRF-pathway. In support, we documented marked increases in plasma CRF levels to accompany meconium passage in term rat fetuses subjected to acute maternal hypoxia (Pediatr. Res. 61:176-9, 2007). Little information is available on the mechanism(s) of meconium passage that occurs within first 24 hours after birth. We examined CRF-R1 receptor expression in day 21 fetal rats and newborn rats within 12 hours after birth.
Methods: GI regions including duodenum, jejunum, ileum, and proximal and distal colon were dissected from rat fetuses on day 21 (term=22; 6 pools, 10 fetuses per pool) and neonates born (n=20) within 12 hr after birth. The GI segments were fixed in Bouin's solution and paraffin embedded. Paraffin sections immunostained with polyclonal antibody to CRF-R1 receptor by standard ABC technique. Immunoreactive materials on the sections were identified by 3, 3' diamino benzidine as a chromagen. Immunoreactivity of GI regions was quantified using Image pro 4.01 software and the intensity (OD/area) is expressed as arbitrary units (AU).
Results: All GI regions, both in e21 fetuses and newborns exhibited positive staining. The intensity of CRF-R1 staining increased significantly from fetal to newborn ages in the duodenum (0.102±0.001 vs 0.120±0.007; $p<0.05$) and jejunum (0.076±0.002 vs 0.205±0.013; $p<0.01$). In contrast CRF-R1 staining decreased significantly from fetal to newborn ages in the ileum (0.208±0.002 vs 0.142±0.004; $p<0.01$), proximal colon (0.226±0.011 vs 0.173±0.002; $p<0.01$), and distal colon (0.282±0.015 vs 0.186±0.008; $p<0.01$).
Conclusion: As compared to the term fetus, newborn rat CRF-1 receptor intensity increased in the proximal small intestine, while it decreased in the distal small intestine and colon. Increased colonic expression of the stimulatory CRF-R1 expression at term suggests that this receptor likely has a critical role in the timing of meconium passage.

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Bisphenol-A Exposure Alters Developmental Gene Expression in the Non-Human Primate. Tamir S Aldad, Chaba Leranth, Hugh S Taylor. *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
OBJECTIVE: Bisphenol-A (BPA) is a xenoestrogen and putative endocrine disruptor used in the manufacture of plastic food and beverage containers. We evaluated the effect of BPA exposure on uterine gene expression in a non-human primate model.
METHOD: A total of nine Vervet monkeys (Cercopithecus aethiops sabaeus) were ovariectomized. Three monkeys were treated with BPA via Alzet pumps (50 microgram/kg/day), two with estradiol benzoate and three received combined treatment with BPA and estradiol. The remaining monkey was untreated and served as a control. Following 28 days of treatment the monkeys were hysterectomized and immunohistochemistry performed to examine endometrial gene expression. We evaluated HOXA10, Proliferating Cell Nuclear Antigen (PCNA), and Caspase III gene expression as markers of differentiation, proliferation, and apoptosis respectively. Differential expression was evaluated by H-score.
RESULTS: Exposure to a combination of estradiol and BPA resulted in increased expression of HOXA10 and PCNA compared to control ($p<0.01$). Lower, but increased, levels of HOXA10 and PCNA gene expression were seen in the specimens exposed solely to estradiol ($p<0.05$). Those specimens exposed to BPA alone demonstrated a small induction in HOXA10 expression ($p<0.05$) with no change in Caspase III or PCNA expression when compared to the control.
CONCLUSION: BPA induced the expression of HOXA10 in the uteri of a non-human primate. Exposure in the presence of endogenous estrogen further augmented estrogenic stimulation confirming that BPA is a xenoestrogen. BPA's effect of developmental gene expression may alter uterine differentiation. BPA acts as an endocrine disruptor by altering estrogen regulation on a gene required for fertility.

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Dynamics of Nuclear Glucocorticoid Receptor Expression in Ovine Fetal Distal Colon: A "Hormonal Switch" for the Maturation of Colonic Contractility and Meconium Passage. Jayaraman Lakshmanan,[1] John D Richard,[1] Guo L Liu,[1] Sharon K Sugano,[1] Ahmet Karadag,[2] Michael G Ross.[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Dept. of Pediatrics, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*
Objective: Endogenous glucocorticoids (GCs) increase with advancing fetal age suggesting that GCs function as a hormonal modulator of organ maturation. Although fetal colonic motility and meconium passage primarily occur near term, the role of GC in colonic maturation is poorly understood. We sought to examine GC-nuclear receptor (GC-NR) expression in ovine fetal distal colon smooth muscle from very-preterm to term gestation to define the cascade of GC initiated biochemical changes as a prerequisite for maturation-associated meconium passage.
Methods: Ovine fetal distal colonic segments removed at very-preterm (VPT: 118-120 d gestation), preterm (PT: 130-132 d), near term (NT: 140-142 d) and term (T: 146-147 d) (n=6 fetuses in each group) were fixed in Bouin's solution and paraffin embedded. Sections were subjected to immunohistochmical analysis with GC-receptor antibody (1:200, sc-8992, SantaCruz biotechnology, CA) using standard ABC regimen. Immunoreactive material identified by 3,3'diaminobenzidine as chromogen. The percentage of GC-NR staining in smooth muscle-enteric unit was analyzed by counting dark brown immunostained nuclei at 10 different fields at 400x magnification. All values are expressed as mean ± SEM.
Results: The GC-receptor antibody immunostained nuclei both in smooth muscle and enteric units and the observed pattern (expressed as percentage of total nuclei) are as follows:

	VPT	PT	NT	T
Muscularis mucosa	23.6±1.4 %	45.5±3.3 %	92.5±1.0 %*	2.0±0.4 %
Circular muscle	15.0±1.5	44.0±6.0	89.0± 2.0*	3.0± 0.5
Longitudinal smooth muscle	15.3±1.5	34.5±5.0	89.0±2.0*	4.0 ±1.0
Submucosal neurons	27.6±3.9	66.7±0.1	88.2±4.8*	8.0±1.4
Myenteric neurons	17.8± 2.4	35.8±2.4	78.8±4.1*	8.0±1.4

* $P<0.001$, NT compared with VPT, PT and T

Conclusion: Nuclear GC expression varies with gestational age with maximal expression occurring near-term in smooth muscle and enteric neurons, in a synchronized manner. A near total disappearance of GC-NR expression occurs at term. These results suggest that peak gastrointestinal GC-NR mediated effects may occur prior to term with secondary signaling effects resulting in distal colonic motility maturation and meconium passage.

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The RNA-Binding Protein HUD Co-Localizes with Choline Acetyl Transferase (pChAT) and Corticotrophin-Releasing Factor (CRF)-Receptor Type I (CRF-R1) in Ovine Fetal Disatal Colon Neurons: Mechanisms of *In Utero* Meconium Passage. Jayaraman Lakshmanan, John D Richard, Guo L Liu, Sharon K Sugano, Octavio Balbuena, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: We have previously speculated that CRF, acting through its receptor CRF-R1, increases gastrointestinal (GI) motility and potentiates in utero meconium passage. Patients with paraneoplastic syndrome with auto-antibodies to HuD, a neuronal RNA binding protein, develop severe GI dysmotility, indicating a role for HuD in GI motility. HuD binds mRNA for actylcholinesterase implying a role in the cholinergic neurotransmitter system. Based on these known functions, we hypothesized that HuD may regulate post-transcriptional activity in fetal colonic cholinergic neurons expressing CRF-R1.

Method: Bouin's solution-fixed paraffin-embedded sections of distal colonic segments were prepared from very preterm (VPT: 118-120 days), preterm (PT: 130-132 days), near term (NT: 140-142 days) and term (146-147 days) ovine fetuses (n=6 at each age). Sections were immunostained with anti-human neuronal protein HuC/HuD antibodies (1:800 to 1:1000) with ABC reagents and examined at 400X. Neurons positive for HuD staining were quantified. Double immunofluorescence and laser confocal analyses evaluated co-expression of HuD in neurons expressing peripheral choline acetyl transferase (pChAT) (a marker for peripheral cholinergic neurons) and CRF-R1 receptor.

Results: HuD immunostaining was seen in either entire cytoplasm (C) or cytoplasm and nuclear regions (C+N) in both submucosal and myenteric neurons. A greater percentage of submucosal (VPT: 55±3, PT: 53±3, NT: 54±3, and T: 57±3 %) than myenteric neurons (VPT: 35±3, PT: 42±3, NT: 38±4, T: 31±2 %) exhibited positive staining at all ages. The percentages of neurons with C+N staining in submucosal neurons were significantly lower at very-preterm gestation. Confocal studies co-localized the HuD staining with pChAT and CRF-R1 receptor immunoreactivity both in submucosal and myenteric neurons.

Conclusion: In the fetal enteric nervous system HuD may function both as a RNA-binding protein and as a nuclear cytoplasmic shuttling protein. Co-localizaion studies suggest that both pCHAT-mRNA and CRF-R1 mRNA species are target transcripts for HUD in myenteric neurons. We speculate upregulation of HuD contributes to in utero meconium passage.

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Distal Colonic Projection Neurons May Utilize Mineralocorticoid Receptors To Prevent Colonic Intrinsic Neurons-Mediated *In Utero* Meconium Passage during Mild Stressors. Jayaraman Lakshmanan,[1] Guo L Liu,[1] John D Richard,[1] Sharon K Sugano,[1] Raina Khan,[1] Octavio Balbuena,[1] Kimberly Chap,[1] Virender Rehan,[2] Michael G Ross.[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Dept. of Pediatrics, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: MR exhibit a greater affinity to glucocorticoids (GC) than do glucocorticoid receptors (GR). Thus low circulating GC levels may preferentially bind MR during mild-stress periods, while the high GC levels may bind GC-receptors. MR antagonism increases GC-mediated responses, suggesting that MRs have a regulatory impact on GC-mediated stress responses. We recently documented that fetal in utero meconium passage is a neurovisceral stress response. To test our hypothesis that MRs play a primary role in mediating suppressive GC effects, we examined the expression patterns of MR in ovine fetal distal colon.

Methods: Bouin's solution fixed paraffin sections of distal colonic segments collected from ovine fetuses (n=6 for each gestational ages) at very preterm (VPT: 118-120 days gestation), preterm (PT: 130-132 days), near term (NT: 140-142 days) and term (T: 146-147days) were subjected to immunohistochemistry with polyclonal antibodies to MR. Digital photos (10 field per colonic ring, 6 rings each gestational age) taken at 400x were used to count the number of intensely stained neurons, referred to as "MR-capped neurons". Differences over time were determined with ANOVA.

Results: MR antibody elicited a punctate staining pattern in all layers of ovine fetal distal colon. Significant immunoreactive intensity was observed in submucosal and myenteric ganglia at all ages: Submucosal ganglia: VPT=0.130 ±0.009, PT=0.154±0.002, NT=0.151±0.005, T=0.169±0.016; A subpopulation of enteric neurons exhibited dense staining ("MR-capped"). Advancing gestation was associated with a significant decreased in the percentage of MR-capped myenteric (VPT = 49±7, PT = 27±5, NT = 19±3, T = 22±1%; $p<0.05$), though not submucosal ganglia neurons.

Conclusion: Results indicate a significant decrease in the percentage of MR Capped myentric neurons with advancing gestation. In view of the potential inhibitory effect of MR on GC-mediated stress responses, these results suggest that the decrease in MR expression may contribute to near term and term *in utero* meconium passage.

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The Ambiguity of Myometrial Progesterone Receptor Expression in Pregnancy and Labour. Alison J Tyson-Capper, Elizabeth A Shiells, Stephen C Robson. *Surgical Reproductive Sciences, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Background and Aims: In contrast to many other species, human parturition is not due to a reduction in circulating levels of progesterone (P) but appears to be related to changes in expression, ratios or signalling events of P receptors (PR-A and PR-B). The literature on PR expression in human myometrium in pregnancy and labour remains conflicting. We hypothesise this is due, at least in part, to differing specificities of the various PR antibodies employed. We carried out a comprehensive analysis of PR expression using ten 'PR-specific' antibodies that recognise different amino acid epitopes within the PR proteins. Two phosphorylation-specific antibodies for PR were also included to define whether phosphorylation status of myometrial PR changes in pregnancy and in labour.

Methods: Western Immunoblotting and semi quantitative RT-PCR were undertaken using protein lysates and RNA prepared from myometrial tissue from: - first (n=4) and second (n=5) trimesters, paired upper and lower segment myometrium from preterm (22-28 wks, n = 4), term not in labour (n = 10), term spontaneous labour (n = 8) and non pregnant (n = 10).

Results: Using the same set of myometrial lysates for each antibody, we found that the specificity of individual PR antibodies, in particular those raised against internal and C-terminal epitopes of the PR proteins, varied considerably resulting in different patterns of expression. There also appeared to be temporal and spatial differences in levels of myometrial PR proteins (90-120kDa/60-70kDa) in pregnancy and in labour with use of the phosphorylation-specific PR antibodies, however, it remains to be resolved which PR isoforms these may be. In contrast, data using four antibodies all of which react with the amino terminal domain of PR consistently indicated that expression of PR, in particular PR-B, decreased significantly at term ($P < 0.001$) and in labour ($P < 0.001$) when compared to non-pregnant levels. A decrease in levels of PR-B protein was also observed when comparing preterm levels to non-pregnant levels. RT-PCR using primers specific to PR-B consistently indicated that levels of PR-B mRNA decreased at term and in labour.

Conclusion: Interpretation of gestation-related changes in myometrial PR expression and phosphorylation status must take into account the PR antibodies used. Further studies are now underway to validate the specificity of the PR antibodies.

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Levels of FSH Receptor (FSHR) in Human Mural Granulosa Lutein (GL) Cells Correlate with the Response to Ovarian Stimulation for IVF. Rebeca Gonzalez Fernandez,[1] Jairo Hernandez,[2] Oscar Pena,[1] Julio Avila,[1] Angela Palumbo.[2] *[1]Bioquimica y Biologia Molecular, Universidad de La Laguna, La Laguna, Tenerife, Spain; [2]The Canary Islands Center for Human Assisted Reproduction, La Laguna, Tenerife, Spain.*

Objective To test the hypothesis that expression of FSHR in mural GL cells from IVF follicles varies with the infertility diagnosis and correlates with the outcome of ovulation induction (OI). **Materials and Methods** 86 women undergoing IVF were classified as: 1. "No ovarian factor", (tubal or male factor and egg donors, NOF;n= 37); 2. Endometriosis (ENDO; n=17); 3. Poor responders (PR; n=19); 4. Polycystic ovary syndrome (PCOS; n=13). Ovulation induction was carried out using a long or microflare or antagonist protocol based on clinical parameters and gonadotrophin doses were selected based on ovarian reserve (day 3 FSH and E2 and basal antral follicle count) and adjusted to the individual response. After ultrasound guided egg retrieval, mural GL cells were isolated from pooled follicular fluids from each patient using a percoll gradient and anti-CD45 immunobeads to eliminate WBCs, viability was assessed by trypan blue. FSHR was measured by RT-PCR as relative expression compared to beta actin. Statistical analysis was performed with the SPSS using Pearson´s correlation, one way ANOVA and Student´s t-test. **Results** FSHR expression in NOF (137±33.6) was statistically significantly higher than in PR (57±15.2) and lower than in PCOS (290±77.2). Both ENDO (85±30.2) and PR levels of FSHR were statistically significantly lower than in PCOS. Analysis of all cycles showed that FSHR expression correlates positively with the number of

total (r=0,26; p< 0,05) and MII (r=0,35; p< 0,01) oocytes and negatively with the units of FSH (r=-0,31; p< 0,01) and LH (r=-0,24; p< 0,05) administered for OI. Separate analysis of NOF showed a positive correlation with the number of total and MII oocytes retrieved and with estradiol levels on day of hCG but not with the total dose of gonadotropins received during OI. FSHR expression correlates negatively with day 3 FSH levels in all patients except in PCOS (r=-0,25; p<0,05). **Conclusions** These results suggest that expression of FSHR in the hyperstimulated ovarian follicle is "average" in normoresponders, high in high responders (PCOS) and low in poor responders (PR and ENDO). Knowledge of the level of expression of FSHR might be useful to individualize gonadotrophin doses and OI protocols thus improving pregnancy rates.

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Comparison of Intracellular ATP Levels between Non-Vitrified and Surviving Post-Vitrification/Thawed Human Oocytes. Somjate Manipalviratn,[1] Zhi-Bin Tong,[1] Eric Widra,[2,3] Alan H DeCherney.[1] *[1]Reproductive Biology and Medicine Branch, NICHD/NIH, Bethesda, MD, USA; [2]Department of Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC, USA; [3]Shady Grove Reproductive Science Center, Washington, DC, USA.*

Objective: To compare intracellular ATP level in non-vitrified and surviving post-vitrification/thawed human oocytes using an ATP bioanalysis assay.

Design: Prospective match-controlled laboratory study

Material and methods: All oocytes were obtained from women undergoing controlled ovarian stimulation for IVF/ICSI. Oocytes were discarded due to nuclear immaturity at the time of planned ICSI. All immature oocytes (GV and MI) were incubated overnight. Oocytes from patients with 2 or more discarded eggs which matured to MII stage were used in this study.

Oocytes from each women were randomly divided into 2 groups. In group 1, the oocytes were placed in 50 µl of ultrapure water and kept at -80°C for further ATP analysis. In group 2, the oocytes were vitrified using 15% ethylene glycol, 15% dimethyl sulphoxide and 0.5 M sucrose as cryoprotectant. These oocytes were kept in liquid nitrogen for 5-7 days before thawing with a rapid thawing method. Oocyte survival was determined by morphological assessment after 90 minutes of incubation then oocytes were placed in 50 µl of ultrapure water and kept at -80°C for further ATP analysis. Intracellular ATP level was determined using luciferin-luciferase bioluminescent assay.

Result: Ninety-one discarded human oocytes were obtained from 23 women. 47 oocytes were vitrified/thawed before measuring for ATP content. The other 44 oocytes were measured for their ATP content without undergoing vitrification/thawing process. Oocyte survival rate after vitrification/thawing is 72.34% (34/47). Mean oocyte ATP levels in non-vitrified oocytes is significantly higher than surviving post-vitrification/thawed oocytes (table 1). Coefficient of variation of luciferin-luciferase bioluminescent assay is less than 5%.

Conclusion: Oocyte ATP level in surviving post-vitrification/thawed human oocytes is significantly lower than non-vitrified oocytes.

Table 1: Oocyte ATP level

	Non-vitrified oocytes	Surviving post-vitrification/ thawed oocytes	Dead post-vitrification/ thawed oocytes
Mean ATP level (pmole/oocyte)	1.090*	0.529*	0.020
SD	0.385	0.553	0.072
N	44	34	13

* p < .001

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KL2 but Not FSH Receptor (FSHR) Expression in Stimulated Human Granulosa Lutein Cells from In Vitro Fertilization (IVF) Patients Correlates with the Occurrence of Pregnancy. Rebeca Gonzalez-Fernandez,[1] Jairo Hernandez,[2] Oscar Pena,[1] Julio Avila,[1] Angela Palumbo.[2] *[1]Bioquimica y Biologia Molecular, Universidad de La Laguna, La Laguna, Tenerife, Spain; [2]The Canary Island Center for Human Assisted Reproduccion, La Laguna, Tenerife, Spain.*

Objective To study the expression of genes involved in cell proliferation and steroidogenesis in the human follicle (KL1, KL2, FSHR, PAPP, P450) and its relationship with IVF outcome. **Materials and methods** 89 patients with different infertility diagnosis underwent ovulation induction with either a long or microflare or antagonist protocol based on clinical parameters; the dose of gonadotrophin used for ovulation induction was selected based on the ovarian reserve (day 3 FSH and E2 and basal antral follicle count) and adjusted to the individual patient response. Ultrasound guided egg retrieval was performed 36 hours after administration of 10.000 IU of hCG. Mural granulosa-lutein cells (GL cells) were isolated from pooled follicular fluids (FF) from each patient using a percoll gradient and anti-CD45 immunbeads to eliminate WBCs, viability was assessed by trypan blue. The genes under study were measured by RT-PCR as relative expression compared to actin. Statistical analysis was performed with the SPSS statistical software using Pearson´s correlation and Mann-Whitney U. **Results** KL2 expression correlates positively with KL1 levels (r=0,86; p< 0,01) and with genes implicated in granulosa cell function such as FSHR (r=0,36; p< 0,01), PAPP (r=0,54; p< 0,01) and P450 (r=0,39; p< 0,01). The number of MII oocytes obtained is positively correlated with both FSHR (r=0,35; p< 0,01) and PAPP (r=0,28; p< 0,05) expression, but not with the other genes. KL2 expression in women who became pregnant during the IVF cycle studied was higher (25,8± 10) than in non pregnant women (16,6 ± 3,1).(N=80, Mann-Whitney U p<0,05). **Conclusions** Patients who become pregnant have increased expression of KL2, which is associated with optimal granulosa cell proliferation and maturation. This is in accordance with the presence of lower apoptosis in granulosa cells in the same patients.

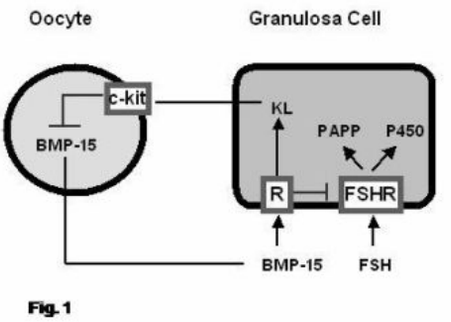

Fig. 1

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Quantification of Pre-Treatment Ovarian Vascularity Using Three-Dimensional Power Doppler To Predict Ovarian Hyperstimulation Syndrome during Assisted Reproduction Treatment. K Jayaprakasan, R Jayaprakasan, H Al-hasie, JS Clewes, BK Campbell, IR Johnson, NJ Raine-Fenning. *School of Human Development, University of Nottingham, Nottingham, United Kingdom.*

Objective: To test the hypothesis that an increased pre-treatment ovarian blood flow is associated with the development of ovarian hyperstimulation syndrome (OHSS) and to evaluate ovarian vascularity as a predictor of OHSS during in-vitro fertilization (IVF). **Methods:** 150 subjects undergoing first cycle of IVF had 3D transvaginal ultrasound in the early follicular phase of the menstrual cycle preceding IVF. 50 of them developed ovarian hyper-response, defined as retrieval of ≥15 oocytes and OHSS. 100 subjects had normal ovarian response with retrieval of 4 to 15 oocytes in the absence of OHSS. Antral follicle count (AFC), ovarian volume (OV), and ovarian vascularity (vascularisation index, VI; flow index, FI and vascularisation flow index, VFI) were measured and an unpaired t-test was used to compare these parameters between the OHSS and the control groups. Multiple logistic regression analysis was used to assess the predictive value of these variables against age, BMI and basal FSH for the development of OHSS. **Results:** The ovarian VI (9.64 ±7.94 vs. 8.64 ± 7.31), FI (37.95 ± 5.5 vs. 37.95 ± 5.5) and VFI (3.77 ± 2.68 vs. 3.51 ± 3.1) were similar in both the groups. AFC and OV were significantly higher (p<0.01) in the OHSS group (28.90 ± 14.55 and 10.40 ± 3.77 cm^3 respectively) than in the control group (19.23 ± 9.91 and 8.94 ± 4.7 cm^3 respectively). AFC was the only significant (p=0.001) predictor of OHSS on multiple regression analysis (Table 1). **Conclusion:** Women developing OHSS during IVF do not demonstrate an increased pre-treatment ovarian blood flow as measured by 3D ultrasound but do have a significantly higher AFC, which is the only significant predictor of OHSS.

Multiple logistic regression analysis evaluating age, basal FSH, BMI and ovarian ultrasound parameters as predictors of OHSS

Parameters	Odds ratio	95% Confidence interval	p value
Age	1.084	0.980, 1.199	0.116
Basal FSH	0.881	0.718, 1.082	0.228
BMI	0.894	0.798, 1.002	0.053
AFC	1.077	1.030, 1.125	0.001
Mean OV	0.979	0.884, 1.085	0.689
Mean VI	0.981	0.807, 1.192	0.845

Mean FI	0.980	0.879, 1.093	0.719
Mean VFI	1.091	0.623, 1.910	0.762

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Human Cumulus Cells Isolated during ART: Steroid Biosynthesis and Gonadotropin Responsiveness in Culture. Jessica K Wickenheisser,[2] William C Dodson.[1] [1]*Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA, USA;* [2]*Obstetrics and Gynecology, The Pennsylvania State University College of Medicine, Hershey, PA, USA.*
Compared to other species, little is known about the steroid biosynthetic capacity and gene expression profiles of human cumulus cells. **Objective:** To examine the ability of human cumulus cells in primary and long-term culture to synthesize steroids and respond to gonadotropin or cAMP-dependent stimulation. **Methods:** Human cumulus cells were isolated from cumulus-oocyte complexes during assisted reproductive technology (ART) and placed in primary culture or propagated to third passage. At subconfluence, cells were transferred into serum-free conditions in the presence of vehicle, forskolin, FSH, or hCG. At 48h, media was collected and progesterone (P4) and estradiol (E2) levels were determined by EIA. Aromatase activity was measured by the tritiated water assay. Aromatase (*CYP19*) and cholesterol side-chain cleavage (*CYP11A1*) mRNA abundance was determined by quantitative real-time PCR (QRT-PCR). Transcriptional regulation of the CYP19 and CYP11A1 promoters was investigated by transient transfection of cumulus cells with promoter luciferase constructs. **Results:** Substantial amounts of P4 and E2 were synthesized by cumulus cells in culture, which were further elevated by forskolin, hCG, or FSH treatment. The presence of CYP19 and CYP11A1 mRNA in cumulus cells was confirmed by QRT-PCR, and the relative mRNA abundance of both transcripts was induced by forskolin, hCG, or FSH treatment. Changes in *CYP19* and *CYP11A1* gene expression in response to cAMP and gonadotropin stimulation were associated with increased transcriptional regulation of both the *CYP19* and *CYP11A1* gene promoters. **Conclusions:** Our studies demonstrate that human cumulus cells are sites of significant P4 and E2 biosynthesis and respond to cAMP- and gonadotropin-stimulation *in vitro*. The ability to examine human cumulus cells in primary and long-term culture provides a unique model system to investigate cumulus cell function, and the paracrine role of cumulus cells in oocyte development, maturation, and subsequent fertilization.

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Differences in the Area of Pancreatic Insulin-Secreting Cells in Newborn Mice Conceived In Vivo Versus In Vitro. Mary Pravdin, Luisa Delle Piane, Francesca Di Sebastiano, Anne Marie Donjacour, Paolo Rinaudo. *Obstetrics, Gynecology and Reproductive Sciences, University of California at San Francisco, San Francisco, CA, USA.*
Background: There is increasing evidence suggesting that events occurring during fetal development may result in increased predisposition to adult conditions, such as diabetes. In Vitro Fertilization (IVF) is a new environmental stressor and the long-term effects of these manipulations are currently unknown. There is some evidence that lower number of insulin-secreting cells at birth signals predisposition to diabetes later in life. In this study, we compare areas of pancreatic insulin-secreting cells in newborn mice conceived in vivo versus in vitro.
Methods: Oocytes were collected from super ovulated CF-1mice and fertilized in vitro with cauda epididymal sperm from B6D2F1/J mice. Fertilized eggs were cultured in Whitten medium under 5% CO2 in humidified air at 37 °C for 96 h. Blastocysts were transferred to the uteri of pseudo-pregnant recipients. Control mice were allowed to conceive in vivo. The newborn animals were sacrificed within 24 hours of birth. Pancreases were sectioned, immunostained with anti-insulin, and total areas and stained areas were compared using t-test with two-tailed distribution. P value of <0.05 was considered significant.
Results: There were total of 12 newborn animals: 5 females (2 IVF, 3 controls) and 7 males (3 IVF, 4 controls). Percentage of total pancreatic area occupied by insulin-secreting cells was lower in female IVF (0.145 mm^2, 0.69%) animals compared to female controls (0.079 mm^2, 0.97%) and higher in male IVF (0.019 mm^2, 0.40%) animals compared to controls (0.138 mm^2, 0.25%). The average of males and females was slightly lower for IVF (0.082 mm^2, 0.54%) than controls (0.109 mm^2, 0.61%). None of these differences were statistically significant.
Conclusions: There was a trend in newborn female mice towards lower amount of insulin-secreting cells in the IVF offspring, suggesting possible predisposition to diabetes later in life. This effect was not observed in newborn males. While our study failed to demonstrate consistent and significant differences in the areas of insulin-secreting cells between newborn mice conceived in vitro and in vivo, the number of animals in the study may have been too small to show significant results. Larger studies are needed to further investigate this question.

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In Vitro Maturation of Oocytes Retrieved from Unstimulated Whole Ovary Specimens in the Mid-Follicular Phase as a Fertility-Preserving Measure. Peter S Uzelac, Phyllis Risch, Kassi Shelton, Steven T Nakajima. *Obstetrics and Gynecology, University of Louisville, Louisville, KY, USA.*
OBJECTIVE: Several fertility preservation methods currently available may not be viable options to certain patients due to their high cost and limited number of centers offering these services. For women undergoing bilateral oophorectomy, in vitro maturation (IVM) of oocytes retrieved from unstimulated whole ovary specimens may represent a more practical solution. Here we describe out initial experience in offering IVM as a fertility-preserving measure to two patients undergoing total abdominal hysterectomy and bilateral salpingo-oophoectomy (TAH-BSO).
METHODS:
Case one was a 29 year old nulligravid with chronic pelvic inflammatory disease unresponsive to medical management. Case two was a 39 year old nulligravid with stage IA endometrial carcinoma. Surgery was planned for the mid-follicular phase in order to collect prophase I oocytes before the onset of late-follicular phase atresia. Upon surgical removal, suction aspiration of all identifiable follicles was performed. Ovaries were then serially sectioned and all tissue was examined for the presence of prophase I oocytes.
RESULTS: Total number of prophase I oocytes retrieved was 9 and 6, maturation rate after 24 hours was 33% (2/6) and 44% (4/9), fertilization rate after 24 hours was 100% (2/2) and 25% (1/4), maturation rate after 48 hours was 50% (2/4) and 40% (2/5) and fertilization rates after 48 hours 50% (1/2) and 50% (1/2), for **c**ase 1 and case 2 respectively.
CONCLUSIONS:
IVM of oocytes retrieved from unstimulated whole ovary specimens may be a simple and inexpensive approach to fertility preservation in women undergoing bilateral oophorectomy. By requiring minimal adjustments to in vitro fertilization protocols, this treatment could easily be implemented in centers which currently have no fertility preservation program. Patient age at time of retrieval may be predictive of developmental potential. Continued patient enrollment and follow-up studies on the developmental potential of embryos derived from this technique are necessary to fully evaluate its potential for a role in fertility preservation.

	Number of Germinal Vessicle Oocytes Retrieved	Oocyte Maturation Rate (24 hr)	Fertilization Rate (24 hr)	Oocyte Maturation Rate (48 hrs)	Fertilization Rate (48 hrs)
Case 1	6	33% (2/6)	100% (2/2)	50% (2/4)	50% (1/2)
Case2	9	44% (4/9)	50% (2/4)	40% (2/5)	0% (0/2)

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Vaginal Progesterone for Luteal Support Increases Clinical Pregnancy Rates in Women with Polycystic Ovary Syndrome Using Letrozole for Ovulation Induction. Christopher P Montville, Maram Khabbaz, Rose Maxwell, Mira Aubuchon, Daniel B Williams, Michael A Thomas. *Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH, USA.*
OBJECTIVE: The routine use of progesterone as luteal phase support in women with a diagnosis of polycystic ovary syndrome (PCOS) undergoing ovulation induction cycles with oral agents has not been fully elucidated. We hypothesize that women with PCOS utilizing either clomiphene citrate or letrozole, an aromatase inhibitor, should administer intravaginal progesterone in the luteal phase to increase pregnancy rates.
DESIGN: Retrospective Chart Review.
MATERIALS AND METHODS: Cycle data from women with PCOS undergoing ovulation induction with clomiphene citrate or letrozole at the University of Cincinnati Medical Center from 2002 to 2007 were evaluated. Diagnosis of PCOS was based on Rotterdam criteria and all other infertility diagnoses were excluded. Clinical pregnancy rates (presence of fetal cardiac activity on ultrasound at 6-7 weeks gestation) in women who received intravaginal micronized progesterone (200 mg BID) following ovulation induction (with and without intrauterine insemination) were compared to those who did not receive progesterone.
RESULTS: No significant differences were noted in demographic parameters, including patient age or BMI. A total of 182 cycles were evaluated in 72 women treated with clomiphene citrate. Clinical pregnancies were documented in

19.5% (18/93) of cycles in the progesterone group compared to 9.9% (9/91) of the non-progesterone group ($p < 0.08$). Forty-three cycles were evaluated in 24 patients treated with letrozole. Clinical pregnancies were documented in 17.2% (5/29) of cycles in the progesterone group compared to none (0/14) in the non-progesterone group ($p < 0.02$).
CONCLUSIONS: Patients with PCOS who used letrozole for ovulation induction had superior clinical pregnancy rates when using intravaginal micronized progesterone compared to women who did not receive luteal phase support. There was a trend toward increased clinical pregnancy rates in PCOS patients utilizing luteal support following clomiphene citrate for ovulation induction. Therefore, in women with PCOS undergoing ovulation induction with oral agents, luteal supplementation with progesterone should be strongly considered, especially in those using letrozole.

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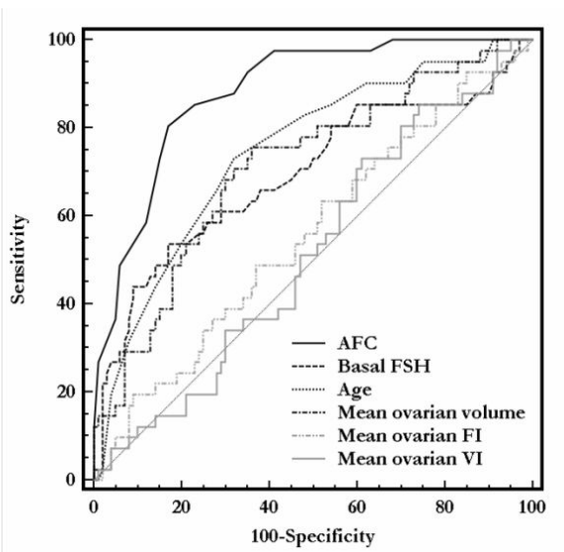
Quantification of Three-Dimensional Ovarian Ultrasound Characteristics of Women Developing Poor Ovarian Response during Assisted Reproduction Treatment. K Jayaprakasan, H Al-hasie, R Jayaprakasan, BK Campbell, JS Clewes, IR Johnson, NJ Raine-Fenning. *School of Human Development, University of Nottingham, Nottingham, United Kingdom.*
Objective: To evaluate the 3D ovarian characteristics of women having poor ovarian response during in-vitro fertilization (IVF). **Methods:** 141 subjects undergoing first cycle of IVF had 3D transvaginal ultrasound in the early follicular phase of the menstrual cycle preceding IVF. 41 of them developed poor ovarian response, defined as either retrieval of <4 oocytes or cycle cancellation. 100 women had normal ovarian response with retrieval of 4 to 15 oocytes. Antral follicle count (AFC), ovarian volume (OV), and ovarian vascularity (vascularisation index, VI; flow index, FI and vascularisation flow index, VFI) were measured and an unpaired t-test was used to compare these parameters between poor and normal responders. Multiple logistic regression analysis was used to assess the predictive value of these variables against age and basal FSH for poor ovarian response. **Results:** The ovarian VI (7.5 ± 5.3 vs. 8.6 ± 7.9), FI (38.9 ± 6.9 vs. 37.9 ± 6.5) and VFI (3.2 ± 2.6 vs. 3.5 ± 3.4) were similar in both poor and normal responders. AFC and OV were significantly lower ($p<0.01$) in poor responders (9 ± 3.3 and 6.3 ± 3.5 cm^3 respectively) than in normal responders (19.2 ± 9.9 and 8.9 ± 4.7 cm^3 respectively). AFC was the best ($p<0.001$) predictor of poor ovarian response on multiple regression analysis (Table 1 & Figure 1). **Conclusion:** The AFC is the single best predictor of poor ovarian response. Ovarian vascularity is not decreased in women who demonstrate a poor response to ovarian stimulation during IVF.

Multiple logistic regression analysis evaluating age, FSH and ovarian ultrasound parameters as predictors of poor ovarian response during IVF

Parameters	Odds ratio	95% Confidence interval
Age	1.040	0.900, 1.202
Basal FSH	1.295	1.050, 1.597*
AFC	0.674	0.571, 0.795**
Mean OV	0.967	0.840, 1.113
Mean VI	0.906	0.415, 1.974
Mean FI	1.152	0.941, 1.411
Mean VFI	0.924	0.140, 6.108

*<0.05; **<0.001



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Gay Men Choosing Parenthood through Assisted Reproduction: Medical and Psychosocial Considerations. Dorothy A Greenfeld. *Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT, USA.*
OBJECTIVE: Gay male couples increasingly seek parenthood through in vitro fertilization using an oocyte donor and a gestational carrier, but no studies describe this unique experience. The purpose of this study was to determine medical and psychosocial issues unique to gay men using ART.
DESIGN: Qualitative analysis of semi-structured interviews with gay male couples seeking parenthood through ART.
MATERIALS AND METHODS: Sixteen gay males (eight couples) entering an ART program were assessed through the use of a semi-structured interview. Characteristics evaluated included age, relationship status, duration of their relationship, psychological health and stability, how the decision was made concerning who would donate the sperm, the decision to use an anonymous or known oocyte donor, and whether their gestational carrier was someone previously known to them or not.
RESULTS: The average age of the men in this study was 40 years. All eight couples were in a committed relationship and had been together for an average of 7.3 years. Five of the couples (69%) had been joined in a civil union which is legal in the state of Connecticut. All subjects were psychologically stable and in good health. Six couples (75%) were very clear about which partner would inseminate the oocytes. Of those couples, two felt that the older partner should donate; two felt that the partner who cared more about a genetic connection to the child should donate; and two felt that the partner with "better genes" should donate. The remaining two couples chose to inseminate equal numbers of oocytes in order to transfer an embryo from each partner. All couples chose an anonymous oocyte donor, two couples chose relatives as their gestational carriers, while the others chose carriers recruited through an agency.
CONCLUSIONS: Participants in this study were determined to become parents through assisted reproduction. They had given thoughtful consideration to the medical and psychosocial issues unique to this process including which partner would be the genetic parent, and why.

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Micro-Dose HCG Increases the Number of Preovulatory Follicles during Clomiphene Superovulation. RB Allen,[1] AZ Steiner,[2] RH Fogle,[1] MJ Kalan,[1] RJ Paulson.[1] *[1]Ob/Gyn, USC Keck School of Medicine, LA, CA, USA; [2]Ob/Gyn, UNC, Chapel Hill, NC, USA.*
Intro:
Micro-dose hCG has been demonstrated to increase ovulation and pregnancy rates following clomiphene administration in women resistant to clomiphene. Since micro-dose hCG stimulates growth in follicles expressing the LH receptor, we hypothesized that its use following clomiphene in women with unexplained infertility would increase the number of follicles that ovulate and subsequently, pregnancy rates.
M&M:
IRB approval was obtained for this prospective pilot study of women with unexplained infertility. On day #3 a baseline ultrasound was performed and serum FSH and E2 levels were measured. Subjects were given 100mg of clomiphene daily from days 3-7 and then returned for serial ultrasounds on day #8. When at least 2 follicles ≥12mm were present, 200 IU of hCG IM daily was initiated. Cycles were monitored by ultrasound every 2 days until a +LH surge occurred. All subjects had previously undergone a cycle using clomiphene alone for superovulation followed by IUI and these cycles were used for comparison.
Results:
5 subjects, aged 33.2±2.5 yrs (mean±SEM) and BMI 25.8±1.6 kg/m^2 with 7.6±3.2 yrs of infertility were enrolled. The mean FSH and E2 levels were 8.9±1.4 mIU/mL and 37.4±5.9 pg/mL, respectively. There was an average of 6.2±0.2 days from starting clomiphene to the attainment of 2 follicles ≥12mm. 4 out of the 5 subjects had 2 follicles ≥12mm by day #8. A mean of 10.4±0.7 days of treatment were required until ovulation occurred. There were twice as many follicles in the micro-dose hCG cycles, although due to the small sample size this did not reach statistical significance (2.8±0.4 follicles, measuring 20.6±0.9 mm in the micro-dose hCG cycles, compared to 1.4±0.3 (p=0.05), measuring 18.6±2.4 mm (p=0.47) in the control cycles). There was no difference in the endometrial thickness at the time of ovulation between the micro-dose hCG cycles and the control cycles: 8.4±2.0 mm vs. 10.0±2.3 mm (p=0.30), respectively. All subjects had at least 2 ovulatory sized follicles in the study cycles. 40% of subjects had an IUI performed, however there were no pregnancies resulting from this study.
Conclusions:

1) The addition of micro-dose hCG to clomiphene may allow more follicles to reach an ovulatory size, which should theoretically increase pregnancy rates
2) This pilot study demonstrates that adding micro-dose hCG may increase the effectiveness of clomiphene when given in a superovulatory protocol.

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Pregnancy Outcomes after GnRH-Agonist Trigger in Patients Undergoing In Vitro Fertilization (IVF) with Gonadotropins and a GnRH-Antagonist. Jessica Salas, Donald Maier, John Nulsen, Claudio Benadiva, Lawrence Engmann. *Obstetrics & Gynecology, University of Connecticut, Farmington, CT, USA.*

Objective: To evaluate the antepartum, intrapartum, and neonatal complications in patients undergoing IVF using a GnRH-antagonist protocol where a GnRH-agonist was used to induce final oocyte maturation.

Materials and Methods: A retrospective review of data from high responders undergoing their 1st or 2nd IVF cycle using a GnRH-antagonist protocol who were triggered with leuprolide acetate (study group) or hcg (control) and achieved at least a singleton pregnancy reaching the third trimester. Patients younger than 40 years old were included. Both groups received luteal phase support with intramuscular progesterone. The study group received estrogen patch supplementation. Outcomes measured were antenatal and intrapartum complications, order of gestation, gestational age at delivery, birth weight, neonatal adverse outcomes, and congenital anomalies. Pearson chi square, Fisher's Exact test, or independent t-test were used as appropriate.

Results: The baseline characteristics were different (Table 1). Maternal antenatal and intrapartum complications were similar in both groups (23.4% vs 39.4%, p=0.23). There were more singletons in the study group (84.9% vs 63.8%, p<0.05). A subgroup analysis of gestational age at delivery, birth weight, neonatal complications and congenital anomalies in singletons showed no difference (Table 2).

Conclusions: This is the first study reporting the maternal and perinatal outcomes after GnRH-agonist trigger. Differences in response to ovarian stimulation may dictate use of GnRH-agonist triggering for prevention of OHSS, which may explain the differences in baseline characteristics. Maternal and neonatal complications remain unaffected.

Table 1

	Lupron (n=33)	hCG (n=47)	p-value
Age	32.5 +/-2.7	34.1 +/-3.16	0.017
D3FSH	5.6 +/-2.1	6.8 +/-2.0	0.016
Oocytes retrieved	20.6 +/-13.3	15.2 +/-8.2	0.026
peak E2	2830 +/-1200	1950 +/-792	<0.001
Embryos transferred	2.0 +/-0.6	2.3 +/-0.3	0.008

+/- std dev

Table 2

	Lupron (n=28)	hCG (n=30)	p-value
Gestational age at delivery (wks)	38.6 +/-2.0	38.2 +/-2.4	0.56
Birth weight (lbs)	7.0 +/-1.2	7.1 +/-1.3	0.73
Neonatal complications (%)	10.7	10.0	0.93
Minor congenital anomalies	3.3%	3.6%	p=0.512

+/- std dev

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Cost Analysis of Outpatient Management of OHSS Via Paracentesis: 'Tap Early and Often' Versus Hospitalization. Belinda J Yauger,[1,2] Melinda B Henne,[1] Alicia Y Armstrong,[1,2] Rebecca Chason,[1] Barbara J Stegmann,[1,2] John T Queenan,[3] James H Segars.[1,2] *[1]Obstetrics and Gynecology, Walter Reed Army Medical Center, Washington, DC, USA; [2]Reproductive Biology and Medicine Branch, NICHD, National Institutes of Health, Bethesda, MD, USA; [3]Obstetrics and Gynecology, University of Rochester Medical Center, Rochester, NY, USA.*

Objective: Moderate to severe ovarian hyperstimulation syndrome (OHSS) occurs in 1-5% of assisted technology cycles and carries the potential for severe complications. Traditional management consists of hospitalization with intravenous fluids (IVF), bedrest, and close monitoring. Early and aggressive paracentesis is an alternative method of treatment for OHSS in an outpatient setting, but the economic consequences of the two treatment regimens have not been compared.

Design: Cost-effectiveness analysis

Materials and Methods: Two scenarios, outpatient management with transvaginal paracentesis and conservative therapy with hospitalization, were compared. Potential initial outcomes were analyzed for the conservative group to include hospitalization either in a ward hospital bed or the intensive care unit (ICU) for an average of seven days. Costs included IVF administration, ultrasound, and daily bloodwork. Initial outcomes for the outpatient management group included no further therapy beyond the initial transvaginal paracentesis, bloodwork, IVFs, and ultrasound versus admission for an average of three days to a ward bed with similar management. The probability of the negative outcome for the conservative group was set at 5% (ICU admission) and 10% for the outpatient group (regular hospitalization).

Results: The cost of conservative therapy including first tier complications ranged from a low of $8,399 to a high of $14,615. The cost of outpatient management with aggressive paracentesis and its first tier complications ranged from a low of $940 to a high of $2,060. This resulted in an estimated cost burden of $7,459 to $12,555 for conservative management with hospitalization. The main improvement factor was that patients in the outpatient management group had a much lower likelihood for prolonged hospitalization than the conservatively managed group.

Conclusions: Aggressive outpatient treatment of moderate to severe OHSS with early paracentesis appears to be a cost-effective strategy.

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Effect of Aromatase Inhibitors on the Endometrium Pattern in Ovulation Induction. Zeynep Alpay, Michael P Diamond, Michael L Kruger, Elizabeth E Puscheck. *Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Hutzel Hospital, Wayne State University, Detroit, MI, USA.*

Introduction: Aromatase inhibitors (AI) are a new method of ovulation induction and is proposed to replace clomiphene citrate (CC) due to their reported advantages. Their superior effect is thought to be due to up-regulation of the estrogen receptors and increase in the sensitivity of the endometrium, resulting in better proliferation despite low estrogen levels in the circulation.

Objective: To compare the effect of different ovulation induction methods, including AI, CC and gonadotropins (GT) on the endometrium in infertility patients.

Methods: We reviewed 321 ovulation induction cycles performed in our institution in the last one-year period retrospectively. There were 149 GT, 145 CC and 27 AI cycles. Age, gravida, day 3 (D3ET) and midcycle (MCET) endometrial thickness, endometrial pattern (EP) on day of HCG injection, endometrial growth during the induction cycle (D-ET), which was calculated by the difference between MCET and D3ET, and the pregnancy rates (PR) were compared. The EP was categorized as Type A (homogenous and hyperechogenic), Type B (intermediate isoechogenic pattern and a poorly defined central echogenic line), and Type C (multilayered triple-line). Chi-square, ANOVA, *t* test and Pearson correlation were used for statistical analysis.

Results: In all cycles, EP was closely related to the D-ET ($P < 0.001$). This correlation appeared to be more pronounced when observed EP was compared with D-ET ($R = 0.311$; $P < 0.001$) rather than with MCET ($R = 0.168$; $P = 0.002$). There was a reverse relationship between the age of the patients and EP ($R = -0.215$; $P < 0.001$). A negative correlation was also found between the gravida and EP ($R = -0.28$; $P < 0.001$). Pregnancy rate was significantly correlated with EP ($R = 0.193$; $P < 0.001$). Pregnancy rate was 2% in Type A EP, 5.2% in B, 16% in C when all cycles were analyzed. Aromatase inhibitors and GT treatments each resulted in 52% Type C pattern in contrast to 33% with CC ($P = 0.014$ for AI *vs.* CC; $P = 0.05$ for GT *vs.* CC). The D-ET was greater in GT cycles compared with AI or CC ($P < 0.001$).

Conclusion: These results show that EP has a strong correlation with PR in ovulation induction cycles. Additionally, AI and GT treatments have similar effects on EP. Both EP and the growth of the endometrium during the induction (D-ET) are good prognostic variables for the successful pregnancy initiation.

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Ovarian Response in Patients Undergoing Ovarian Stimulation after Myomectomy. Hyacinth Browne,[1,2] Desiree McCarthy-Keith,[1,2] Barbara Stegmann,[1,2] Alicia Armstrong.[1,2] *[1]Reproductive Biology and Medicine Branch, NICHD, NIH, Bethesda, MD, USA; [2]Reproductive Endocrinology and Infertility, Walter Reed Army Medical Center, Washington, DC, USA.*

Objective: The impact of myomectomy on ovarian function has not been well-studied. Other surgical treatments of fibroids, such as hysterectomy and uterine artery embolization, have shown an increase of FSH into the peri-menopausal range. The objective of this study is to examine ovarian response in infertile women undergoing ovarian stimulation after abdominal myomectomy.

Design: Retrospective analysis.

Materials and Methods: A retrospective analysis of all infertile women with known fibroids who had a failed ART cycle, from January 2000 to 2007, followed by an abdominal myomectomy and a subsequent ART cycle was

performed. Women served as their own controls. Ovarian function pre and post-myomectomy was assessed by age, day 3 and 10 FSH levels, days of stimulation, total gonadotropins used, peak estradiol level, number of oocytes retrieved, embryos obtained, and high-grade embryos, and pregnancy outcome. Quantitative results are presented as mean ± SD.

Results: Four women had a failed ART cycle and underwent an abdominal myomectomy prior to a subsequent ART cycle. The mean age was 35 and 36 pre- and post- myomectomy, respectively. All subjects had uterine factor infertility. Two of these women also had tubal factor infertility, and one had endometriosis and male factor infertility. We found no difference in ovarian response pre and post-myomectomy.

	Pre-Myomectomy	Post-Myomectomy
Age (years)	35 +3.6	36 +3.6
Day 3 FSH (U/L)	6.9 + 1.7	6.1 + 0.96
Day 10 FSH (U/L)	6.1 +1.9	6.2+ 2.6
Days of stimulation	10+1.2	10.3 +0.96
Total gonadotropins used (amps)	49 +12.7	47.8+15.5
Peak estradiol (pg/ml)	4230.5 +433.5	3636.3+247.2
Number of oocytes retrieved	14+ 4.6	20.3+ 11.7
Number of embryos obtained	8.3 + 2.1	10.3 + 6.9
Number of high grade embryos	3.5 + 2.1	4+ 3.4
Clinical pregnancy	0	1

Conclusion: As expected, abdominal myomectomy did not adversely affect ovarian response in infertile women undergoing ovarian stimulation after a failed ART cycle. Larger, randomized prospective studies are needed to accurately assess whether myomectomy has a negative impact on ovarian response.

Support: This research was supported by the Intramural Research Program of the Reproductive Biology and Medicine Branch, NICHD, NIH and Walter Reed Army Medical Center.

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Serum Anti Mullerian Hormone (AMH) Hormone Is Predictive of Oocytes Retrieved at In Vitro Fertilization Cycles. David H Barad,[1] Andrea Weghofer,[1,2] Norbert Gleicher.[1] *[1]Center for Human Reproduction, New York, NY, USA; [2]Allgemeines Krankenhaus of the University of Vienna Medical School, Vienna, Austria.*

Objective: Anti-mullerian hormone (AMH) is produced by developing primordial follicles. AMH levels are stable through the menstrual cycle and therefore can be drawn randomly. The objective of this study was to assess the association of anti-mullerian hormone (AMH) and oocytes obtained at IVF retrieval.

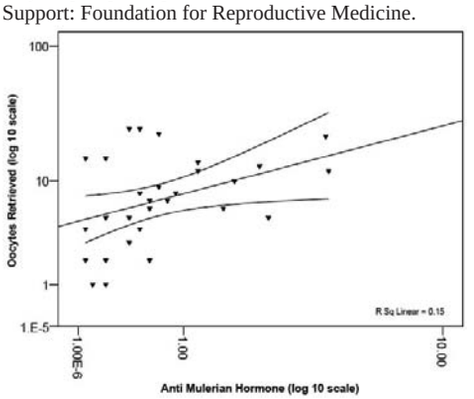
Design: Cross-sectional cohort study.

Materials and Methods: The study cohort is comprised of thirty women in 30 cycles of In vitro fertiization (IVF). Serum levels antimullerian hormone (AMH) were drawn before starting an ovulation induction cycle for IVF. Standard ovulation induction was performed with leuprolide flare and 450 mIU/ml per day of follicle stimulating hormone. We performed linear regression of log convert4d oocyte number against the log converted AMH level. Serum AMH was measured using an enzymatically amplified two-site immunoassay DSL-10-14400 active MIS/AMH ELISA. Statistical analysis was performed using SPSS version 15.0. Continuous values are presented as mean std error.

Results: The log number of oocytes retrieved was directly related to the log value of AMH (P = 0.036).

Conclusions: Our data demonstrate an association between serum AMH and oocytes produced in response to ovulation induction. Using AMH levels in addition to FSH levels in evaluating for ovarian reserve and pregnancy outcome may help improve predictions of reproductive performance.

Support: Foundation for Reproductive Medicine.



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A Single Human Chorionic Gonadotropin Measurement and Maternal Age Predicts In Vitro Fertilization Outcome. Travis W McCoy, Steven T Nakajima, Henry CL Boher, Jr. *Department of Obstetrics, Gynecology, and Women's Health, University of Louisville, Louisville, KY, USA.*

Context: Prediction of outcome after in vitro fertilization (IVF) can be difficult due multiple factors. Human chorionic gonadotropin (hCG) levels correlate with pregnancy outcome, but data that can be used to easily counsel patients on their possible outcome is lacking.

Objective: To investigate the use of hCG levels along with other significant factors to predict the likelihood of an IVF pregnancy progressing to the point of detection of cardiac activity by ultrasound

Design: Retrospective data analysis of 1377 IVF cycles performed from January 1997 to July 2007 resulting in 665 pregnancies. Multiple logistic regression analysis modeling was performed to determine the factors most predictive of an ongoing early pregnancy and to assess possible confounding variables.

Setting: An academic fertility center.

Patients: Patients undergoing in vitro fertilization using autologous fresh embryos.

Intervention: None

Main Outcome Measure: Pregnancy continuation to the documentation of cardiac activity by ultrasound.

Results: Maternal age, day 14 (post-oocyte aspiration) hCG level, and day 16 hCG levels were significant in predicting pregnancy outcome. Day 14 and day 16 hCG levels were highly correlated and can be considered proxies for each other. The most accurate predictive model used only a single day 14 hCG level and maternal age. The type of fertilization method used, the cycle number for that patient, and the number of embryos transferred were not found to be significantly different. Ongoing pregnancy rates were directly proportional to day 14 hCG level, and inversely proportional to maternal age. The incidence of multiple pregnancies also increased proportionally to the initial hCG level. 99% of ongoing pregnancies had hCG level > 23 mIU/mL.

Conclusions: A single day 14 post-oocyte aspiration hCG level and maternal age are the most predictive of an ongoing IVF pregnancy. There was no difference in outcome between fertilization methods or number of embryos transferred. There is no benefit to obtaining serial hCG levels after the initial one.

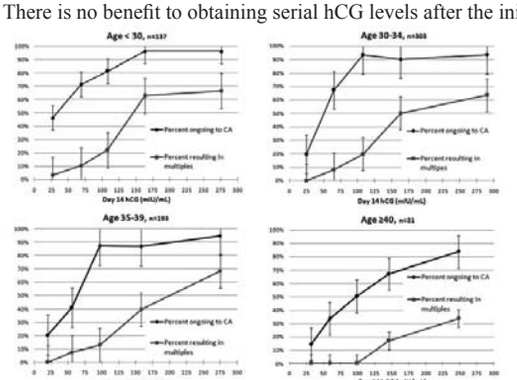

Figure 2. Relationship of day 14 hCG level and rates of ongoing pregnancies and multiple gestations, by age group

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Mechanism for Impaired Follicular Growth in Hyperandrogenic State: DHT Inhibits FSH Signaling Pathways in Granulosa Cells. Pradeep P Kayampilly, KMJ Menon. *Department of OB/GYN and Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI, USA.*

Proliferation and growth of granulosa cells in ovarian follicles under the influence of FSH and other paracrine factors is critical for normal ovulation. This orderly progression is disrupted under hyperandrogenic states such as PCOS. The present study examined how androgens disrupt these processes by examining the effect of dihydrotestosterone (DHT) on FSH-mediated mitogenic signaling pathways using primary cultures of granulosa cells. Granulosa cells harvested from 3 day estradiol primed rats were treated with DHT (90 ng/ml) for 24 hours. The cells were then treated with FSH (50 ng/ml) for different time intervals and the activation of critical molecules involved in FSH-induced mitogenic signaling pathways were examined. Two hour FSH treatment resulted in three fold increase in the expression of cyclin D2 mRNA, a marker of cell proliferation. Exposure to DHT completely abolished this stimulation. DHT treatment also reduced FSH-mediated PKA activity as well as ERK phosphorylation. This inhibition occurs at a post cAMP level, since FSH-mediated cAMP production was not affected by DHT treatment. Inhibiting

ERK activation with U0126 (10 μM)) reduced FSH stimulated cyclin D2 mRNA expression by 50%. FSH has also been shown to stimulate mTOR, a regulator of growth and proliferation of many cell types. Inhibiting mTOR activation with 10nM rapamycin for 15 min significantly reduced FSH-mediated cyclin D2 mRNA expression. DHT exposure for 24 hours also inhibited FSH stimulated mTOR signaling, as shown by a 40% reduction in the phosphorylation of its down stream target p70S6kinase. Furthermore, pretreatment with DHT resulted in significantly reduced FSH-mediated TSC-2 phosphorylation, which is an upstream regulator of mTOR pathway. Further studies revealed that FSH mediated TSC-2 phosphorylation and mTOR signaling is regulated by ERK, but independent of Akt. Based on these results we conclude that elevated 5α reduced metabolites of androgens inhibit FSH-stimulated PKA-ERK pathway resulting in the inhibition of multiple mitogenic signaling pathways leading to defective follicle maturation culminating in anovulation. Thus, rescuing the ERK activation might serve as a potential therapeutic target to restore normal granulosa cell proliferation in hyperandrogenic states. (Supported by NIH Grant HD-38424).

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Searching PCOS-Genes: Results of a Genome Screen for PCOS in a Dutch Founder Population. Olivier Valkenburg,[1] Annemarie G Mulders,[1] Aida Bertoli-Avella,[2] Ben A Oostra,[2] Joop SE Laven. *[1]Department of Gynecology and Obstetrics, ErasmusMC, Rotterdam, Zuid-Holland, Netherlands; [2]Department of Internal Medicine, ErasmusMC, Rotterdam, Zuid-Holland, Netherlands.*

CONTEXT: Although the etiology of PCOS is not yet fully understood, evidence has accumulated for a complex genetic background i.e. a combination of multiple genetic and environmental factors. To reduce genetic heterogeneity, this study was conducted in a genetically isolated population in the Netherlands .

OBJECTIVE: In order to identify genomic loci that are associated with PCOS, a whole genome screen using highly polymorphic microsatellite markers was performed in a founder population.

SUBJECTS and METHODS: 72 PCOS patients (2003 Rotterdam criteria) were identified in the founder population (Rucphen, the Netherlands) of ± 20.000 inhabitants. Patients underwent a standardized screening procedure that included clinical, ultrasound and endocrine evaluation. All patients plus 141 unaffected first-degree family members were genotyped using 366 polymorphic markers on (microsatellites) from the ABI PRISM® Linkage Mapping Set MD-10 (average spacing 10 cM). Association-analysis was performed with the transmission disequilibrium test (TDT, genehunter software). Linkage analysis was performed in 11 clusters of 2 or more closely related patients (Simwalk2).

RESULTS: There was an average number of 10.1 alleles per polymorphic marker. The TDT identified two non-adjacent markers on chrome 6 (D6S262 and D6S308) that showed significant association with PCOS (P≤0,01). Other markers that showed significant association were positioned on chromosomes 1 (D1S450), 3 (D3S1565), 4 (D4S406) , 7 (D7S530), 8 (D8S264) and seventeen (D17S784) (all P values ≤ 0.01). Linkage analysis in 11 sub-pedigrees revealed no significant linkage for these, or other, loci with PCOS. Moreover the results of a prior report of linkage of a marker on chromosome 19 (D19S884) could not be confirmed.

CONCLUSIONS: The lack of consistent results suggests the absence of a consistent genetic background in this select group of PCOS patients. This supports the hypothesis of a complex genetic background for PCOS that allows relatively small contributions of multiple risk-genes to be involved in the pathogenesis of this syndrome. In this founder-population the genetic heterogeneity was not sufficiently reduced to find risk-loci in a genome wide screen using highly polymorphic markers with an average spacing of 10 cM.

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Endometrial and Sub-Endometrial Blood Flow Is Reduced in Women with Polycystic Ovaries Who Have Clinical Hyperandrogenaemia. NJ Raine-Fenning,[1] PM Lam,[2] IR Johnson.[1] *[1]School of Human Development, University of Nottingham, Nottingham, United Kingdom; [2]Department of Obstetrics & Gynaecology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong S.A.R., China.*

Objectives: To objectively quantify uterine and endometrial blood flow in women with polycystic ovarian syndrome (PCOS) and to examine if this was different in women with different phenotypic expressions of the disease.

Methods: Transvaginal 2D and 3D ultrasound was performed in 36 women with PCOS, as defined by the Rotterdam criteria, and sub-group analysis conducted based on the subjects' body mass index (BMI), ovulation status, and hirsutism score. ANOVA was used to compare the mean values between the groups.

Results: PCOS women with clinical hyperandrogenaemia had significantly lower endometrial and subendometrial blood flow than their anovulatory normoandrogenic counterparts (Table 1). There were no differences between lean and obese women or between anovulatory and ovulatory women with PCOS. The pulsed wave Doppler parameters were similar in all three phenotypic groups.

Conclusions: Hirsute women with PCOS have impaired endometrial perfusion compared to their normoandrogenic counterparts which is only evident with 3D ultrasound and not conventional pulsed wave Doppler.

Endometrial and uterine blood flow in clinically hyperandrogenic women with PCOS

Subjects' characteristics	Hirsute	Normoandrogenic
Age (years)	30.5 ± 1.1	33.8 ± 1.7
BMI (kg/m2)	27.48 ± 0.79	25.22 ± 1.11
Endometrium		
VI (%)	0.572 ± 0.064 *	1.009 ± 0.129
FI (0_100)	33.305 ± 1.410	34.878 ± 2.879
VFI (0_100)	0.198 ± 0.027 *	0.363 ± 0.058
Sub-endometrium		
VI (%)	1.778 ± 0.245 *	3.083 ± 0.447
FI (0_100)	39.695 ± 1.135	40.741 ± 2.526
VFI (0_100)	0.717 ± 0.105 *	1.283 ± 0.204
Uterine artery		
PSV (cm/s)	35.21 ± 2.52	27.20 ± 1.98
PI	2.64 ± 0.17	2.67 ± 0.18

VI = vascularisation index; FI = flow index; VFI = vascularisation flow index; PSV = peak systolic velocity; PI = pulsatility index (* P<0.01)

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Risk of Early and Late Obstetric Complications in Women with PCOS. Nusayba A Bagegni,[1] Jill Blaine,[1] Anuja Dokras.[2] *[1]Obstetrics & Gynecology, University of Iowa Hospitals & Clinics, Iowa City, IA, USA; [2]Obstetrics & Gynecology, University of Pennsylvania, Philadelphia, PA, USA.*

Background: There is conflicting evidence on the association between PCOS and early and late obstetric complications. It is unclear if the reported risks are independent of BMI, preexisting hypertension and diabetes. We examined the risk of early and late obstetrical complications in a large group of women with PCOS compared to controls.

Methods: We reviewed pregnancy records of women with PCOS (Rotterdam criteria, n=130) and controls (tubal infertility, n=130) after in vitro fertilization at University of Iowa from 1994-2006. The Wilcoxon rank sum test and Fisher's exact test were used to evaluate differences between variables and logistic regression analysis was used to determine the independent risk of PCOS.

Results: Subject demographics and medical history are shown in Table. The first trimester miscarriage rate was 18% in women with PCOS and 15% in controls. After logistic regression analysis PCOS was not associated with miscarriage (p=0.495). The prevalence of gestational DM (GDM) was similar in both groups 12% PCOS vs 11% controls. PCOS was not associated with GDM after adjusting for age and BMI (p=0.678). However, BMI was significantly associated with GDM after adjusting for age and PCOS (p=0.012). Risk of both pre-eclampsia and PIH was 10% in PCOS and 5% in controls, but not statistically significant after adjusting for age, BMI and twin gestation. Preexisting HTN showed a significant association with preeclampsia (p<0.01). There was no significant difference in preterm delivery, Cesarean section, twin gestation, intrauterine fetal death and intrauterine growth restriction in the 2 groups.

Conclusion: Despite adequate power, our study did not detect an increased risk of miscarriage in women with PCOS. Obesity was a significant contributor to late obstetric complications, namely GDM. These findings may warrant aggressive counseling of women with PCOS on the potential benefits of weight loss prior to pregnancy.

Prepregnancy Clinical Variables

Variable	PCOS (n=130)	Controls (n=130)
Age, mean (SD)	31.1(4.3)	33.0 (3.7)†
BMI, mean (SD)	31 (8)	28.9 (5.5)
Caucasian	87%	88%
Nulliparity	78 (60%)	20 (15%)†
>2 Spontaneous Abortions	7 (5%)	19 (15%)
Current Smoker	4 (3%)	12 (10%)
Hypertension	13(10%)	8(6%)
IGT	30(29%)	12 (20%)
DM	5(5%)	1(2%)

DM=diabetes mellitus, IGT=impaired glucose tolerance, p<0.05, †p<0.01

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Variation in Prevalence of Polycystic Ovaries in Regularly Cycling Women across the Reproductive Lifespan. Erica Johnstone,[1] Sheng-Fang Jiang,[2] Barbara Sternfeld,[2] Mitchell Rosen,[1] Marcelle Cedars.[1] *[1]OB, GYN & RS, UCSF, San Francisco, CA, USA; [2]Division of Research, Kaiser Permanente, Oakland, CA, USA.*

Objective: To assess the prevalence of polycystic appearing ovaries (PCAO) as defined by the Rotterdam criteria; and to determine whether metabolic parameters differ between regularly cycling women with PCAO and those with normal ovaries.

Background:

Studies have demonstrated a population frequency of PCAO of 21-33%, with 7-24% among women with regular cycles. Most were performed in a young reproductive age population; none examined the impact of age. All were performed prior to adoption of the Rotterdam criteria. Recent studies have shown an increased prevalence of metabolic syndrome among women diagnosed with polycystic ovarian syndrome (PCOS). However studies focused on women in their 20s found no increase in BMI or fasting insulin with PCAO but regular menses.

Participants:

222 women aged 25-45 with regular cycles (every 25-35 days) enrolled in the OVA study, a population-based study of ovarian aging.

Methods:

Each subject underwent a transvaginal ultrasound for assessment of ovarian volume and antral follicle count (AFC). Outcomes collected included waist measurements and HDL, LDL, total cholesterol, triglycerides, fasting glucose and insulin.

PCAO were determined by the Rotterdam Criteria (AFC of ≧ 12 on one ovary or ovarian volume > 10cc). Student's t-test and Chi-square tests were used to assess differences between those with and without PCAO for continuous and categorical variables, respectively.

Results:

The prevalence of PCAO decreased with increasing age (Table 1). Of the 69 women with PCAO, 62% met the AFC criterion only, while 19% met the volume criterion only, and 19% met both. Women with and without PCAO did not differ in waist measurements, or in fasting lipids, insulin, or glucose.

Conclusions:

The Rotterdam criteria, while less subjective than those described by Adams in 1986, have led to an increased prevalence of PCAO among women with regular menses, over 1/2 of women in their 20s. Women with PCAO do not differ from those with normal ovaries in metabolic parameters associated with PCOS. Consideration should be given to adopting an age-adjusted criterion for AFC, or a combination of AFC and ovarian volume, for diagnosing PCOS.

Table 1. Prevalence of polycystic ovarian morphology by age

Age	N	Number with PCAO	Percentage
25-29	28	18	64
30-34	56	19	34
35-39	74	25	34
40-45	64	7	11
Total	222	69	31

p <.0001

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HDL Levels and Menstrual Cyclicity Improve with a High Protein Diet for Weight Loss in Polycystic Ovarian Syndrome. H Cook-Andersen, R Alvero, KL Davidson, WD Schlaff, SA Foley. *Advanced Reproductive Medicine, University of Colorado Health Science Center, Denver, CO, USA.*

BACKGROUND: PCOS, especially accompanied by obesity, has been reported to be associated with a characteristic dyslipidemia comprising elevated triglycerides (TGs) and depressed HDL, especially the HDL-2 fraction. This is an atherogenic profile; in fact, studies suggest low HDL-2 may correlate most strongly with cardiovascular disease risk. Weight loss is a mainstay of treatment and improves all manifestations of the disease, but the optimal diet to recommend remains undetermined. Preliminary studies show a high protein diet may improve total HDL levels and insulin responses. However, the effect of weight loss and dietary composition on HDL-2 levels in PCOS has not been investigated.

OBJECTIVE: To evaluate the fasting lipid profile in newly diagnosed obese PCOS patients and to determine the effects of a high-protein diet with or without metformin on weight loss, HDL-2 and other lipoproteins, and menstrual cyclicity.

METHODS: In this pilot retrospective observational study, the fasting lipid profile of 42 obese women newly diagnosed with PCOS was determined. They were then placed on a high protein (80-100 g/day), low carbohydrate (30-50 g/day) diet with or without metformin (76 and 24%, respectively) and followed monthly for an average of 13 months (range 5-31).

RESULTS: At diagnosis, 66% had low HDL and 89% had low HDL-2; only 29% had elevated TGs. On the diet, the patients demonstrated an average weight loss of 24.7 lbs (212.01 to 187.4, p<0.001) and decreased BMI of 4.2 kg/m2 (34.2 to 30.0, p<0.001). HDL levels increased significantly (18% increase from 46.6 to 55.0, p<0.001), especially the HDL-2 fraction (34% increase from 10.9 to 14.6, p<0.001). Triglycerides decreased as well (135.0 to 107.2, p<0.027). LDL decreased but did not reach statistical significance. 29 resumed menstrual cycles, 11 were started on oral contraceptives, and 1 had a hysterectomy. 2 pregnancies occurred. No difference was seen with metformin use.

CONCLUSION: A majority demonstrated decreased HDL and HDL-2 at diagnosis. The high protein diet resulted in significant weight loss and improvement in HDL-2 levels, as well as improvements in total HDL, TGs and menstrual cyclicity. Metformin produced no added benefit. Prospective trials based on these data will help determine the optimal diet to reduce the significant short- and long-term morbidity in the large population of women with PCOS.

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Relationship between Resistin, Inflammatory Markers and Insulin Resistance in Women with Polycystic Ovary Syndrome. Emily K Bradbury, Rebecca A Chilvers, Summer James, Xin Ma, Manubai Nagamani. *Division of Reprod Endocrinology, Dept of OB/GYN, University of Texas Medical Branch, Galveston, TX, USA.*

Resistin is an adipokine that has been associated with obesity and insulin resistance in animal models. Studies on the role of resistin on insulin resistance in humans have been controversial. Recently resistin has been shown to exert atherosclerotic effects and elevated resistin levels have been observed in women with coronary heart disease (CHD). Women with polycystic ovary syndrome (PCOS) are at high risk for CHD. Our present study investigates potential association of resistin and markers of inflammation, C-reactive protein (CRP) and insulin resistance in women with PCOS.

Methods: Thirty two women with PCOS participated in the study. All were hirsute and had irregular cycles. Nineteen women with normal ovulatory cycles who matched the PCOS patients in BMI served as controls. Fasting glucose, insulin, resistin and CRP levels were measured in all women. After a high carbohydrate diet for 3 days, a standard oral glucose tolerance test (OGTT) was performed. Blood samples were collected for glucose and insulin before and 1, 2 and 3 hrs after 75g oral glucose. The area under the curve (AUC) for insulin and glucose was calculated. Women with overt diabetes were excluded from the study.

Results: Fasting insulin levels (24.9 $\pm$ 2.2 µU [±SE] /ml) and insulin response to oral glucose (AUC) (527.9 $\pm$ 52.2µU/ml) were higher ($P < 0.001$) in women with PCOS compared to controls. All were insulin resistant with HOMA-IR value > 3.9 mol x µU / L^2. Resistin levels in women with PCOS (15.6 ±1.3 ng/ml) was significantly ($P < 0.02$) higher compared to control women (10.7 ± 1.4 ng/ml). CRP levels in women with PCOS (9954.2 ± 918 ng/ml) was also significantly ($P < 0.001$) higher than the controls (2396.2 ± 579 ng/ml). There was a significant positive correlation between resistin and CRP levels ($r = 0.362$, $P < 0.04$). There was no correlation between resistin levels and fasting insulin levels or insulin AUC. There was also no correlation between resistin levels and fasting glucose or glucose AUC.

Conclusions: Our results indicate that (1)women with PCOS and insulin resistance have increased resistin levels, (2) there is a strong association between resistin and CRP (3) elevated resistin and CRP levels may predict women who are at increased risk for CHD (3)) It is unlikely that resistin plays a major role on insulin resistance in women with PCOS.

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Differences in Fat Distribution and Hormone Levels in the Ovulatory and Anovulatory Phenotypes of Polycystic Ovary Syndrome (PCOS). Vuk P Jovanovic,[1] Enrico Carmina,[2] Prati Vardhana,[1] Michel Ferin,[1] Rogerio A Lobo.[1] *[1]Department of Obstetrics and Gynecology, Columbia University, New York, NY, USA; [2]Department of Internal Medicine, University of Palermo, Palermo, Italy.*

Current diagnostic criteria for PCOS includes both ovulatory (OV) and anovulatory or "Classic" (C) phenotypes. In an effort to further characterize differences and /or similarities between these 2 phenotypes, we studied 48 hyperandrogenic women with PCOS (age 27.3±6.4, BMI 27.1±5.6) and 20 age matched controls (age 27.7±4.5, BMI 26.8±3.9). Women with PCOS

were divided into weight matched (OV) n=14 and (C) n=34 groups. Fat and weight distribution were assessed by DEXA (Total fat, R1 fat, trunk fat) as well as fasting levels of LH, E2, MIS/AMH, kisspeptin and testosterone, the adipocytokines (leptin, adiponectin, visfatin and retinol-binding-protein-4 RBP4) and serum glucose, insulin and CRP. The hyperandrogenic PCOS groups had characteristically altered hormone profiles compared to matched controls. Although total fat mass was comparable, women with C-PCOS had a significantly larger waist circumference (93.2 14.9 *vs.* 86 85 cm, p<0.05) trunk fat, R1 fat and %trunk and %R1 fat compared to OV-PCOS (p<0.05). Leptin, RBP4 and visfatin did not significantly differ among the PCOS subgroups although adiponectin was lower in the C-PCOS group (p<0.05). QUICKI was significantly lower in C-PCOS (0.33±0.02 *vs.* 0.35±0.02, p<0.05) and insulin was higher (14.9±5.76 *vs.* 9.7±3.5, p<0.01). Serum LH was also higher (11.3±7.8 *vs.* 6.5±1.7, p<0.05) but kisspeptin, testosterone and estradiol were similar. MIS/AMH (8.3±5.2 *vs.* 6.8±4.9 ng/ml, not significant) and CRP (2.78±1.5 vs. 1.3±0.5, p<0.05) were higher in C- PCOS. Significant correlations (p<0.05) were noted among kisspeptin/RBP4 (r 0.426), MIS/Testosterone (r 0.451), Insulin/ %trunk fat (r 0.616, p<0,01), total fat/leptin (r 0.772, p<0.01), %trunk fat/adiponectin (r -0.522). In conclusion, women with C-PCOS when compared to similarly hyperandrogenic women with OV-PCOS with similar BMI, have increased abdominal fat, and appear to have differences in serum LH, CRP and increased insulin resistance. The latter, as well as subtle differences in the adipocytokines may explain these differences in anthropometric findings.

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Study on the Zona Pellucida Gene (ZP4) Sequence in Patients with Polycystic Ovary Syndrome. Blazej Meczekalski,[1] Robert Nawrot,[2] Witold Nowak,[3] Anna Gozdzicka-Jozefiak,[2] Alina Warenik-Szymankiewicz.[1]
[1]Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, ul Polna 33, Poland; [2]Depertment of Molecular Virology, A. Mickiewicz University, Poznan, ul Umultowska 89, Poland; [3]Department of Molecular Biology Techniques, A. Mickiewicz University, Poznan, ul Umultowska 89, Poland.

Problems of normal oogenesis and folliculogenesis but also disturbed oogenesis and folliculogenesis in polycystic ovaries are not fully understood. Oocyte specific genes play an essential role in oogenesis and folliculogenesis. There are suggestions about possible role of some oocyte specific genes in etiopathophysiology of PCOS. Zona pellucida 4 gene (ZP4) is recently identified gene, which belongs to zona pellucida genes such as ZP1, ZP2 and ZP3. The role of ZP4, contrary to above-mentioned other ZP genes is not well described.

The aim of this study was to analyze ZP4 coding sequence and expression in patients with polycystic ovary syndrome.

Material included blood received from 29 patients (mean age 24,2 +/- 3,23 years; mean BMI 31,4 +/- 4,54 kg/m^2) with polycystic ovary syndrome. All patients with PCOS were diagnosed with the use of ESHRE/ASRM criteria from 2003.

DNA was isolated from blood cells(after separation of blood cells from serum) using a DNA isolation kit (Qiagen). Genomic DNA was used for in vitro amplification by PCR with a specific set of primers complementary to the coding sequence of the ZP4 gene.

Products from each PCR reaction were examined by SSCP method. Samples with changes detected by SSCP in comparison to control probes were cloned into plasmid vector and then automatically sequenced

From a total of 29 patient samples with PCOS, we identified 5 nucleotide changes in the ZP4 coding seguence : 4 silent nucleotide changes in exons 1,2,5 ,7, and 1 nucleotide change in the exon 5 (position 114, T>G). The mutation in exon 5 (T>G) results in substitution of cystein for glycin of amino acid in position 223 of ZP4 protein. In summary, our data demonstrate that ZP4 nucleotide changes account for 15% of patients with PCOS.

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Relationship between Serum Mullerian Inhibiting Substance Levels and Insulin in Women with Polycystic Ovary Syndrome. Rebecca A Chilvers, Shilla Chakrabarthy, Summer James, Xin Ma, Manubai Nagamani. *OB/GYN, University of Texas Medical Branch, Galveston, TX, USA.*

Müllerian-inhibiting substance (MIS) is a member of the transforming growth factor-β superfamily of growth factors. It is expressed exclusively in granulosa cells and is believed to play a role in the regulation of follicle selection and maturation. Women with PCOS have anovulation and most of them have hyperinsulinemia. The purpose of our study is to investigate possible association between insulin and MIS in the dysregulation of folliculogenesis in PCOS.

Methods: Twenty one women with PCOS who had anovulatory cycles and hyperandrogenism were recruited for the study. Sixteen women with ovulatory cycles and matched the PCOS patients in age and BMI served as controls. An oral glucose tolerance test (OGTT) was performed in all women. Blood samples were obtained at fasting and 1, 2 and 3 hours after glucose ingestion for measurement of glucose and insulin. To investigate the effect of hyperinsulinemia on MIS secretion, MIS levels were measured in ten patients during the OGTT. Fasting MIS, testosterone, DHEAS, FSH, and LH levels were measured in all patients.

Results: Fasting insulin levels (16.4 ± 2.4µU/ml) vs 9.9 ± 1µ U/ml [± SE]) (P < 0.03) and area under the curve (AUC) of insulin (267.9 ± 33.0 vs 134.0 ± 12.2µU/ml) (P <0.001) were significantly increased in women with PCOS compared to control women, while the glucose levels were normal indicating insulin resistance. MIS levels were significantly (P < 0.001) increased in women with PCOS (6.5 ± 0.9 ng/ml) compared to controls (1.9 ± 0.2 ng/ml). There was no correlation between age and MIS levels in PCOS patients while there was a highly significant negative correlation between the age and MIS levels in the control women (r = -0.822, P< 0.0001). There was significant negative correlation between MIS and fasting insulin levels (r = - 0.462, P < 0.03) and insulin AUC during the OGTT (r = -0.485, P <0.02). There were no changes in MIS levels during OGTT.

Conclusions: Results of our study indicate that in women with PCOS, (1) there is an increase in secretion of MIS, (2) higher insulin levels are associated with lower MIS levels, (3) acute increase in insulin levels has no effect on MIS levels, (4) lower MIS levels in women with severe hyperinsulinemia could be due to associated increased follicular atresia and a decrease in the ovarian reserve. Further studies are needed to investigate the role of insulin on MIS secretion.

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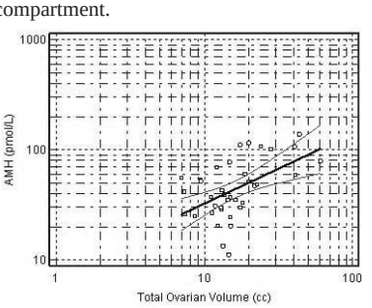
Regulation of Serum Anti-Müllerian Hormone (AMH) in Patients with Polycystic Ovary Syndrome: Relevance of Insulin Resistance Indices. Hiroyuki Asakura, Noriko Tanaka, Kyoko Nishio. *Ohgimachi Ladies' Clinic, Osaka, Japan.*

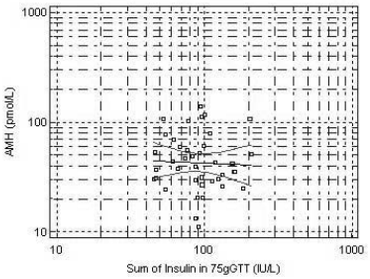
Objective: Elevation of serum anti-Müllerian hormone (AMH) levels among polycystic ovary syndrome (PCOS) patients has been reported. However, the regulatory factors of AMH in PCOS remain unknown. We examined correlations between AMH values and various indices of insulin resistance in PCOS women.

Methods: 40 infertile women compatible with Rotterdam criteria for PCOS were recruited under informed consent. The subjects underwent 75g oral glucose tolerance test (75gGTT, sampling at 0, 60, 120 minutes), and 2-step ELISA for AMH (sensitivity 0.7 pmol/L, inter & intra-assay C.V. :12.3%, 14.2%, respectively). p<0.05 was considered as statistical significance.

Results: Average AMH level of the subjects (Age:31.9±3.9 years, BMI: 21.1±3.0 kg/m^2, mean±S.D.) was 50.4±31.1pmol/L, which was higher than normo-ovulatory women (16.7±10.5 pmol/L, n=16). AMH levels had significant positive correlation with total ovarian volume by ultrasound (18.2±11.4 cc), but not with BMI, waist-hip ratio (0.85±0.06), and levels of serum LH (5.5±3.6 IU/L), total testosterone (67.3±32.8 ng/dL). Although fasting glucose levels (83.1±4.9 mg/dL) were positively correlated with total ovarian volume (r=0.41), AMH levels had no significant correlation with fasting insulin (4.3±2.3 IU/L), fasting glucose/insulin ration (22.8±8.4), HOMA-IR (0.90±0.51), and the sum of insulin levels (98.6±42.1 IU/L)during 75gGTT.

Conclusions: Increased serum AMH level of PCOS and its positive correlation with total ovarian volume implies that determination of serum AMH level would aid in confirming diagnosis of the ovulatory disorder. Absence of relationship between AMH and various clinical indices of insulin resistance suggests alternative regulatory factors of AMH gene expression in the follicular compartment.





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Sphingomyelin and Phosphotydylcholine Levels in Normal and PCOS Women. Ozgul Muneyyirci-Delale,[1,4] Mudar Dalloul,[1] Xian-Cheng Jiang,[2] Mahua Chakraborty,[2] Vijaya Nacharaju,[1] Ibraham Joulak,[1] Nezar Dalloul,[1] Lian-fu Yang,[1] Ashadeep Chandrareddy,[1] Rudolph Parris,[1] Hans vonGizycki.[3]
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Introduction: Atherogenesis is initiated by the interaction of cholesterol-rich lipoproteins with the arterial wall. It has long been known that sphingomyelin (SM) accumulates in human atheroma and that the major source is plasma lipoproteins. The concentration of SM relative to total phospholipids (principally phosphotydylcholine (PC) plus SM) is an important determinant in the assessment of risk-factors for atherosclerosis. Polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder of young women, associated with obesity, insulin resistance, diabetes, hyperlipididemia and hypertension. Women with PCOS are thus associated with conditions predisposing to cardiovascular disease.

Methods: After obtaining consent (as required by IRB) blood was collected from 13 normal cycling women and 30 women with PCOS. Serum levels of SM and PC were determined using two rapid and sensitive enzymatic assays. PC levels did not influence SM measurements and vice-versa. All data analysis (T-test and ANOVA for linear correlations) was done using SPSS Version 15, © 2006.

Results: Sphingomyelin was not correlated to the body mass index (BMI) of the patients, where as phosphotydylcholine was positively correlated to BMI. Mean SM levels were 57.34 ± 10.51µg and 82.28 ± 23.61µg in normal and PCOS women, respectively. The PCOS women had significantly higher SM levels ($p = 0.001$). PC levels in PCOS patients (203.35 ± 62.97µg) was significantly lower than the levels (239.81 ± 62.17µg) in normal women ($p = 0.017$). The ratio of SM/(SM+PC) in PCOS patients (0.29) was higher than in the normal group (0.19).

Conclusions: Women with PCOS are associated with higher levels of SM and lower levels of PC, hence, high ratio of SM to total lipids. SM levels are known to be related to the development of atherosclerosis, and several lines of evidence suggest a preventive role for PC. The combination of high SM and low PC levels in PCOS indicates a risk for atherosclerosis in this population of women.

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Isolation of GnRH Gene Sequences Critical for Expression in Male Reproductive Tissues. Amisra A Nikrodhanond, Keeley L Mui, Helen H Kim.
OB/GYN, University of Chicago, Chicago, IL, USA.

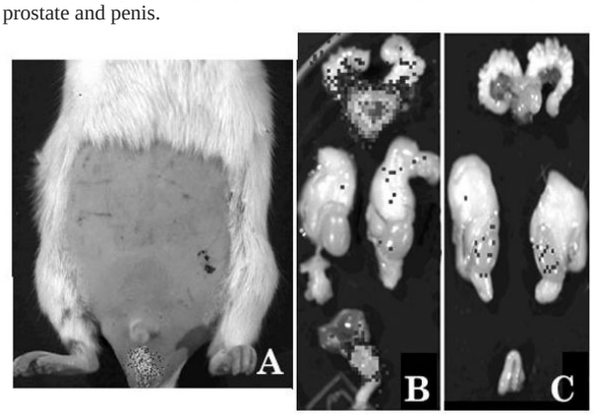
Background: Although primarily a neuroendocrine hormone, Gonadotropin-releasing hormone (GnRH) is also produced in reproductive tissues, where it acts locally. The study of GnRH regulation in these tissues is limited by low levels of expression.

Objective: To elucidate mechanisms that regulate GnRH expression in male tissues, our objective was to identify regions of the GnRH gene that target expression *in vivo*.

Methods: Transgenic mice were generated with 2 different fragments of the mouse GnRH gene promoter (-3446/+28 and -249/+28 bps), fused to the luciferase reporter gene. In these mice, luciferase activity (detected as light) reflects GnRH promoter activity. Using bioluminescent imaging, GnRH promoter activity was assayed in live GnRH-LUC mice and in their reproductive tissues *ex vivo*. To confirm imaging results, luciferase activity was also measured as relative light units (RLU) in tissue homogenates. For each DNA construct, 5 male mice were examined.

Results: With whole-body imaging, bioluminescence was detected in the genital region of the GnRH-LUC transgenic mice (Fig.A). In mice that incorporated the -3446LUC transgene, examination of reproductive tissues *ex vivo* revealed bioluminescence in the prostate and penis, but not in the seminal vesicles, epididymis, or testes (Fig.B). In contrast, in the -249LUC mice, bioluminescence was detected in the testes, but not in other tissues (Fig.C). Examination of luciferase activity in tissue homogenates from -3446LUC mice confirmed GnRH promoter activity in the prostate (3876 ± 624 RLU) and penis (576 ± 151 RLU) and absence in the testes (39 ± 11 RLU). In the -249LUC mice, however, luciferase activity was detected only in the testes (1283 ± 97 RLU) and not in the prostate (65 ± 25 RLU) or penis (48 ± 10 RLU).

Conclusions: Our studies demonstrate that GnRH is present in male reproductive tissues, but may be differentially regulated in the testes vs. prostate/penis. Promoter elements, contained within the proximal -249 bp of the mouse GnRH gene, are sufficient to mediate testicular GnRH expression while -3446 bp of the gene promoter are necessary to direct expression to the prostate and penis.



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Characterization of Mouse Ringo/Speedy Homologues. Z Walton, S Uckac, O Guzeloglu-Kayisli, MD Lalioti, D Sakkas, E Seli. *Ob & Gyn, Yale U., New Haven, CT, USA.*

Introduction: Ringo/Speedy (Ringo/Spy) is a recently discovered cyclin-dependent kinase (CDK) activator that functions similar to cyclins in controlling the cell cycle. Ringo/Spy plays a crucial role during meiotic maturation in Xenopus by inducing meiotic G2/M progression and Germinal Vesicle Breakdown (GVBD). More recently, Padmanabhan and Richter demonstrated that Ringo/Spy mRNA is repressed in the Xenopus oocyte cytoplasm by Pumilio 2. Upon meiotic reactivation, Pumilio 2 loses its interactions with Ringo/Spy permitting its translation. Ringo/Spy is then expressed, leading to activation of CPEB by phosphorylation, which in turn elicits polyadenylation and translation activation of the mRNA for a critical oocyte maturation factor, the mos kinase. In this study, we investigated the expression of Ringo/Spy in mouse.

Methods: Ten different somatic tissues, testes, and ovaries were tested by reverse transcription-polymerase chain reaction (RT-PCR) for the expression of Ringo/Spy homologues and their alternative splicing variants. Ringo/Spy mRNA expression was also tested in prophase I (PI) and metaphase II (MII) oocytes, 1-cell, 2-cell, 4-cell, 8-cell embryos and blastocysts. Amplification with actin primers provided a positive control and allowed semi-quantitative analysis.

Results: We analyzed the two previously identified homologues of Ringo/Spy (A and B). The two alternative splicing variants of Ringo/Spy A (A1 and A2) were separately studied for their expression profile.

We also identified an additional alternative splicing variant of Ringo/Spy B and evaluated similarly. Ringo/Spy A (1 and 2) were expressed in testes, ovaries, and certain somatic tissues including brain, and spleen. Ringo/Spy B (both variants) were only expressed in testis. Ringo/Spy A or B were not present in mouse oocytes or early embryos.

Conclusions: Our findings indicate that the previopusly described Ringo/Spy homologues A and B are not expressed in mouse oocytes or early embryos suggesting that either an alternative CDK-activator or a yet to be identified homologue of Ringo/Spy may be mediating meiotic G2/M progression in mouse. Testis specific expression of Ringo/Spy B, and previously described role of Ringo/SPy in meiosis suggests a role for this protein in male gametogenesis in mouse.

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Significant Influences of Derangements in the Homocysteine Pathway on Human Sperm Quality. Jolanda C Boxmeer,[1] Marij Smit,[2] Elaine Utomo,[1] Johannus C Romijn,[2] Marinus JC Eijkemans,[3] Jan Lindemans,[4] Joop Laven,[1] Nick S Macklon,[1,5] Eric AP Steegers,[1] Regine PM Steegers-Theunissen.[1,6,7,8] *[1]Obstetrics and Gynecology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Andrology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [3]Public Health, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [4]Clinical Chemistry, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [5]Reproductive Medicine and Gynecology, University Medical Center, Utrecht, Netherlands; [6]Epidemiology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [7]Pediatric Cardiology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [8]Clinical Genetics, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*

Objective: To investigate associations between biomarkers of the homocysteine pathway and semen parameters, including the NA fragmentation index (DFI).

Methods: From 251 men of couples undergoing IVF or ICSI treatment semen samples were collected. Fertility was defined as a semen concentration ≥ $20x10^6$ cells and proven fertility. Semen parameters were assessed according to WHO and strict criteria and DFI was measured. In seminal plasma total homocysteine (tHcy), folate, cobalamin and pyridoxal'5'phosphate (PLP) levels were determined. Correlations were adjusted for the possible confounders as age, BMI, smoking, alcohol intake and varicocele.

Results: In seminal plasma cobalamin correlated independently with folate and tHcy (both r = 0.26; $p \leq 0.001$). Sperm count significantly correlated with sperm volume (β 0.25; $p \leq 0.001$), percentage progressive motility (β 0.39; $p \leq 0.001$) and percentage normal morphology (β 0.39; $p \leq 0.001$). DFI correlated with sperm volume (β 0.23; $p \leq 0.001$) and percentage progressive motility (β -0.41; $p \leq 0.001$). In subfertile men, DFI was correlated with the percentage normal morphology (β -0.33; $p \leq 0.01$). In seminal plasma tHcy and cobalamin correlated with sperm count (β 0.21; $p \leq 0.01$, β 0.15; $p \leq 0.05$, respectively). The B vitamins were inversely correlated with sperm volume (folate:β -0.20; $p \leq 0.01$, cobalamin:-0.16; $p \leq 0.05$,PLP: -0.19; $p \leq 0.01$). In fertile men, folate levels in seminal plasma were correlated with DFI (β -0.36; $p \leq 0.05$).

Conclusions: Low levels of folate in seminal plasma may be detrimental for the stability of DNA in sperm cells. High levels of sperm DNA damage are associated with poor sperm motility and morphology.

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Characteristics and Outcomes of Glyburide Non-Responders in Pregnant Women with Type II or Gestational Diabetes Mellitus (GDM) Initially Treated with Glyburide. Jennifer Aguayo, Gladys A Ramos, Alethea Hanley, Carri R Warshak, Thomas R Moore. *Reproductive Medicine, University of California, San Diego, San Diego, CA, USA.*

Objective: To determine the risk factors that may predict inadequate response to first-line glyburide monotherapy in pregnant women with Type 2 diabetes mellitus (DM) or GDM and to assess if non-responders are at increased risk of adverse pregnancy and neonatal outcomes.

Study Design: This was a retrospective cohort of 146 women diagnosed with Type II or GDM initially treated with glyburide at a single institution from 2001-2004. Maternal characteristics, adequate glycemic control defined as more than 66% of fasting glucose <95 mg/dL and one-hour post-prandials <135 mg/dL, and neonatal outcomes were assessed. Non-responders were defined as failure to achieve adequate glycemic control on maximum daily doses of glyburide or intolerance due to side effects, necessitating switch to insulin therapy. Statistical methods included bivariate analyses.

Results: Of the 146 women initially treated with glyburide, 25 (17%) failed to achieve adequate glycemic control or did not tolerate glyburide. Reasons for glyburide non-response were maximum dose (20 mg/d) reached (56%), maternal hypoglycemia (28%) and other side effects (16%). There were no statistically significant differences between non-responders and responders with respect to family history of diabetes (75% vs. 60%, p=0.25), prior history of GDM (24% vs. 32%, p=0.63) and macrosomia (>4000 g) (28% vs. 24%, p=0.80) or 1 hour glucose challenge test (204±51 vs. 180±36mg/dL, p=0.07). Non-responders had a higher rate of obesity (BMI≥30) (71% vs. 44%, p=0.02) and earlier gestational age at initiation of therapy (24±7.7 vs. 29±7.7 wks, p=0.01). There were no differences between the groups in the mean 36-week fasting (96±17 vs. 90±14 mg/dL, p=0.15) and post-prandial glucose values (133±26 vs. 127±19 mg/dL, p=0.87). No significant differences were observed in incidence of pre-eclampsia, primary cesarean delivery or birth weight. Neonatal outcomes including ponderal index, neonatal hypoglycemia, NICU admission, and birth injuries also did not differ between the groups.

Conclusion: Women who are obese and who require earlier initiation of glyburide therapy are at increased risk of non-response to glyburide monotherapy. However, despite non-response, these women can be managed with subsequent insulin therapy to achieve similar glycemic control and pregnancy and neonatal outcomes.

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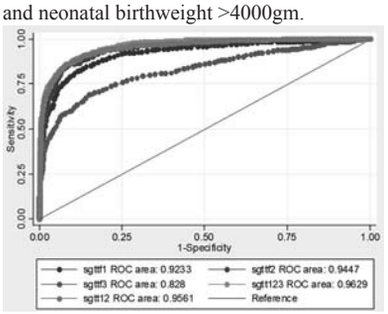
A Different Diagnostic Strategy Using the 100gram, 3-Hour Glucose Tolerance Test for the Diagnosis of Gestational Diabetes Mellitus. Yvonne W Cheng, Ingrid Block-Kurbisch, Jennifer Lydell, Aaron B Caughey. *Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA.*

Objective: To examine whether a simplified strategy using the 100gm, 3-hour glucose tolerance test (GTT) may be useful for the diagnosis of gestational diabetes mellitus (GDM).

Methods: This is a retrospective cohort study of women with singleton pregnancy who received the 50-gm, 1-hour glucose challenging test (GCT) for initial screening and the 100gm, 3-hour GTT as confirmatory tests for the diagnosis of GDM between 1988 and 2001. Various combinations of the 100gm, 3-hour GTT results were examined and compared to the diagnostic criteria for GDM established by the Carpenter and Coustan criteria using the receiver-operator characteristic (ROC) curves. Perinatal outcomes of women who would have had a false-positive or false-negative test results were compared to those who did not have GDM using the Carpenter and Coustan criteria. Potential confounding factors were controlled for using multivariable regression models.

Results: 2,535 women had 100gm, 3-hour GTT results available for analysis during the study period. Using GDM diagnosed by the Carpenter and Coustan criteria as reference, various diagnostic strategies was compared using ROC curves (Figure 1). Summation of the 1-hour and 2-hour GTT results with a diagnostic threshold of 334mg/dL yielded the most optimal balance between sensitivity (82.1%) and specificity (93.2%). When compared to women without GDM, women who were diagnosed with GDM by C&C criteria but not by summation of 1-hour and 2-hour GTTs had higher odds of operative vaginal delivery (aOR=1.81, 95% confidence interval [CI] 1.03-3.27) and neonatal birthweight >4000gm (aOR=1.91, 95% CI 1.03-3.57).

Conclusion: Using only the summation of only the 1-hour and 2-hour GTT results of the 100gm 3-hour GTT offers an alternative test strategy which may be more convenient and less costly for the diagnosis of GDM. However, women who would have GDM by the Carpenter and Coustan criteria but not by the summation method have higher odds of having an operative vaginal delivery and neonatal birthweight >4000gm.



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Outcome of Induction of Labor Indicated for Term PROM among Women with or without a Uterine Scar. Yifat Ochshorn, Avital Skornick Rapaport, Adi Reches, Joseph B Lessing, Ariel Many. *Obstetrics&Gynecology, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.*

Objective: We aimed at comparing the outcome of induced term deliveries presenting with PROM with or without a previous uterine scar.

Methods: The computerized files of 973 women delivered following induction of labor due to term PROM, were reviewed. Perinatal outcome parameters such as the mode of delivery, indication for cesarean section, rate of low 5' Apgar scores and NICU admissions were compared between women with and without a previous cesarean.

Results: During the study period 973 women delivered in our institution following PROM and induction of labor , 49 of them had a previous uterine scar. Parturients of both groups (with and without a scar) were similar with

regard to age, gestational age at delivery and parity. Cesarean section rate was higher for the previous scar group (28% vs 17%, p<0.05). The most common indication for cesarean was arrest of dilatation and/or descent among women with previous scar accounting for 43% and 27% (p<0.05) for women with and with no uterine scar, respectively. No differences were noted in neonatal outcome parameters such as rate of low 5' Apgar scores and NICU admission rate between the two groups.

Conclusion: Induction of labor due to PROM culminates in higher cesarean rate in women with one previous scar compared with parturients with no scar. Perinatal outcome is similar between the two groups.

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Acid Base Balance and Immediate Perinatal Outcome of Vertex Compared with Breech Presentation in Elective Cesarean Section. Avital Skornick Rapaport, Yifat Ochshorn, Joseph B Lessing, Yuval Yaron, Michael Kupferminc, Ariel Many. *Obstetrics&Gynecology, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.*

Backround: Apgar scores, umbilical blood pH and bicarbonate are generally lower and Pco_2 levels are higher in vaginally delivered breech neonates compared to cephalic deliveries. Although cesarean delivery improved Apgar scores there is a debate wether it improved the acid base status. This retroprospective study compared umbilical cord blood acid-base values and perinatal outcome of elective cesarean breech-delivery with those of elective cephalic cesarean delivery and to determine whether a different metabolic status and perinatal outcome should be expected in neonates in breech presentation.

Study design: The study group included singleton pregnancies delivered by elective cesarean section at term between January 2003 and March 2006. Computerized files of singleton breech presentation elective cesarean sections were compared to those of singleton vertex neonates delivered by elective cesarean section. Demographic data included: maternal age, pregnancy week at delivery and parity. Perinatal outcome measures checked were: birth weight, Apgar scores at 1'and 5', umbilical cord venous and arterial pH and base excess.

Results: During the period between January 2003 and March 2006 there were 1411 singleton elective cesarean sections, 404 of them were breech and 1007 vertex. The mean age, gravida and parity were significantly different between groups (33.5 vs.31.8, 2.95 vs. 2.19. and 1.26 vs.0.64 respectively, p<0.001). The birth weight was significantly different - 3414.8 gram for the vertex and 3189.9 gram in the breech deliveries (p<0.001) There were no differences in either Apgar scores or umbilical pH between the breech and vertex neonates delivered by cesarean section at term (38-40+6w). Venous and arterial PO_2 and PCO_2 levels were significantly different, though the differences were very small and we doubt if these differences have any clinical importance.

Conclusion: Although vaginal breech deliveries are associated with increased risk of asphyxia during delivery, elective cesarean breech deliveries are not at increased risk for lower pH levels or Apgar scores.

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The Clinical Significance of Bleeding during the Second Trimester of Pregnancy. Arie Koifman,[1] Amalia Levi,[2] Yaron Zaulan,[1] Avi Harlev,[1] Eyal Sheiner.[1] *[1]Obstetrics & Gynecology, Soroka University Medical Center, Ben Gurion University of the Negev, Beer-Sheva, Israel; [2]Epidemiology and Health Services Evaluation, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer-Sheva, Israel.*

Objective: This study aimed at investigating clinical importance and pregnancy outcome in women suffering from bleeding during the second half of their pregnancies.

Methods: A population based study including all deliveries which took place in the Soroka University Medical Center between the years 1988-2005 were examined. Comparison was performed between patients with and without second trimester bleeding Pregnancies terminated before 22 weeks, multiple gestations and women lacking prenatal care were excluded . Stratified analysis, using the Mantel-Haenszel technique, and a multiple logistic regression model were performed .

Results: During the study period, 175,093 singleton deliveries occurred in our institute. Of these, 2010 (1.1%) were complicated with bleeding upon admission during the second half of pregnancy. The cases were attributed to placental abruption (63.5%; n=1276) and placenta previa (36.5%; n=734). Independent risk factors associated with bleeding, were oligohydramnios, polyhydramnions, (odds ratio [OR]= 1.6; 95% confidence interval [CI] 1.2-2.0; P=001 and 1.5;1.2-1.8;P<0.01 respectively), suspected intra uterine growth restriction (IUGR, 3.2; 2.6-4.0; P<.001), gestational age, previos abortions and maternal age. These patients subsequently were more likely to deliver by cesarean section (CS, 72.9% vs. 12.1%, OR=19.5; 95%CI 17.6-19.5; P<0.001). Perinatal mortality among patients admitted due to second half bleeding was significantly higher as compared to patients without bleeding (P<.001).

Conclusion: Bleeding upon admission during the second half of pregnancy is an independent risk factor for perinatal mortality. Careful surveillance, including fetal monitoring, is suggested in these cases in order to reduce the adverse perinatal outcome.

Crude and adjusted odds ratios for perinatal mortality among patients with vaginal bleeding.

Characteristics		OR	95% CI	P
Crude OR for perinatal mortality		7.9	6.6-9.4	<0.001
OR adjusted for:				<0.001
	IUGR	7.5	6.3-9.0	<0.001
	Oligohydramnios	7.6	6.3-9.1	<0.001
	Premature rupture of membranes	7.9	6.6-9.7	<0.001
	Cord prolapse	7.7	6.5-9.3	<0.001
	Preterm delivery	2.5	2.0-3.0	<0.001

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Can Neonatal Anthropometrics Predict Perinatal Outcomes? Tania F Esakoff, Yvonne W Cheng, Teresa Jones, Aaron B Caughey. *Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA.*

Objective: To determine if neonatal ponderal index (PI), body mass index (BMI), or birthweight can be used to predict perinatal outcomes.

Study Design: This is a retrospective cohort study of 14,591 pregnancies. The predictors included neonatal PI, BMI, or birthweight while the outcomes included hypoglycemia, shoulder dystocia, acidemia and hyperbilirubenemia. ROC curve analyses were utilized to evaluate the relationship between sensitivity and specificity for each of the predictors and outcomes, with an area under the curve (AUC) significantly greater than 0.5 indicating a screening test that is better than chance.

Results: Neonatal PI, BMI and birthweight are poor predictors of NICU admission, acidemia and hypoglycemia with all AUC values not statistically significantly different than chance alone. They are a reasonable predictor of shoulder dystocia, with birthweight functioning the best (p<0.001).

Conclusions: In general, neonatal anthropometric measurements do not appear to be good predictors of short term neonatal outcomes. Although they are a reasonable predictor of shoulder dystocia, they are not useful clinically for this outcome since they cannot be calculated until after birth. In future studies, new models of neonatal anthropometrics should be created to better predict adverse neonatal outcomes.

Neonatal anthropometric measurements and their relationship to perinatal outcomes expressed as AUC and 95% confidence intervals

	PI	BMI	BWT	p-value
NICU admit	0.48 (0.44-0.512)	0.46 (0.43-0.50)	0.45 (0.41-0.48)	NS
Acidemia	0.48 (0.44-0.53)	0.48 (0.44-0.52)	0.48 (0.44-0.52)	NS
Hypoglycemia	0.46 (0.42-0.50)	0.47 (0.43-0.52)	0.49 (0.45-0.54)	NS
Shoulder Dystocia	**0.66 (0.62-0.69)**	**0.74 (0.70-0.77)**	**0.79 (0.76-0.82)**	**P<0.001**

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Favorable Outcome in Women with Previous Spontaneous Preterm Birth without Progesterone Therapy during Pregnancy: A Prospective Cohort Study. Maurice GAJ Wouters, Annemieke C Bolte, Herman P van Geijn. *Obstetrics & Gynecology, VU Medical Center, Amsterdam, Netherlands.*

OBJECTIVE: Women with a history of one or more spontaneous preterm births are considered to be at a high risk of recurrence. Prenatal progesterone administration is associated with a 35% reduction (from 32.9% to 25.7%) in the recurrence rate of preterm birth less than 37 weeks' gestation [Dodd JM, Flenady V, Cincotta R, Crowther CA. Prenatal administration of progesterone for preventing preterm birth. *Cochrane Database of Systematic Reviews* 2006, Issue 1. Art. No.: CD004947. DOI: 10.1002/14651858.CD004947.pub2].

METHODS: From April 2004 until June 2007, pregnant women with a history of at least one spontaneous preterm delivery were offered protocol-based prenatal care including first- or second trimester bacterial vaginosis screening, repeated ultrasound cervical length assessment, and supportive care by specialized nurses. Women with a positive test for bacterial vaginosis were treated with oral metronidazol therapy, and women with a cervical length below 2.5 cm (before 25 weeks of gestation) underwent a vaginal cerclage.

Progesterone administration was not provided. Data on obstetrical and medical history, pregnancy, delivery and neonatal outcome were prospectively collected and evaluated.
RESULTS: In total, 104 pregnant women were prospectively followed-up. Eighty-seven women had experienced a preterm delivery in their last pregnancy, 52 of whom had delivered before 32 weeks. Three women were diagnosed previously with a bicornual or septal uterus. Twelve women received metronidazol therapy and 18 women underwent a vaginal cerclage. Eighty-four women (80.8%) delivered at 37 weeks or beyond. One-hundred and three women (99.0%) delivered no earlier than 30 weeks, and only one woman delivered at 23 weeks. Two women gave birth to a twin (at 30 and 35 weeks, respectively). Two neonatal deaths due to pulmonary hypoplasia were recorded.
CONCLUSION: Women at high risk of preterm delivery who were offered protocol-based prenatal care not including progesterone therapy had a better pregnancy outcome than would be expected from previous studies. Our findings may question the reported beneficial effects of prenatal progesterone administration.

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Contemporary Outcomes of Expectantly Managed Mid-Trimester Preterm Premature Rupture of Membranes. Tracy A Manuck, Alexandra G Eller, M Sean Esplin, Robert M Silver. *Obstetrics & Gynecology, University of Utah Health Sciences, Salt Lake City, UT, USA.*
Objective: Historically, outcomes following expectant management of mid-trimester preterm premature rupture of membranes (PPROM) have been uniformly poor. Thus, many patients elected pregnancy termination. However, outcomes may be improved with recent advances in neonatal medicine. Our purpose was to assess outcomes in expectantly managed early PPROM in an era of improved maternal and neonatal care.
Study Design: This is a retrospective cohort of patients from 2 tertiary healthcare systems from 2002-2007 experiencing PPROM ≤24.0 weeks gestation. Patients electing immediate termination of pregnancy, carrying fetus(es) with lethal anomalies, or delivering within 12 hours of PPROM were excluded. Survival without major morbidity was the primary outcome. Data were analyzed using student t-test and chi-square as appropriate.
Results: A total of 92 women carrying 117 fetuses (73 singleton, 15 twin, 2 triplet, 2 quadruplet) met inclusion criteria. Only the fetus in the "ruptured sac" was studied. PPROM occurred at a mean of 20.7 (+/-2.6, range 13.6-24) weeks. The average latency period was 31.8 (+/-29.4, range 0.5-105) days, with a mean delivery gestational age of 25.2 (+/-4.3, range 15.4-34) weeks.

Gestational Age @ ROM (wks)	n	IUFD, delivered <23.0 wks, or later elected termination	Delivered ≥23.0 wks, NICU death	Delivered ≥23.0 wks, discharged home w/major morbidity*	Delivered ≥23.0 wks, no major morbidity
<17.0	8	75.0%	12.5%	12.5%	0%
17-18.9	13	53.9%	15.4%	7.7%	23.1%
19-20.9	22	27.3%	22.7%	31.8%	18.2%
21-22.9	23	30.4%	13.0%	34.9%	21.7%
23-24.0	26	0%	23.1%	46.8%	30.1%
Overall	***92***	***28.2%***	***18.5%***	***33.7%***	***19.6%***

*Grade 3 or 4 IVH, pulmonary hypoplasia, or NEC requiring surgery

Overall, 47 infants (51.1%) survived to hospital discharge; 20 (40.8% of these survivors, 21.5% of all babies) were discharged without the major morbidities evaluated. Results are similar if data from only singletons are analyzed. One mother developed sepsis following expectant management. All mothers were discharged within the first 4 postpartum days.
Conclusion: In this cohort, >50% of babies survived and were discharged home after early PPROM. Of these, 41% did not develop grade 3 or 4 IVH, pulmonary hypoplasia, or NEC requiring surgery. Outcomes improve as gestational age at the time of rupture increases. These data are valuable in counseling women with early PPROM.

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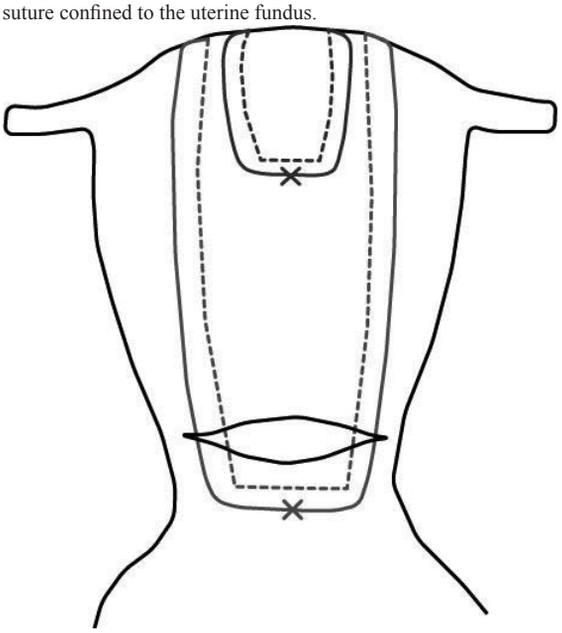
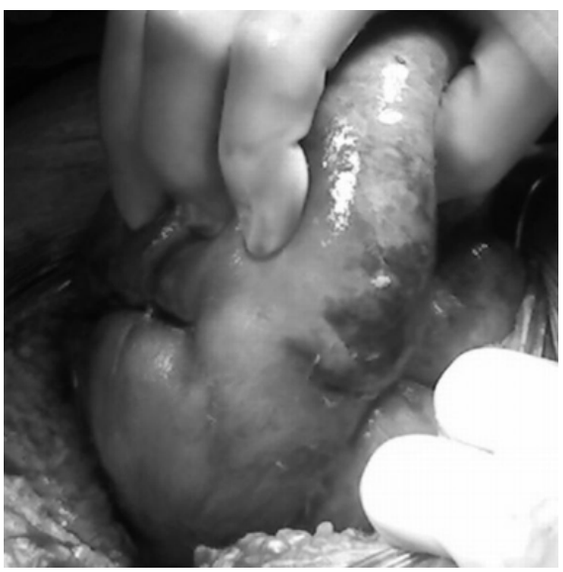
Uterine Necrosis: A Complication of Uterine Compression Sutures. Amy G Gottlieb,[1] Santosh Pandipati,[2] Karlotta M Davis,[3] Ronald S Gibbs.[1] *[1]Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, University of Colorado Health Sciences Center, Aurora, CO, USA; [2]Maternal-Fetal Medicine, Northwest Perinatal Center, Portland, OR, USA; [3]Obstetrics and Gynecology, Division of Urogynecology, University of Colorado Health Sciences Center, Aurora, CO, USA.*
This communication provides the first report of fundal uterine necrosis following placement of multiple uterine compression sutures. Only two previous published cases report occurrence of uterine necrosis following application of a uterine compression suture -- both identified as the B-Lynch technique -- and neither of these cases report necrosis confined to the fundus. In our case, uterine atony was refractory to pharmacologic therapy and manual compression of the uterus appeared to decrease the amount of blood loss. Therefore, we applied a traditional B-Lynch which effectively contracted the majority of the uterus, while the fundus remained atonic. A second horizontal, square suture was then placed between the cornua and carried over the fundus (see figure one: red reflects B-Lynch suture; blue represents second fundal, square stitch). The patient subsequently experienced significant, persistent fevers despite antibiotic therapy. A repeat laparotomy was performed, at which time we found uterine necrosis confined to the uterine fundus (see figure 2: photograph taken at the time of hysterectomy). The placenta showed no histologic evidence of chorioamniotis -- hence, the patient's main risk factor for fundal necrosis was the compression sutures. Physicians should be aware of the risk of uterine necrosis, especially after placement of multiple compression sutures and, more specifically, after placement of a compression suture confined to the uterine fundus.





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Serotonin Syndrome Is Associated with Triptan Monotherapy. Offie P Soldin,[1] Joseph M Tonning.[2] *[1]Medicine, Oncology and Physiology, Georgetown University Medical Center for the Obstetric Pharmacology Research Unit (OPRU), Washington, DC, USA; [2]Center for Drug Evaluation and Research (CDER), Division Drug Risk Evaluation, Food and Drug Administration, Silver Spring, MD, USA.*

Background: Migraines are far more common in women than in men. The incidence of migraines increases in girls after puberty, reaching an incidence of 25% in women who experience migraine at least once a year around middle age. Migraine has been postulated as one of the major risk factors for stroke during pregnancy and the puerperium.Triptans are a class of serotonin receptor agonists used in the treatment of migraine headaches. Triptans administered in combination with other drugs have been known to precipitate serotonin syndrome, a rare but potentially life-threatening condition clinically manifested by a triad consisting of mental-status changes, autonomic hyperactivity, and neuromuscular abnormalities.

Objective: To determine whether triptan monotherapy is associated with serotonin syndrome

Methods: Using data mining techniques we analyzed the entire Food and Drug Administration's (FDA) Adverse Event Reporting System (AERS) database.

Results: After excluding reports of concomitant serotonergic medication or other potentially confounding medication, eleven reports remained of serotonin syndrome associated with triptan use without concomitant serotonergic medication. The mean age for these eleven cases was 39.9 years; nine cases occurred in females and two occurred in males. There were no apparent instances of overdose among these eleven cases.

Conclusions: Serotonin syndrome is a rare but serious occurrence with the use of triptan monotherapy. Such cases were seen with eletriptan, rizatriptan, sumatriptan, and zolmitriptan. Because of the spontaneous nature of voluntary reporting to AERS, the actual number of occurrences of serotonin syndrome in patients using triptans is probably higher and cannot be assessed from AERS data. Users of triptan in combination with an SSRI or SNRI should be warned of this rare but serious adverse effect. During periods of drug initiation, dose escalation, or the addition of another serotonergic agent, patients should be particularly vigilant for symptoms of concern and seek urgent medical attention if any occur. The offending agents should be withdrawn and the patients closely monitored and treated with supportive measures as required.

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Rising Maternal Mortality in the Midst of Modern Technology. Oormila P Kovilam,[1] Jane Khoury,[2] Padmini C Sekar,[3] Ralph C Buncher.[3] *[1]Obstetrics & Gynecology, University of Cincinnati, Cincinnati, OH, USA; [2]Center for Epidemiology and Biostatistics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [3]Environmental Health, University of Cincinnatic, Cincinnati, OH, USA.*

Introduction: Maternal mortality rate (MMR) is an index of overall wellbeing of the community and safe motherhood should be given utmost priority in obstetric care. As per 1990, WHO estimates 1% of maternal mortality occurs in developed countries[1]. This rate has established without much decline in recent years. The MMR for Ohio for 1987 to 1996 was reported by CDC to be 6.3 per 100,000 live births with 95% confidence interval 5.1 to 7.6.

Objective: Our objective was to audit the trend in maternal mortality in the state of Ohio for the last 20 years.

Methods: Information from the death certificates completed by attending physicians, medical examiners, coroners, funeral directors filed with Ohio state registration offices were analyzed. We only used women who were residents of the state of Ohio at the time of death, and cause of death was coded as a "Complication of Pregnancy, Childbirth and the Puerperium", ICD9 codes 630 to 677 and ICD10 codes O0 to O9. Five year maternal mortality pattern and long term trend was assessed.

Results: The number of maternal deaths recorded as due to pregnancy complications varied over the years from a high of 20 in 1981 to a low of 4 in 1996 and 1997. In general the five-year mortality rate was decreasing over time until the last four years 2000 to 2003, when we may be seeing an upward trend compared to 1995 to 1999 (p=0.07). The rates and associated 95% confidence intervals (CI) are shown in the table below.

Years	MMR	95% CI
1980-1984	7.9	4.6-11.1
1985-1989	6.0	3.2-8.8
1990-1994	5.8	3.0-8.6
1995-1999	4.5	1.6-7.4
2000-2003	8.4	3.3-13.5

Conclusion: The rate of maternal mortality is on the rise after a period of downward trend. We should develop a multidisciplinary approach to analyze and target the root causes of maternal death.

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Women with Factor V Leiden or Prothrombin G20210A Mutation Do Not Have More Intra-Uterine Fetal Deaths with Maternal or Fetal Placental Circulation Disorders. Fleurisca J Korteweg,[1] Nienke Folkeringa,[1,4] Jan Jaap HM Erwich,[1] Jozien P Holm,[1] Albertus Timmer,[2] Nick JGM Veeger,[3] Joke M Ravise,[1] Jan van der Meer.[4] *[1]Obstetrics, University Medical Center Groningen, Groningen, Netherlands; [2]Pathology, University Medical Center Groningen, Groningen, Netherlands; [3]Epidemiology, University Medical Center Groningen, Groningen, Netherlands; [4]Hematology, University Medical Center Groningen, Groningen, Netherlands.*

Introduction. Women with thrombophilia are at higher risk of VTE during pregnancy due to the acquired hypercoagulable state. It is likely that not only maternal veins but also placental vessels are more prone to the development of thrombosis. Our objective was to compare maternal and fetal placental circulation disorders in women with intra-uterine fetal death (IUFD) and thrombophilia and women without thrombophilia.

Methods. In a Dutch multi-centre study on IUFD, during the period 2002-2007 we studied 750 singleton deaths > 20 weeks of gestation for which the diagnosis of IUFD was determined before labour. Factor V Leiden and prothrombin G20210A were tested at induction of labour. Panel classification of cause according to the Tulip classification[1] was performed by assessors after individual investigation of structured patient information. We studied the cause of death group "maternal and fetal placental circulation disorders": placenta bed pathology with abruption or infarction as origin of mechanism and placental parenchyma pathology with fetal thrombotic vasculopathy and massive perivillous fibrin deposition as origin of mechanism.

Results. Of the 689 women tested for factor V Leiden, 46 (6.7%) were carriers. Of the 261 deaths caused by "maternal and fetal placental circulation disorders" 18 (6.9%) mothers were carriers of factor V Leiden. Of the 428 deaths with another cause of death 28 (6.5%) mothers were carriers of factor V Leiden (p=0.88). Of the 691 women tested for prothrombin G20210A, 21 (3.0%) were carriers. Of the 264 deaths caused by "maternal and fetal placental circulation disorders" 9 (3.5%) mothers were carriers of prothrombin G20210A mutation. Of the 427 deaths with another cause of death 12 (2.8%) mothers were carrier of prothrombin G20210A mutation (p=0.65).

Conclusion. In women with IUFD and factor V Leiden or prothrombin G20210A mutation "maternal and fetal placental circulation disorders" did not seem to cause IUFD more often than in non-carriers.

1. Korteweg et al. BJOG 2006.

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Thrombophilia in 750 Male Partners of Women with Intra-Uterine Fetal Death. Fleurisca J Korteweg,[1] Nienke Folkeringa,[1,4] Jan Jaap HM Erwich,[1] Jozien P Holm,[1] Albertus Timmer,[2] Nick JGM Veeger,[3] Jan van der Meer.[4] *[1]Obstetrics, University Medical Center Groningen, Groningen, Netherlands; [2]Pathology, University Medical Center Groningen, Groningen, Netherlands; [3]Trial Coordinating Center, Dept of Epidemiology, University Medical Center Groningen, Groningen, Netherlands; [4]Hematology, University Medical Center Groningen, Groningen, Netherlands.*

Introduction. Growing evidence suggests that women with thrombophilic defects may be at higher risk of fetal loss. Although some paternal components to the predisposition of preeclampsia have been demonstrated it is not known whether paternal components contribute to intra-uterine fetal death (IUFD). Our objective was to investigate the relation between paternal thrombophilic defects and IUFD.

Methods. In a Dutch multi-centre study on IUFD, from 2002-2007 we studied 750 singleton deaths > 20 weeks of gestation for which the diagnosis of IUFD was determined before labour. We tested male partners of women with IUFD for antithrombin (AT), Protein C, Protein S type I and III, factor V Leiden, prothrombin G20210A (factor II) and factor VIII: Ag. Standard tests were performed in one laboratory. Normal ranges were determined in healthy male blood donors. We compared prevalence of thrombophilic defects to reference values from the literature.

Results. Of the 642 men tested for factor V Leiden, 26 (4.0%) were carrier versus 618 (96.0%) non-carriers. Prevalence of factor V Leiden in the normal population is 5% (p=0.27). 642 men were tested for prothrombin G20210A, 6 (0.9%) were carriers and 636 (99.1%) non-carriers. All were heterozygous. Prevalence of prothrombin G20210A mutation in the normal population is 3%

(p=0.002). Decreased levels of antithrombin, Protein C, Protein S type I and III and increased levels of factor VIII: Ag were observed significantly more often in male partners of women with IUFD compared to the normal population.
Conclusion. In our IUFD group the prevalence of male factor V Leiden carriers was comparable to the normal population, prevalence of prothrombin G20210A mutation was lower. The difference in other factors imply an as yet unexplained association of male thrombophilia with fetal death in their partner.

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Establishment of Morphological Abnormalities after Stillbirth Is Not a Useful Selection Criterion for Chromosomal Analysis. Jan Jaap HM Erwich,[1] Fleurisca J Korteweg,[1] Katelijne Bouman,[2] Albertus Timmer,[3] Joke Ravise,[1] Jozien P Holm.[1] *[1]Obstetrics and Gynecology, University Medical Center Groningen, Groningen, Netherlands; [2]Genetics, University Medical Center Groningen, Groningen, Netherlands; [3]Pathology, University Medical Center Groningen, Groningen, Netherlands.*
Introduction. Cause of death is unexplained in about two thirds of stillbirths, consisting of intra uterine fetal deaths (IUFD) and intrapartum deaths. Aneuploidy is an important cause of death. Uniform evidence based stillbirth protocols for chromosomal analyses are not available. Some studies advise chromosomal analyses in a selected population only due to substantial costs. One of the selection criteria is morphological abnormalities present at birth. Our objective was to determine the value of evaluation of morphological abnormalities at birth by the physician attending the delivery for an IUFD cohort.
Methods. In a Dutch multi-centre study on IUFD, during a 5 year period (2002-2007) we studied 750 singleton deaths > 20 weeks of gestation for which the diagnosis of IUFD was determined before labor. Morphological abnormalities of the fetus determined by the trainee or gynaecologist at birth were all classified by a perinatal geneticist according to the phenotypic abnormality classification by Merks et al[1].
Results. Tissue cultures were performed for 508 (67.7%) IUFD's, in 246 cases (48.4%) a karyotype was obtained. An abnormal karyotype was observed in 32 (13.0%) deaths. Of the 246 karyotypes 180 were determined post-mortem. In six of 25 IUFD's (24.0%) with an abnormal karyotype established post-mortem no morphological abnormalities were seen at birth whereas in the group with a normal karyotype (n=155) established post-mortem, morphological abnormalities were observed in 31 IUFD's (20.0%). Overall, in 139 out of 180 cases (77%) morphology matched with the karyotype, resulting in only a marginal strength of agreement, with a kappa of 0.36 (95% CI 0.21-0.51).
Conclusion. Establishment of morphological abnormalities after fetal death by the physician attending the delivery of an IUFD is not a sensitive selection criterion for performance of chromosomal analysis.
[1] Merks JH, van Karnebeek CD, Caron HN, Hennekam RC. Phenotypic abnormalities: terminology and classification. Am J Med Genet A 2003 Dec 15;123(3):211-30.

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Fetal Macrosomia and Erb's Palsy in Women with Gestational Diabetes (GDM) and in Healthy Controls. Kari Teramo, Lauri Suhonen, Risto Kaaja, Vilho Hiilesmaa. *Department of Obstetrics and Gynecology, University Central Hospital, Helsinki, Finland.*
Objective Fetal macrosomia, the most important complication of GDM, increases the risk of shoulder dystocia, Erb's palsy and intrauterine hypoxia. We compared frequencies of fetal macrosomia and Erb's palsy in two GDM cohorts with different severity of glycemic disturbance and in healthy controls.
Methods We studied 898 consecutive GDM women with singleton childbirth and 2 or 3 abnormal values in 2-h OGTT with 75 g of glucose. Abnormal plasma glucose values of the OGTT were: fasting ≥5.1, 1-h ≥10.0 and 2-h ≥8.7 mmol/l. Insulin treatment was started when ≥2 values were ≥5.5 preprandially or ≥7.8 mmol/l postprandially in the 24-h glucose profile done within 7 days of diagnosis. If the same woman had more than 1 childbirth during the study period, only the last pregnancy was included. The control group consisted of 798 women from a nearby town with singleton childbirth in the same hospital. Women with diabetes were excluded from controls. Macrosomia was defined as birth weight (BW) >2.0 SD above the mean of a standard population. Erb's palsy was diagnosed by pediatric surgeons.
Results GDM women were older, more obese, had more childbirths and more often a previous child with BW >4000g than controls. Insulin was started in 367 GDM women (41.9%). C/S rate was 18.7% in controls, 27.0% in diet-treated and 42.0% in insulin-treated GDM women. Macrosomia rate was 2.3% in controls, 4.6% in diet-treated and 18.4% in insulin-treated GDM women (p<0.001 compared with controls or diet-treated GDM women). The frequency of macrosomia did not differ between the diet-treated GDM and control women. Erb's palsy occurred in 0.3% of controls, in 1.7% of diet-treated and in 1.6% of insulin-treated GDM women. The frequency of Erb's palsy was significantly higher in both GDM groups than in controls (p=0.013). By regression analysis, previous child with BW >4000g (p<0.0001), previous C/S (p=0.0006), mother's age (p=0.012), BMI (p=0.015), insulin treatment (p=0.02) and fasting plasma glucose of the 24-h profile (p=0.027) were significant independent predictors of fetal macrosomia.
Conclusions The 24-h glucose profile done shortly after the diagnosis of GDM clearly distinguishes between low-risk (diet-treated) and high-risk (insulin-treated) GDM pregnancies for fetal macrosomia. Unfavourable fetal body composition of diet-treated GDM women is the likely explanation for the high rate of Erb's palsy in this group.

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A Maternal Atherogenic Lipid Profile Increases the Risk of Congenital Heart Defects in the Offspring. Huberdina PM Smedts,[1] Eveliene M van Uitert,[1] Olivier Valkenburg,[2] Joop SE Laven,[2] Marinus JC Eijkemans,[3] Jan Lindemans,[4] Eric AP Steegers,[1] Regine PM Steegers-Theunissen.[1,5,6,7] *[1]Obstetrics and Gynecology, Division of Obstetrics and Prenatal Medicine, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Obstetrics and Gynecology, Division of Reproductive Medicine, Erasmus MC; [3]Public Health, Erasmus MC; [4]Clinical Chemistry, Erasmus MC; [5]Epidemiology and Biostatistics, Erasmus MC; [6]Clinical Genetics, Erasmus MC; [7]Pediatric Cardiology, Erasmus MC - Sophia Children's Hospital.*
BACKGROUND: Maternal hyperglycemia and hyperhomocysteinemia in early pregnancy contribute to the development of congenital heart defects (CHD). In adult life both metabolic derangements also increase the risk of atherosclerosis. Deranged lipid levels are strong determinants for cardiovascular diseases.
OBJECTIVE: To investigate whether derangements in the maternal lipid profile are also associated with CHD in the offspring.
METHODS: At around 16 months after delivery of the index-child a case–control study was conducted among 261 mothers of a child with CHD, 179 outflow tract defects (OTD), 82 non-outflow tract defects (non-OTD), and 325 control mothers of a non-malformed child. Pregnant or lactating mothers or those who changed their diet compared to the periconception diet were excluded from analysis. Primary outcome parameters were total cholesterol (C), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglycerides (TG), Apolipoprotein B (ApoB) and Apolipoprotein A-I (ApoA-I). Biomarkers (geometric means) were compared by the Student's *T*-test. Risk estimates were adjusted for age, diabetes, BMI, and periconception folic acid containing supplement use.
RESULTS: Case-mothers showed higher concentrations of C, 4.9 vs. 4.7 mmol/L; $P<0.05$, LDL-C, 3.2 vs. 3.0 mmol/L; $P<0.05$), and ApoB, 84.0 vs. 80.0 mg/dL; $P<0.01$, compared with controls. HDL-C, ApoA-I and TG were comparable between the groups. CHD risk increased with a rising ApoB concentration ($P= 0.001$). ApoB concentrations above 85.0 mg/dL almost two-fold increased CHD risk, OR 1.8 (95% CI: 1.3 to 2.6), and resulted in a population-attributable risk of 21.1%.
CONCLUSIONS: A more atherogenic maternal lipid profile significantly contributes to the risk of CHD offspring. Thus, having CHD offspring may also be an early predictor for the mother to develop adult (cardio) vascular disease.

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Higher Rates of Maternal Complications in Twin Pregnancies. Ngoc T Phan, Sarah E Little, Yvonne W Cheng, Aaron Caughey. *Obstetrics and Gynecology, UCSF, San Francisco, CA, USA.*
OBJECTIVE: To compare maternal outcomes in women with twin versus singleton pregnancies.
STUDY DESIGN: This is a retrospective cohort study of all singleton and twin pregnancies who received prenatal care and delivered at a single institution. Maternal outcomes were compared between twin and singleton gestations. Bivariate comparisons were made using the chi-square test and confounding variables were controlled using multivariate logistic regression.
RESULTS: With the exception of chorioamnionitis, women with twin gestation had a significantly higher rate of *all* maternal complications examined in this study.

OUTCOMES	Twin (n=841)	Singleton (n = 22,262)	*P* value
Cesarean delivery	44%	18%	<0.001
Preeclampsia	17%	5%	<0.001
Gestational diabetes	6%	4%	0.01
Placenta previa	1.4%	0.7%	0.01
Placental abruption	3%	1%	<0.001
Postpartum hemorrhage	58%	22%	<0.001
Transfusion	7%	0.9%	<0.001
Chorioamnionitis	7%	9%	0.14
Endomyometritis	6%	3%	<0.001

CONCLUSION: Women experiencing twin gestations have higher rates of morbidity and perinatal complications than women with singleton gestations. This evidence should be used to counsel women considering assisted reproductive technologies or selective reduction of multiple gestations. Additionally, these data can be used when establishing labor expectations in patients with twin gestations.

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Perinatal Outcomes of Twin Pregnancies: Differences by Race/Ethnicity. Ngoc T Phan, Yvonne W Cheng, Allison S Bryant, Aaron Caughey. *Obstetrics and Gynecology, UCSF, San Francisco, CA, USA.*

OBJECTIVE: To compare the perinatal outcomes in twin gestations by race/ethnicity.

STUDY DESIGN: This is a retrospective cohort study of 798 twin pregnancies delivered at a single institution. Perinatal outcomes of twin pregnancies were compared between women of Asian, Black, Latina and White race/ethnicity.

RESULTS: The rates of gestational diabetes, placental abruption and preterm delivery were significantly different between the groups. Racial/ethnic differences were not observed for other maternal or neonatal complications in patients with twin gestation.

MATERNAL OUTCOMES	White	African American	Latina	Asian	*P*-Value
Cesarean delivery	46%	46%	45%	41%	0.763
Preeclampsia	18%	15%	26%	13%	0.058
Gestational Diabetes	4%	3%	10%	10%	<0.05
Placenta previa	1%	3%	0%	3%	0.195
Placental abruption	3%	7%	0%	4%	<0.05
Preterm delivery (<32 wks)	15%	25%	11%	14%	<0.05
Low birth weight (<2500g)	51%	56%	58%	47%	0.264

CONCLUSION: Women of different race/ethnicity experience different rates of complications in the settings of twin gestation for some perinatal outcomes. Specific racial/ethnic counseling regarding perinatal outcomes should be considered in the setting of a twin gestation.

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Impact of Fetal Gender on Pregnancy Complications and Neonatal Outcome. Barbra de Vrijer,[1] Mesfer Al-Shahrani,[1] Debbie Penava,[1] Orlando Da Silva,[2] Bryan S Richardson.[1] *[1]Obstetrics and Gynaecology, University of Western Ontario, London, ON, Canada; [2]Pediatrics, University of Western Ontario, London, ON, Canada.*

OBJECTIVE: To determine the impact of fetal gender on pregnancy complications and neonatal outcome.

M**ETHODS:** Hospital-based cohort study of singleton births ≥25 weeks without major congenital anomalies at a regional tertiary care perinatal center from January 1, 1996, to December 31, 2005 (n= 16657 male and 15792 female infants). Logistic regression was performed to determine reasons for caesarean section at term and neonatal outcome.

RESULTS: Term male newborns had a 5-minute Apgar score <7 more frequently (M; 1.2% vs. F; 0.9%, p<0.05), were more likely to be admitted to the NICU (M; 7.7% vs. F; 5.5%, p<0.001) and had higher incidences of RDS and need for mechanical ventilation. Term pregnancies with male fetuses were more likely to have PROM, intrapartum fever and receive intrapartum antibiotics. A woman with severe pre-eclampsia/eclampsia between 29-33 weeks or placenta previa at 33-36 weeks had a greater likelyhood of carrying a female fetus (M; 11.6% vs. F;18.5%, p<0.05 and M; 2.7% vs. F; 4.5%, p<0.05 respectively). Males displayed higher rates of preterm delivery (M; 10.9% vs. F; 9.3%, p<0.001) and very preterm males (25-28 weeks) had higher incidences of proven sepsis. Birth and placental weights were similar in the very preterm group, but higher in males in all later GA groups. Males had higher CS rates (M; 20.6% vs. F; 18.1%, p<0.001) secondary to a higher incidence of term CS (M; 19.1% vs. F; 16.7%, p<0.001). In this group, more CS were performed in women pregnant with male fetuses for failure to progress and fetal distress (M; 43.5% vs. F; 37.5%, p<0.01 and M; 21.9% vs. F; 17.5%, p<0.01, respectively). SGA, LGA, birth weight >4000g and chorioamnionitis were independently associated with CS, but only birth weight >4000g was independently associated with male fetuses delivered by CS (OR 1.91, 95% CI 1.78, 2.04, p<0.001).

CONCLUSION: Women pregnant with male fetuses are at increased risk for pregnancy complications such as preterm delivery, intrapartum fever and caesarean section for FTP, likely secondary to birthweight >4000g. Only minor differences were determined in neonatal complications in the preterm groups; only neonatal sepsis showed an independent association with male gender. However, term male infants were more likely to have Apgar scores <7, be admitted to the NICU, develop RDS and need mechanical ventilation.

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Predictors of Fetal Growth Restriction and Preterm Birth in Pregnant Adolescents. Simon J Wheeler,[1] Lucilla Poston,[1] Cindy J Hutchinson,[1] Annette Briley,[1] Tom A Sanders,[2] Jane Thomas,[2] Rebecca L Jones,[3] Philip N Baker.[3] *[1]Maternal & Fetal Research Unit, St Thomas's Hospital, King's College London, London, United Kingdom; [2]Nutritional Sciences Division, King's College London, London, United Kingdom; [3]Maternal & Fetal Health Research Centre, University of Manchester, St. Mary's Hospital, Manchester, United Kingdom.*

Background: Pregnancy during adolescence has been consistently shown to carry a higher risk of small-for-gestational age (SGA) and preterm birth (PTB). Clinicians would benefit from specific predictors of these pregnancy outcomes in order to provide an appropriate level of maternity care in the most cost-effective manner.

Methods: In a longitudinal study, 500 pregnant adolescents were comprehensively assessed from booking to parturition, for growth, socioeconomic and nutritional parameters. Blood samples, collected at 28-32 weeks' gestation, were assayed for a wide range of nutritional biomarkers. Logistic regression was used to determine significant associations between studied variables and pregnancy outcomes. Sensitivity, specificity, positive and negative predictive values, and likelihood ratios were calculated and thresholds were tested to find the values most appropriate for clinical use.

Results: Of the 478 infants for whom birthweight centiles could be calculated, 84 (17.6%) were born SGA and 43 (9.0%) were preterm. Six variables were associated with SGA birth: body mass index <19.0kg/m2 (p=0.002) or >30kg/m2 at booking (p=0.032), low gestational weight gain until 28-32 weeks (p<0.0001), low red cell folate (p=0.001), low plasma LDL cholesterol (p=0.003), and maternal smoking (p=0.001). Five variables were significantly associated with PTB: maternal booking weight <51kg (p=0.013), high serum homocysteine (p=0.012), high plasma LDL cholesterol (p=0.012), low serum leptin (p=0.024) and maternal receipt of benefits (p=0.016).

Conclusion: The predictors identified in this study are simple and widely available. They appear to be most useful when found together and when associations with outcomes are non-linear. This was particularly true for SGA-associated variables. The efficacy of the variables as independent predictors of poor pregnancy outcome needs to be confirmed in a larger cohort before these finding can be applied to antenatal care. However, these data suggest that many poor outcomes in adolescent pregnancies can be predicted, and thus potentially mitigated, by the beginning of the third trimester.

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Is Uterine Artery Doppler Velocimetry Influenced by Prior Poor Obstetric History? Kimberly W Hickey,[1] Yesmean Hassan,[2] Sarah H Poggi,[3] Alessandro Ghidini.[3] *[1]Maternal Fetal Medicine, Georgetown University Hospital, Washington, DC, USA; [2]Georgetown University Medical School, Washington, DC, USA; [3]Perinatal Diagnostic Center, Inova Alexandria Hospital, Alexandria, VA, USA.*

Background: Abnormal first trimester uterine artery (UtA) Doppler velocimetry has been correlated with adverse pregnancy outcomes related to poor placentation including fetal growth restriction, fetal demise, and hypertensive disorders of pregnancy. While parity, in general, is known to favorably influence UtA Doppler indices, it is unclear if a prior poor obstetric outcome will influence UtA Doppler indices early in a subsequent pregnancy.

Study Design: Multiparous women undergoing first trimester screening of singleton pregnancies were enrolled into a cases (prior poor pregnancy outcome; n=22) vs. controls (normal prior pregnancy outcome; n=22). Poor pregnancy outcome was defined as: preeclampsia, fetal growth restriction, abruption, or fetal demise and did not include spontaneous preterm deliveries. Demographic, clinical, and sonographic data (including UtA indices and assessment of notching) were obtained. Statistical analysis included Student's t test and Chi Square.

Results: The two cohorts were not significantly different in terms of maternal age, ethnicity, race and medical history. Prior adverse obstetric outcome was signficantly associated with recurrence of poor obstetric outcome (0% vs. 50 %;

p=0.001). First trimester UtA Doppler indices were similar in the two cohorts in terms of the resistance and pulsatility indices (RI 0.63 vs. 0.67, p=0.39; PI 1.29 vs. 1.44, p=0.42). However, bilateral notching was much more common in the cohort of prior adverse outcomes (22% vs. 45% p=0.05) as well as in patients destined to have a subsequent poor outcome (p=0.001).

Conclusions: Not surprisingly, prior poor obstetric outcome was strongly associated with recurrent adverse obstetric outcome. UtA notching was robustly associated with prior poor obstetric history as well as a recurrent poor outcome. This clinical history had no discernable influence on RI or PI. Reassurance may not be offered based on first trimester UtA RI and PI.

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Anti-Retroviral Therapy Is Associated with Increased Blood Loss and Uterine Atony for Patients Undergoing Primary Cesarean Section. Carey Eppes, Alice Cootauco, Melissa Russo, Jessica Bienstock. *Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA.*

Objective:

Anti-retroviral therapy has been associated with gastrointestinal smooth muscle dysfunction. It has also been hypothesized that myopathies can occur secondary to anti-retroviral therapy due to mitchondrial alterations. In our experience, we have noticed an increased incidence of uterine atony in our HIV patients on anti-retroviral therapy. We sought to examine the incidence of postpartum hemorrhage and uterine atony in patients currently on anti-retroviral therapy.

Study Design:

A retrospective case-controlled study was conducted on all HIV positive pregnant women on anti-retroviral therapy undergoing a primary low segment transverse cesarean section from 1996 through 2007. These patients were obtained from an IRB approved database containing HIV positive patients within our institution. Patients' medical records were abstracted for demographic data, use of uterotonics, preoperative and postoperative hematocrits, and incidence of blood transfusions. Controls were matched for age, parity, gestational age and surgical indication. Data was analyzed using the t-test, Fischer's exact test and chi square.

Results:

There were no differences in demographics, incidence of chorioamnionitis or magnesium sulfate use between groups. Patients on anti-retroviral therapy had a statistically greater decrease in hematocrit and estimated blood loss compared to controls. They also had an increased need for uterotonics and blood transfusions.

Conclusion:

Anti-retroviral therapy may impact uterine smooth muscle contractility in pregnancy, as evident by the increased incidence of uterine atony and change in hematocrit. Additional research is needed to elucidate the mechanism. Clinicians should be aware of the potential for uterine atony and excessive blood loss in patients on anti-retroviral therapy.

Demographics and outcomes

	Anti-retrovirals	Control	P Value
Maternal Age (years)	28	28	0.87
Median parity (range)	1 (0-7)	1 (0-8)	
Gestational Age (weeks)	37.4	37.7	0.56
Fetal Weight (grams)	2834	3012	0.12
Estimated Blood Loss (cc)	**866**	**779**	**0.025**
Hematocrit Decrease	**5.7**	**4.7**	**0.038**
Uterotonics (%)	**13%**	**0.5%**	**0.036**
Transfusions (%)	**9.3%**	**0.7%**	**0.002**
Chorioamnionitis (%)	0.5%	0.7%	0.58
Magnesium Sulfate (%)	9.3%	9.9%	0.88

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Relationships between Nutritional Status and Pregnancy Outcomes in Pregnant Adolescents: The About Teenage Eating (ATE) Study. Lucilla Poston,[1] Simon J Wheeler,[2] Cindy Hutchinson,[1] Annette Briley,[1] Tom A Sanders,[2] Jane Thomas,[2] Rebecca L Jones,[3] Philip Baker.[3] *[1]Maternal & Fetal Research Unit, St Thomas's Hospital, King's College London, United Kingdom; [2]Nutritional Sciences Division, King's College London, United Kingdom; [3]Maternal & Fetal Health Research Centre, University of Manchester, St. Mary's Hospital, Manchester, United Kingdom.*

Background: Adolescent pregnancy is frequently associated with adverse outcomes, especially small-for-gestational age (SGA) deliveries. Some studies have implicated maternal nutritional status in these poor outcomes.

Methods: In a prospective longitudinal study, 500 ethnically-diverse, pregnant adolescents were studied from booking to parturition, with collection of anthropometric and nutritional variables. Blood samples (28-32 weeks' gestation; n=305 subjects) were assayed for a spectrum of nutritional biomarkers. Logistic regression was used to determine significant associations between studied variables and pregnancy outcomes.

Results: Median age at recruitment was 17.8 years (IQR:17.1-18.4). Outcome data was available for 478 subjects. 214 (44.8%) had uncomplicated pregnancies. Median birthweight was 3,200g (IQR: 2,831-3,530g) and median birthweight centile was 37.8% (IQR: 14.3-64.2%). 84 (17.6%) infants were born SGA and 43 (9.0%) were preterm. Spontaneous vaginal deliveries occurred in 71.0% of cases. There were 14 (2.9%) cases of pre-eclampsia and 34 (7.1%) admissions to neonatal care. 31.9% of subjects reported smoking at booking. Iron deficiency anaemia was prevalent in 49.4% of subjects by 28-32 weeks and was strongly associated with higher infant birthweight centile (p<0.0001). 48.4% had serum 25-hydroxy vitamin D concentrations <15ng/ml, although this was not associated with any outcomes. Low folate status, as indicated by low red cell folate (p=0.001), low serum folate (p=0.032) and high serum homocysteine (p=0.027) concentrations, was associated with higher rates of SGA birth.

Conclusion: Adolescent pregnancy in inner-city populations is associated with a high risk of SGA and preterm birth, increasing the likelihood of health problems in later life and perpetuating social disparities in health. The association between anaemia and higher birthweight is unexplained. Impaired maternal folate status may contribute to impaired fetal growth. We suggest that SGA birth in this population could be reduced by the use of antenatal supplements or the mandatory fortification of flour with folic acid.

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Association of Adequacy of Prenatal Care Utilization and Maternal Health Status with Birth Outcomes in North Carolina, 1999-2003. Lisa M Kaiser,[1] Marie Lynn Miranda,[2] Jon Hussey,[3] Paul Buescher,[3] Dohyeong Kim,[2] Geeta K Swamy.[1] *[1]Department of Obstetrics and Gynecology, Duke University School of Medicine, Durham, NC, USA; [2]Children's Environmental Health Institute, Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC, USA; [3]Department of Maternal and Child Health, School of Public Health, University of North Carolina, Chapel Hill, Chapel Hill, NC, USA.*

Objective: Studies have shown that inadequate prenatal care (PNC) is associated with increased risk of negative birth outcomes in pregnant women. Adequacy of PNC may be particularly important for women with chronic health conditions. Therefore, we examined the association between adequacy of prenatal care and birth outcomes in women with and without preexisting health conditions.

Methods: Following Duke University IRB approval, a retrospective cohort study of the North Carolina Detailed Birth Records from 1999-2003 was conducted (N=546,673 singleton births). The odds of negative birth outcomes associated with adequacy of prenatal care were calculated for each maternal health group using multivariate logistic regression with adjustment for confounding factors. The outcomes studied were low birth weight (<2500g), preterm birth (<37 weeks), and infant mortality. The Adequacy of Prenatal Care Utilization Index (APNCU) was used to assess levels of prenatal care utilization.

Results: Increased odds of low birth weight, preterm birth, and infant mortality were associated with women receiving inadequate or adequate plus prenatal care. Increased odds of adverse birth outcome were also found in the intermediate care category, but to a lesser degree. This association was observed in both women without chronic health conditions and those with pre-existing health problems, while controlling for other risk factors.

Conclusion: Inadequate utilization and intensive utilization of prenatal care are associated with increased odds of adverse birth outcomes, regardless of maternal health status. The association between inadequate prenatal care and negative birth outcome may be mediated directly by the effects of prenatal care or indirectly by other characteristics of the subset of women who do not adequately utilize prenatal care. Intensive utilization of prenatal care may confer increased odds of adverse birth outcomes due to the association of women with high-risk pregnancies seeking increased amounts of prenatal care.

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Cigarette Smoking: Does Quitting during Pregnancy Improve Perinatal Outcomes? Yvonne W Cheng, Sanai Nakagawa, Aaron B Caughey. *Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA.*

Objective: To examine whether quitting cigarette smoking during pregnancy influences maternal and neonatal outcomes.

Methods: This is a retrospective cohort study of women with singleton pregnancy who delivered in the United States in 2003. Smoking status and

amount of cigarette use pre-pregnancy and during pregnancy were self-reported and subgrouped into nonsmokers, quitting during pregnancy, and continued smoking throughout pregnancy. Categorical outcomes were compared using chi-square test. Multivariable logistic regression analyses were used to control for potential confounders (continued smoking compared to quitting during pregnancy).

Results: Women who quit smoking during pregnancy had higher rates of pregnancy-associated hypertension and cesarean delivery but lower rates of preterm delivery and neonatal birthweight <2500gm compared to non-smokers. Compared to women who quit smoking during pregnancy, those who continue to smoke have lower odds of pregnancy-associated hypertension and cesarean delivery, but higher odds of preterm delivery, neonatal birthweight <2500gm, and Apgar score <7 at 5 minutes (see Table)

Conclusion: Quitting cigarette smoking during pregnancy appears to reduce undesirable neonatal outcomes, though an increase in pregnancy associated hypertension. These findings should be emphasized to women who are smoking during pregnancy.

Perinatal Outcomes Associated with Cigarette Smoking Status During Pregnancy

	Quit Smoking (n=16,164)	Cont Smoking (n=27,329)	aOR 95% CI
Preg Assoc HTN	5.08 %	3.32 %	0.76 0.69-0.84
Cesarean	26.20 %	24.25 %	0.93 0.88-0.97
PTD <37 wks	10.35 %	12.52 %	1.17 1.09-1.25
Birthweight <2500g	6.49 %	9.83 %	1.78 1.62-1.96
5min Apgar <7	2.31 %	2.37%	1.17 1.01-1.35

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Intrauterine Pressure Measurements after Balloon Tamponade for Postpartum Hemorrhage. Victor Dabelea,[1] Peter M Schultze,[1,2] Robert S McDuffie.[1,2] *[1]Obstetrics and Gynecology, Exempla Saint Joseph Hospital, Denver, CO, USA; [2]Obstetrics and Gynecology, Kaiser Permanente, Denver, CO, USA.*

Intrauterine balloon tamponade has been demonstrated to control severe postpartum hemorrhage. Our hypothesis is that control is dependent on achieving intrauterine pressure sufficient to control venous rather then arterial bleeding. The purpose of this study is to assess pressures achieved in cases of postpartum hemorrhage after balloon tamponade.

Methods. From Aug 2006 to Aug 2007, intrauterine pressures after balloon tamponade were measured in 18 patients with severe postpartum hemorrhage. To achieve effective tamponade the balloon was inflated initially up to 150-200ml with incremental increases of volume by 50 ml until bleeding stops. To measure intrauterine balloon pressures we used a standard pressure transducer (Edwards Lifesciences) connected to the balloon port and to a pressure monitor (GE Dash 3000). The pressure transducer was mounted on the bedrail at uterine level, primed with sterile saline and then connected to the balloon port of the catheter. The system was zeroed to atmospheric pressure, the stopcock of the intrauterine balloon port was opened and the pressure was recorded (P1: the total pressure on the fluid). To verify the accuracy of measurements we used another balloon catheter (reference), inflated with the same amount of saline, connected to the pressure transducer and zeroed to atmospheric pressure. The reference balloon measured P2 (the intrinsic balloon pressure caused by distension at that volume). The system was then zeroed to the reference balloon. The pressure transducer was then reconnected with the intrauterine balloon and the actual intrauterine pressure was recorded (P3). We calculated the correlation coefficient between P3 and systolic, diastolic and mean blood pressure using a linear regression (StatsDirect statistical software).

Results. Recorded intrauterine pressure (P3) ranged from 17 to 40 mmHg (mean 27.9). P3 closely approximated the difference between P1 and P2. No significant correlation was found between P3 and systolic blood pressure (r=0.39, p= 0.1), diastolic blood pressure (r=0.03, p=0.8) or mean blood pressure (r=0.23, p=0.3).

Conclusion: The effective intrauterine pressure necessary to stop hemorrhage does not significantly correlate with blood pressure. The amount of pressure necessary to stop bleeding is consistent with tamponade of venous bleeding.

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Prediction of Neonatal Hypoxic Ischaemic Encephalopathy from Umbilical Artery Blood Gas Analysis at Delivery. Christopher R White,[1] Dorota A Doherty,[2] Roland Kohan,[3] John P Newnham,[1] Craig E Pennell.[1] *[1]School of Women's and Infants' Health, The University of Western Australia, Perth, Western Australia, Australia; [2]Women and Infants Research Foundation, King Edward Memorial Hospital, Perth, Western Australia, Australia; [3]Department of Neonatal Paediatrics, King Edward Memorial Hospital, Perth, Western Australia, Australia.*

Objective:

There is a growing body of evidence to suggest that peripartum assessment of fetal or neonatal lactate levels are as good as or better than standard blood gas analysis in the prediction of neonatal outcome. In this study we have evaluated the ability of umbilical cord blood gases and lactate levels in the prediction of neonatal hypoxic-ischaemic encephalopathy (HIE).

Results:

Over the study period 2003-2006 there were 42 cases of HIE from the 19,426 deliveries at KEMH (2.2 per 1000). There was no significant variation in the annual rate of HIE over the study period (p=0.689). Cord arterial blood gases and lactate levels were available for 35 of the 42 cases of HIE. Twenty-five percent of cases of HIE had cord blood gases within the normal range suggesting a hypoxic-ischemic insult several hour prior to the delivery. Cord arterial pH, base excess and lactate were all excellent predictors of HIE with areas under receiver operator curves exceeding 0.9 (see Table). Cord lactate levels had the highest sensitivity at predicting HIE of the biochemical markers of fetal acidosis.

Conclusion:

Arterial pH, base excess and lactate are all effective predictors of HIE. Together with other compelling evidence in the literature[i], these data support the routine use of cord arterial and venous gases at all births. Further, these data emphasise the potential role that lactate may play in smaller centres where lactate samples can be obtained on all deliveries at 100th the cost of standard blood gas analysis.

[i] MacLennan, A. (1999). A template for defining a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. *BMJ* **319**, 1054-1059.

ROC Curves of Umbilical Artery Values HIE Cases

	Area Under Curve (95% CI)	Optimal Cut-Off	Sensitivity	Specificity
pH	**0.906** (0.854-0.958)	7.180	80.00%	88.05%
pO_2	0.629 (0.513-0.745)	10.65	44.83%	84.54%
pCO_2	0.814 (0.725-0.903)	77.25	53.33%	97.37%
Base Excess	**0.925** (0.882-0.968)	-9.15	82.35%	90.85%
Lactate	**0.917** (0.861-0.972)	4.79	93.33%	79.12%

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Detection of Enlarged Yolk Sac on Early Ultrasound Is Associated with Adverse Pregnancy Outcomes. Danielle M Berdahl,[1] Jill Blaine,[1] Bradley Van Voorhis,[1] Dokras Anuja.[2] *[1]Obstetrics and Gynecology, University of Iowa, Iowa City, IA, USA; [2]Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA.*

Objective

The outcomes of pregnancies associated with enlarged yolk sac diameter are currently unclear. Our study was designed to determine if enlarged yolk sac diameter on early ultrasound was independently associated with adverse pregnancy outcomes in early and late gestation.

Methods

Retrospective study from 2002-06 at University of Iowa identifying all transvaginal ultrasounds (TVUS) with YS ≥5 mm (cases, n=92) and YS <5 mm (controls, n=105) matched for maternal age. Demographic information, maternal BMI, smoking status, previous pregnancy outcomes, history of hypertension, diabetes and fertility treatments was obtained. Pregnancy outcomes including miscarriage, obstetric and fetal complications were obtained. Pregnancies with higher order multiples were excluded. Results were analyzed using the chi square test, Wilcoxon rank-sum test and Fisher's exact test to compare variables in the 2 groups. Logistic regression analysis was used for miscarriage and PTD.

Results

TVUS was performed between 5-12.5 weeks gestation. Table shows differences in demographics and medical history between the 2 groups. Pregnancies with YS size ≥5 mm had an increased risk of first trimester loss of 32% vs. 13% in controls, OR 3.18 (CI 1.51-6.68, p=0.002) after regression analysis with above factors. Age ≥36 years was also independently associated with risk of miscarriage OR 4.25 (CI 1.8-10.1, p<0.001). When pregnancy survived the

first trimester, an increased risk of preterm delivery was noted in the ≥5mm YS diameter group 29% (18) vs. controls 11% (10) , OR of 3.28 (CI 1.3-8.27, p=0.012). The prevalence of twin gestation, preeclampsia, cesarean section and birth defects was similar in both groups.

Conclusion

Pregnancies with YS size ≥5 mm may require close monitoring and counselling about the increased risk for first trimester loss and preterm delivery. Further research is necessary to elicit mechanism for PTD risk.

Pre-clinical variables

Variables	YS Diameter ≥ 5 (n=92)	YSdiameter <5 (n=105)
Age, mean (SD)	30.3±6.4	30.5±6.2
BMI median (25th-75th)	24.8 (21-30)	24.9 (22-31)
gravidity	0.83	1.83
≥2 spontaneous abortions	13 (14%)	19 (19%)
In vitro fertilization	23 (25%)	23 (22%)
Current smoker	4 (3%)	12 (10%)
Hypertension	3 (3%)	5 (5%)
Diabetes	4 (4%)	2 (2%)
PCOS	8 (10%)	4 (4%)
YS diameter	5.87	3.54

p<0.02

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Universal Umbilical Cord Sampling Is Associated with Improved Perinatal Outcome. Christopher R White,[1] Dorota A Doherty,[3] Roland Kohan,[2] John P Newnham,[1] Craig E Pennell.[1] *[1]School of Women's and Infants' Health, The University of Western Australia, Perth, Western Australia, Australia; [2]Department of Neonatal Paediatrics, King Edward Memorial Hospital, Perth, Western Australia, Australia; [3]Women and Infants Research Foundation, King Edward Memorial Hospital, Perth, Western Australia, Australia.*

Objective:

Current evidence suggests that umbilical cord pH at delivery provides the most sensitive reflection of birth asphyxia. Paired umbilical artery/vein blood gases have been routinely collected at King Edward Memorial Hospital over the last 5 years. The objective of this study was to determine: local reference ranges; accuracy of sampling; and the rates of metabolic acidosis.

Results:

Of the 19,426 births (2003-2006), accurate paired results were available on 64% of births. Over the study period there was a progressive improvements in accuracy rates of paired sampling (p<0.05). The median (2.5^{th}, 97.5^{th} centile) values for cord arterial blood gases were: pH 7.27 (7.08,7.37); pO2 16.3mmHg (4.6,31.0); pCO2 55.1mmHg (39.0,79.0); base excess -3.0 (-10.8, 2.7); and lactate 3.7mmol/L (1.8,7.8). There was a progressive improvement in all blood gas measures over the 4 years of this study (all p<0.05). Moreover, there were significant reductions in all measures of metabolic acidosis (see Table). The progressive improvement in the measures of metabolic acidosis remained significant after multivariate analysis including obstetric, fetal, and demographic factors associated with metabolic acidosis.

Conclusion:

The introduction of universal umbilical cord blood gas analysis to all births is associated with significant improvements in all markers of metabolic acidosis. Together with other compelling evidence in the literature, these data support the routine use of cord arterial and venous gases at all births; however, improved accuracy rates on paired sampling requires an ongoing education program

Umbilical Artery Indicators of Acidosis, Percentage of Total

	pH < 7.0	pH < 5th Centile*	Base Excess < 5th Centile*	Lactate > 95th Centile*
2003	0.62%	5.06%	5.06%	8.27%
2004	0.58%	5.58%	5.93%	5.99%
2005	0.82%	4.36%	5.22%	2.81%
2006	0.52%	4.08%	3.82%	3.31%
2003-06 Reduction	16%	27%	25%	60%

* 5th, 95th centile was calculated on all accurate paired samples with pH 5th centile 7.12; base excess 5th centile -9.3; lactate 95th centile 6.7mmol/L

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Duration of Intrapartum Group B Streptococcal (GBS) Prophylaxis and Concentration of Penicillin G (PCN) in Fetal Serum at Delivery. Emma L Barber, Guomao Zhao, Irina A Buhimschi, Jessica L Illuzzi. *Obstetrics and Gynecology, Yale University, New Haven, CT, USA.*

OBJECTIVE: Intrapartum PCN prophylaxis aims to prevent early-onset GBS sepsis by interrupting vertical transmission from colonized mothers to their newborns. However, despite its wide clinical use, systematic pharmacokinetic evidence in support of the current PCN dosage regimen is lacking. Current CDC guidelines recommend intensified surveillance and testing of infants exposed to <4h of prophylaxis. Our goal was to examine the relationship between maternal time of exposure to PCN and fetal serum PCN levels among maternal-fetal dyads exposed to short durations of PCN prophylaxis (<4h) compared to those exposed to longer durations.

STUDY DESIGN: Ninety-eight laboring GBS positive women were administered 5 million units (MU) of intravenous PCN to be followed by 2.5 MU every 4 hours until delivery (CDC 2002). Subjects with renal disease, multiple gestation, and preterm delivery (<37wks) were excluded. Umbilical cord blood samples were collected at delivery and PCN levels measured by high-performance liquid chromatography. Intra and inter-assay coefficients of variation were <3%.

RESULTS: The PCN concentrations (mean±SD) by duration of prophylaxis were: <1 h, 11.6±4.5µg/ml (n=10); 1-2h, 9.7±3.4µg/ml (n=15); 2-3h, 6.6±3.8µg/ml (n=15);3-4h, 3.6±1.8 µg/ml (n=17);4-8h, 6.9±3.7 µg/ml (n=11);>8h 4.1±2.6 µg/ml (n=24); and for those without a second dose after 4h, 2.3±0.9 µg/ml (n=6). Fetuses exposed to short duration (<4h) had higher levels of PCN than those exposed to >4 h (p=0.003). In multivariable linear regression analysis, fetal PCN levels were determined by total duration of exposure, time since last dose, dosage, and number of doses, but not maternal BMI. PCN levels in cord serum increased linearly until 1 hour; thereafter, they decreased rapidly, but all groups were significantly above the minimum inhibitory concentration (MIC) for GBS (0.1 µg/mL)(p<0.002). Furthermore, every sample individually remained 10-179 fold above the MIC.

CONCLUSION: In this study, even short durations of prophylaxis achieved levels above the MIC, suggesting a benefit to prophylaxis even in precipitous labors. The data also suggests that the current CDC designation of infants exposed to <4h of PCN prophylaxis as particularly at risk for GBS sepsis may be inaccurate from a pharmacokinetic standpoint.

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Placental Release of 3-Hydroxy Anthranilic Acid (3-OH AA) as a Marker for Oxidative Stress Is Increased with Labour. Ariadna Grigorou,[1] Udani Ratanayake,[2] Lisa Hutton,[2] David Walker,[2] Bryan S Richardson.[1] *[1]Obstetrics & Gynaecology, University of Western Ontario, London, Canada; [2]Physiology, School of Biomedical Sciences, Monash University, Melbourne, Australia.*

Objective: 3-OH AA is a metabolite of tryptophan with pro-oxidant and pro-apoptotic properties and has been shown to increase in umbilical cord blood in pregnancies with intra-uterine infection. Since labour-related events may also activate inflammatory pathways, we sought to determine the placental release of 3-OH AA into the umbilical circulation in labouring vs non-labouring patients at term.

Methods: Twenty-six patients were studied (term labour n=18, and term elective cesarean section n=8) with blood sampling from a clamped segment of umbilical cord after delivery of the fetus and from the cord at its insertion into the placenta after delivery of the placenta, with subsequent measurement of blood gases/pH and 3-OH AA (isocratic HPLC using fluorometric detection with assay sensitivity at 1 pmol).

Results: 3-OH AA measurements from respective umbilical and placental cord vessels were all variably higher in the labouring group vs the elective cesarean group patients (Table 1). For labouring group patients, the 3-OH AA levels from the umbilical vein were significantly higher than those from the umbilical artery, indicating net release from the placenta into the fetal circulation. Placental vein levels were also significantly higher than those from the umbilical vein, indicating continued placental release of 3-OH AA into the cord blood after delivery of the fetus.

Conclusion: Labour at term is associated with changes in the placental metabolism of tryptophan resulting in the increased release of 3-OH AA into the fetal circulation with the potential for pro-oxidative and apoptotic effects in many tissues, including the brain.

Table 1. 3-OH AA plasma measurements (uM)

	Umbilical			Placental			Umbilical vs Placental
	Vein	P	Artery	Vein	P	Artery	Vein
Labour	0.86±0.12	**	0.56±0.08	1.53±0.23	ns	1.03±0.22	**
P	ns		ns	**		*	
CSx	0.67±0.12	ns	0.41±0.16	0.69±0.16	ns	0.48±0.15	ns

Data presented as means ±SEM; *p<.05, **p<.02, ns=not significant

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Is Ischemic Placental Disease a Maternal or Fetal Syndrome? Cande V Ananth,[1] John C Smulian,[1] Anthony M Vintzileos.[2] *[1]Department of Obstetrics, Gynecology, and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA; [2]Department of Obstetrics and Gynecology, Winthrop-University Hospital, Mineola, NY, USA.*

Objective: Ischemic placental disease (preeclampsia, small for gestational age, SGA and placental abruption) is a major contributor to pregnancy-related morbidity. Although the placenta is considered a fetal organ, it is accepted that ischemic placental disease (IPD) can present clinically with either fetal or maternal manifestations. We hypothesized that the pattern of diagnosis (maternal versus fetal) varies by gestational age and would provide insights in to origins of indicated and spontaneous preterm birth.

Methods: This was a retrospective cohort study utilizing the maternally-linked reproductive history data for Missouri residents (1989-97), restricted to singleton live births. Women who experienced spontaneous onset of labor and subsequently delivered preterm were classified as spontaneous preterm birth. Medically indicated preterm birth included women who delivered preterm through a labor induction or (prelabor) cesarean delivery. IPD was classified as maternal (preeclampsia only), fetal (SGA only) or both (preeclampsia with SGA or abruption, and all 3 conditions).

Results: Among term births with IPD, 22.6% presented as maternal disease only, 69.5% as fetal disease, and the remainder (7.4%) as both. Among spontaneous preterm births with IPD, a greater proportion were of fetal presentation (43.8%), whereas among indicated preterm birth with IPD, 44.1% had both maternal and fetal presentations, 29.9% with maternal and 26.1% with fetal presentations.

Disctibution of maternal and fetal presentations in term and preterm births (PTB)

Ischemic Placental Disease	Term births (n=75,137) (%)	Spont PTB (n=7,818) (%)	Indicated PTB (n=4,720) (%)
Maternal presentation			
Preeclampsia only	22.6	32.3	29.9
Fetal presentation			
SGA birth only	69.5	43.8	26.1
Maternal and fetal presentations			
Abruption only	2.4	12.7	18.1
Preeclampsia + SGA	4.5	8.1	18.2
Preeclampsia + Abruption	0.2	0.6	2.1
SGA + Abruption	0.8	2.2	4.5
All 3 conditions	0.1	0.4	1.2

Conclusions: IPD at preterm gestations has a higher chance of clinical manifestations at delivery that involve both the mother and the fetus. At term, isolated fetal manifestations predominate. This suggests differing clinical IPD presentations are influenced both by gestational age and likely by thresholds for obstetrical interventions.

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Fetal Hemoglobin Concentration in Amniotic Fluid Is Elevated in Preterm Labor or Prelabor Rupture of Membranes. Edi Vaisbuch,[1] Juan Pedro Kusanovic,[1] Offer Erez,[1] Francesca Gotsch,[1] Shali Mazaki-Tovi,[2] Chong Jai Kim,[3] Jung-Sun Kim,[3] Tinnakorn Chaiworapongsa,[2] Samuel S Edwin,[1] Nandor G Than,[1] Chia-Ling Nhan-Chang,[2] Moshe Mazor,[5] Pooja Mittal,[2] Sonia Hassan,[2] Roberto Romero.[1] *[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA; [2]Dept Ob/Gyn, Wayne State Univ, Detroit, MI, USA; [3]Dept Pathology, Wayne State Univ, Detroit, MI, USA; [4]Dept Ob/Gyn, Soroka Univ Medical Center, Beer-Sheva, Israel.*

Objective: Hemoglobin (Hg) and its catabolic products have been observed in cases of amniotic fluid (AF) discoloration, which is a risk factor for intra-amniotic infection/inflammation (IAI). The study aimed to determine the association between AF fetal Hg concentration and gestational age, term and preterm labor and IAI.

Study design: This cross-sectional study included: 1) mid-trimester (n=60); 2) term not in labor (TNL) (n=21); 3) term in labor (TIN) (n=47); 4) preterm labor (PTL) who delivered at term (n=89); 5) PTL without IAI (n=74); 6) PTL with IAI (n=78); 7) preterm prelabor rupture of membranes (PPROM) with (n=48) and without IAI (n=48). AF fetal Hg concentrations were determined by ELISA.

Results: 1) Fetal Hg was detected in 80.4% of all AF and 31.7% of mid-trimester samples; 2) Women at TNL had a higher median AF fetal Hg concentration than patients at mid-trimester (19.5 ng/ml, IQR 0-49 vs 0.0 ng/ml, IQR 0.0-19.9, p=0.008); 3) No differences were found in median AF fetal Hg concentration among patients with and without labor at term (TIL: 19.1 ng/ml, IQR 0-36.2; p=0.4); 4) Median AF fetal Hg concentration was not significantly different among the 3 PTL subgroups [PTL with IAI: 114.6 ng/ml, IQR 33.2-283, PTL who delivered preterm: 109.6 ng/ml, IQR 42.-247.1, PTL without IAI who delivered at term: 134.21 ng/ml, IQR 56.3-255.7, p=0.71(Kruskal Wallis)]; 5) In PPROM, there were no differences among patients with and without IAI (148.4 ng/ml, IQR 82.8-811.1 vs 109.7 ng/ml, IQR 51.4-293.1, respectively; p=0.074); 6) Median AF fetal Hg concentrations were significantly higher in PTL or PPROM, with or without IAI than in pregnant women at term, with and without labor (p<0.001 for all comparisons).

Conclusions: 1) Immunoreactive AF fetal Hg increases with gestational age; 2) Among women with PTL or PPROM, the median AF fetal Hg concentration is not associated with IAI; 3) The median AF fetal Hg concentration is higher in pregnancies complicated with PTL or PPROM than in term pregnancies.

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The Impact of Latency Time to Delivery after Preterm Premature Rupture of Membranes (PPROM) on Neonatal Outcome. Dan Nayot,[1] Deborah Penava,[1] Barbra de Vrijer,[1] Orlando da Silva,[2] Bryan S Richardson.[1] *[1]Obstetrics and Gynaecology; [2]Pediatrics, University of Western Ontario, London, ON, Canada.*

Objective: There continues to be controversy as to the management of PPROM with conservative management to advance gestational age (GA) versus aggressive management with early induction to avoid chorioamnionitis. We have therefore used the perinatal and neonatal databases of a large regional patient population to determine the association of pregnancy variables with latency time to delivery after PPROM and the impact of latency duration on adverse neonatal outcomes.

Methods: The perinatal/neonatal database of St. Joseph's Health Care, London, Ontario was used to obtain demographic and neonatal outcome information for all patients with PPROM >25 and <37 weeks gestation, singleton and no major anomalies, delivering between January 1, 1996 and December 31, 2005. Patients were grouped according to GA at PPROM stratified for latency time <72 hrs vs >72 hrs with incidences for those pregnancy related variables and neonatal outcomes available from the database then compared with the use of logistic regression analysis.

Results: There were 1535 patients who met the inclusion criteria of whom 10%, 19%, and 71% had PPROM at 25-28 wks , at 29-32 wks, or at 33-36 wks, respectively, and with these PPROM groupings showing a stepwise decrease in the percentage of patients with latency to delivery >72 hrs, at 67%, 42%, and 10%, respectively. Pregnancy related variables and neonatal outcomes for these patient groupings are as shown in Table 1.

Conclusion: Despite a 2 to 3 fold increase in the incidence of chorioamnionitis with latency to delivery >72 hrs, a policy of conservative management to advance GA after PPROM will result in decreased severe infant morbidity until 32 weeks, and moderate infant morbidity until 36 weeks.

Table 1. Pregnancy variables and neonatal outcomes

	25-28 wks		29-32 wks		33-36 wks	
	<72h	>72h	<72h	>72h	<72h	>72h
PPROM GA (wk)	26.5	26.6	30.9	30.5**	35.2	34.0**
PIH (%)	4.2	2.1	14.6	2.5**	13.2	2.7**
IUGR <3% (%)	10.4	4.1	10.5	2.5*	4.5	2.7*
Chorio (%)	25	38	15	35**	2.6	10.6**
MMI (%)	95	90	67	49**	9.1	4.4**
SMI (%)	65	35**	9.4	4.9*	0.4	1.8

MMI=Moderate Morbidity Index with one or more of neonatal RDS, IVH 1/2, ROP 1/2, or sepsis; SMI=Severe Morbidity Index with one or more of neonatal BPD, IVH 3/4, PVL, NEC, ROP 3/4, or demise; *p<.05, **p<.01 with neonatal outcomes adjusted for selected pregnancy variables

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Prolonging the Latency Period Using Multiple Tocolytic Agents in Twin Gestations with Intact and Ruptured Membranes. Caroline L Stella,[1] Fariba Pajoohi,[1] Hind Moussa,[1] Maged M Costantine,[1] Annette Bombrys,[1] Mounira Habli,[1] William Kossenjans,[2] Rose Maxwell,[1] Helen Y How,[1] Baha M Sibai.[1] *[1]Obstetrics & Gynecology, University of Cincinnati, Cincinnati, OH, USA; [2]University of Cincinnati Clermont College, Cincinnati, OH, USA.*

Introduction: There is minimal data regarding multiple tocolytic agent use in twin gestations for prolonging latency period in women with preterm labor (PTL) with either intact or premature rupture of membranes (PROM).

Objective: To determine if multiple tocolytic agents are effective and safe in prolonging latency period in twin gestations in PTL with intact membranes or PROM.

Methods: A retrospective review of twin gestations admitted for PTL with single/multiple tocolytic use was performed from 1998-2006 at The University

Hospital. Outcomes studied were prolongation of latency period with intact membranes and PROM using magnesium sulfate ($MgSO_4$), nifedipine, terbutaline and ≥2 tocolytic agents. Secondary outcomes examined were maternal complications. Statistical analysis performed were t-test and λ^2. Comparisons were expressed as odds ratios (95% CI).

Results: In a 9-year period, 183 twin gestations were admitted in PTL and administered ≥1 tocolytic agent(s) with mean gestational age of 28.8 weeks. When ≥2 tocolytic agents were used, maternal complications were: 7 (9.5%) with intact membranes and 1 (2.8%) with PROM had pulmonary edema (OR=2.67, CI 0.58-12.29); 11 (14.1%) with intact membranes and 2 (5.6%) with PROM had chorioamnionitis (OR=1.3, CI 0.41-4.15); 2 (2.6%) with intact membranes and 2 (5.6%) with PROM had postpartum hemorrhage (OR=0.43, CI 0.05-3.62).

Conclusion: There is no difference in prolongation of latency period achieved in twin gestations in PTL with intact membranes or PROM using ≥2 tocolytic agents. Similarly, there is no difference between the 2 groups with latency period ≥ 48 hrs. There is an increased likelihood of pulmonary edema and chorioamnionitis with use of ≥2 tocolytic agents. However, results are not significant due to Type II error.

Mean latency period and latency ≥48 hrs using single or multiple tocolytic agent(s)

	Intact Membranes (n=126)			PROM (n=57)		
Tocolytic Agent(s)	N	Mean Latency (days)	Latency ≥48 hrs n (%)	N	Mean Latency (days)	Latency ≥48 hrs n (%)
MgSO4	24	14.9*	18 (75%)	12	5.1*	7 (58%)
Nifedipine	9	28.1	7 (78%)	4	7.0	3 (75%)
Terbutaline	4	26.5	3 (75%)	2	21.0	2 (100%)
≥2 tocolytic agents	88	19.1	64 (73%)	39	22.8	31 (79%)
p<0.05						

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Utility of High Sensitivity C-Reactive Protein in Term Labor and Preeclampsia. Sindhu K Srinivas, Jamie Bastek, Ella Ofori, Michal A Elovitz. *OB/GYN CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

Introduction: High sensitivity CRP (hsCRP) is a serum marker of inflammation and has proven clinical utlility in predicting cardiovascular disease (CVD). Considering the hypothesized association between preeclampsia (PRE) and inflammation and CVD, it is plausible that hsCRP may have utility in predicting PRE. Prior to widespread utilization of this marker, the affect of labor on levels of CRP needs to be clarified. We assessed the association between labor and hsCRP levels in term deliveries and between elevated hsCRP and adverse perinatal outcomes. A secondary analysis comparing hsCRP in women with preeclampsia (PRE) to those without was performed.

Methods: Women presenting for term delivery or PRE were prospectively identified as part of a case-control study. Clinical data and serum were collected for all subjects. A standard immunoturbidimetric assay was used to measure hsCRP levels. Women presenting for induction of labor or planned cesarean delivery (non-labor) were compared to women presenting in labor. A secondary analysis comparing non labor women with and without PRE was performed. Serum was collected prior to labor induction in the non-labor group. Non-parametric comparisons were made using Wilcoxon rank sum tests. MVLR was used to evaluate dichotomous outcomes and control for confounders.

Results: 344 women were included (non-labor group (n=146), labor group (n=120), non-labor PRE (n=78)). The median and mean hsCRP levels were 6 and 9 and 15 and 21 in the non-labor and labor groups respectively (p=0.005). Elevated levels of hsCRP in these term deliveries were not associated with chorioamnionitis (p=0.3), maternal postpartum complications (endometritis, hemorrhage, transfusion) (p=0.67), mode of delivery (p=0.3), or admission to the NICU (0.96). Levels of hsCRP were significantly greater in the non-labor PRE group compared to non labor without PRE (mean 34.5 vs.20.1, median 27 vs.6, p<0.001).

Conclusion: Use of hsCRP as a biomarker may improve clinical prediction of obstetrical complications such as PRE. Levels of hsCRP are affected by labor and this should be taken into account when studying the utility of this biomarker. Further, CRP levels are elevated in women with PRE even after excluding patients in labor. Further investigations to determine if CRP elevation in term labor is associated with adverse outcomes may be warranted. The role of hsCRP as a valid and discriminating biomarker in PRE should be assessed.

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Clinical Predictors of Histologic Chorioamnionitis. William M Curtin,[1] Heather Florescue,[1] Leon A Metlay,[2] Philip J Katzman.[2] *[1]Obstetrics & Gynecology, University of Rochester School of Medicine & Dentistry, Rochester, NY, USA; [2]Pathology & Laboratory Medicine, University of Rochester School of Medicine & Dentistry, Rochester, NY, USA.*

OBJECTIVE: To compare clinical characteristics of term parturients with and without histologic chorioamnionitis and to identify factors that predict acute placental inflammation.

STUDY DESIGN: Case control study of 351 consecutively submitted placentas in term parturients from 2005. Slides reviewed for the presence (cases) or absence (controls) of histologic acute chorioamnionitis and classified according to Amniotic Fluid Infection Nosology Committee guidelines. Review of the electronic labor record facilitated collection of maternal demographic, intrapartum, and newborn data. Data analyzed with t-tests, chi-square tests, and calculated odds ratios with 95% CIs as appropriate. A forward stepwise logistic regression analysis was used to identify predictors of histologic chorioamnionitis.

RESULTS: Of 351 submitted placentas, 210 had histologic chorioamnionitis (cases) and 141 did not (controls). The groups were similar with respect to age, race, GBS status, and mode of delivery. Gestational age, birthweight, duration of labor and ruptured membranes, and number of vaginal exams were greater in the cases (p≤ .01). The cases were more likely to have had epidural anesthesia (OR 2.9), internal monitoring (OR 2.2), fever (OR 4.8), maternal tachycardia (OR 1.9) and fetal tachycardia (OR 3.8) and less likely to have had induction of labor (OR 0.49). Newborns in the histologic chorioamnionitis group were more likely to have been observed for sepsis (OR 3.8). The results of the logistic regression analysis are given in the table.

CONCLUSION: Risk factors for pathologically documented acute chorioamnionitis are similar to those for clinical chorioamnionitis. The unexpected finding that induction of labor was actually associated with a reduction in histologic chorioamnionitis may be worthy of further study.

Independent Predictors of Histologic Chorioamnionitis

Variable	OR (95%CI)
Induction of labor	0.28 (0.17-0.48)
Internal Monitoring	1.8 (1.1-3.0)
No. vaginal exams	1.16 (1.04-1.3)
Fever	3.0 (1.6-5.6)
Fetal tachycardia	2.7 (1.4-5.1)

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Maternal Outcomes in Patients with Severe Sepsis/Septic Shock during Pregnancy – Postpartum Period: A Single Center Experience. Mounira A Habli, Sareena Singh, Jade Richardson, Annette Bombry, Helen How, Baha Sibai. *Obsterics And Gynecology, University Of Cincinnati, Cincinnati, OH, USA.*

OBJECTIVE

Little data exists regarding maternal and neonatal outcomes in severe sepsis/septic shock in pregnancy. Our objective is to evaluate the etiology, maternal and neonatal outcomes in patients with severe sepsis/septic shock.

STUDY DESIGN

A retrospective study of 23 pregnant women complicated by severe sepsis /septic shock between 2000-2006. Severe sepsis defined as sepsis associated with acute respiratory distress syndrome (ARDS) or cardiovascular dysfunction(CVD) or with 2 or more other organ dysfunction.All patients were resuscitated with fluids and treated with broad spectrum antibiotics and supportive care as needed. Outcome data were: etiology, management, maternal complications, duration of ICU stay and perinatal survival.

RESULTS

Patients were young (mean age=23.6± 7.6 years) with a mean gestational age at delivery 29.5±8.9 weeks. Etiologies were pyelonephritis(n=8), septic abortion(n=4), endomyometritis (n=3), chorioamnionitis (n=3), ruptured appendix(n=3), pneumonia(n=1) and one unknown. Eighteen (78%) were diagnosed during antepartum and 5 (22%) postpartum period.There were 2(9%) maternal deaths and high rate of major morbidities (Table). Among the 18 antepartum patients,there were,4 abortions,4 IUFD,2 neonatal death for a perinatal survival rate of only 39%.

CONCLUSION

Pregnancies complicated with severe sepsis/septic shock are associated with substantial maternal and perinatal morbidities. The low maternal mortality rate in our study as compared to previous reports is attributed to early diagnosis and aggressive management of maternal complications. Fetal loss rate, however continues to be high when septic shock develops antepartum.

Maternal Morbidities n=23	
ARDS-n (%)	12 (52)
Acute renal failure-n (%)	10 (43)
Cardiovascular dysfunction-n (%)	15 (65)
Hepatic failure-n (%)	3 (13)
Disseminated intravascular coagulopathy-n(%)	6 (26)
Duration of ICU stay (days, mean+SD)	9.21±12.8
Hospital stay (days, mean+SD)	16.4±15.2

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Mifepristone (RU486) Leads to Undampenned Immune Activation during Infection. Ozlem Equils,[1] Preethi Rajagopalan,[2] James A McGregor,[3] Shireen Vali.[2] [1]*Pediatrics, Cedars-Sinai Medical Center-UCLA, Los Angeles, CA, USA;* [2]*Cellworks Group Inc, Saratoga, CA, USA;* [3]*Obstetrics and Gynecology, Keck School of Medicine, Los Angeles, CA, USA.*

Objective: RU486 is an antiprogesterone, antiglucocorticoid agent commonly used in medical abortion. Recently RU486 abortion has been implicated in the sepsis and death of women with *Clostridium sordelii* infection; however the molecular mechanisms are not clearly understood.

Methods: Sepsis is defined as uncontrolled immune activation, where blood IL-6 and TNFa levels are elevated. We developed a dynamic computer (in silico) model of pregnancy (uterine myometrial environment -infection/inflammation and endocrine crosstalk). Mathematical differential equations were used to describe the interactions between molecules. Infection was represented as increased levels of activated, nuclear transcription factor NF-kB. In the model RU486 inhibited both the glucocorticoid receptor and progesterone receptors. Simulations were run adding different concentrations of RU486 (1.5 uM= dose used in patients, 0.5 uM, 0.05uM) at different time points during infection (before, at the time of or after NF-kB activation). RU486 degradation kinetics was also included. The effect of RU486 on NF-kB induced IL-6 and TNFa levels was assessed.

Results: Infection induced NF-kB activation led to increased IL-6 and TNFa levels. There was a subsequent increase in cortisol that led to dampening of NF-kB activation, IL-6 and TNFa levels. In the presence of RU486, IL-6 and TNFa levels continued to rise. The effect of RU486 on NF-kB induced IL-6 and TNFa was dose dependent and was more prominent in slow metabolizers who had RU486 in the system for a longer time. Addition of RU486 after the onset of NF-kB activation led to increased IL-6 and TNFa levels above those observed without RU486. The addition of misoprostol (prostaglandin E1 analogue), at the concentrations used together with RU486 for medical abortion, did not add to the effect of RU486 on NF-kB induced IL-6 or TNF.

Conclusions: RU486 has dose dependent effects on infection induced immune activation and may contribute to the pathogenesis of *C sordelii* induced sepsis syndrome.

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Etiologic Agents Isolated in Cases of Fatal Neonatal Sepsis. Joan E Moss,[1] Rosa I Mateo,[2] Hal K Hawkins,[3] Nima Goharkhay,[1] Monica Longo,[1] George R Saade,[1] Judith F Aronson,[3] Elena Sbrana.[1] [1]*Dept. of Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, TX, USA;* [2]*Infectious Diseases Division, Shore Health System, Cambridge, MD, USA;* [3]*Dept. of Pathology, The University of Texas Medical Branch, Galveston, TX, USA.*

OBJECTIVE:

To determine the leading etiologies of fatal neonatal sepsis observed in a large hospital autopsy service, and the most common associated pathologic observations on the newborns at autopsy.

STUDY DESIGN:

Over a 5 year time period, autopsy data were collected from cases of neonates that died with a clinical diagnosis of sepsis. Maternal obstetrical histories were reviewed from the hospital clinical records, and clinical laboratory data were collected. Gross and microscopic observations were recorded on all cases examined. Statistical analysis was conducted using Sigma Stat on a Microsoft Windows platform.

RESULTS:

Pseudomonas aeruginosa was identified as underlying etiology in 29.4% of the infants autopsied. In the above cases, all with late onset sepsis, bronchopneumonia was identified as the immediate cause of death, and the most common findings were hemorrhagic bronchopneumonia, pleural adhesions, pleural effusions, alveolar hemorrhage, diffuse alveolar damage, atelectasis, and multiple abscesses. The second most common organim was group D *enterococcus*, cultured in 17.6% of newborns. However, in most of these cases, multiple infectious agents were isolated from the same specimens, and the contribution of each etiologic agent to the final multi-organ failure was unclear.

Group B *Streptococcus* (GBS) was cultured only in 11.8% of cases, all with early onset sepsis, and it was identified as the underlying cause of respiratory failure in the infants that tested positive.

Klebsiella or Staphylococcal infections were observed in about 12% of cases, and were accompanied by other bacterial co-infections 50% of the time. *Serratia* was isolated from a single newborn, deceased at 2 weeks of age, and was associated with a clinical history of severe necrotizing enterocolitis.

CONCLUSIONS:

In an autopsy case series, *Pseudomonas aeruginosa* was identified as the leading etiologic agent of late onset neonatal sepsis, being responsible for 30% of cases examined. Other important isolates were group D *enterococcus*, *Klebsiella*, *S. Aureus*, and GBS. Despite universal testing and early diagnosis and treatment, GBS persist as an important underlying etiology of early onset neonatal septicemia.

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Neonatal Innate Immune Response to Listeria Infection. Josiah S Hawkins,[1] Prameela Karimi,[2] Yanxia Wang,[3] Jianlin Chen,[3] Christopher Y Lu.[3] [1]*Obstetrics and Gynecology;* [2]*Pediatrics;* [3]*Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA.*

Background:

The increased susceptibility of neonates to infection remains a major clinical problem. We previously found that after intraperitoneal (IP) Listeria monocytogenes infection, neonatal mice have an LD_{50} that is 5 orders of magnitude lower than adults. We also found that the inflammatory response of neonatal mouse macrophages, but not neutrophils, in the peritoneal fluid was deficient, and that this correlated with low levels of macrophage chemokines MCP-1 and RANTES. Given that the liver and spleen are important organs in Listeria infection, we sought to characterize the innate immune response in these organs.

Methods:

A sublethal dose of Listeria was injected intraperitoneally into Balb/C 4-6 week old adult mice and 3-5 day old neonatal mice. Liver and spleen was collected at 0, 24, 48 and 72 hours, sectioned serially for staining with hematoxylin-eosin and primary antibodies (rabbit anti-Listeria monocytogenes IgG; MHC class II rat anti-mouse IgG, a marker for activated macrophages; F4/80 rat anti-mouse IgG, a marker for macrophages) and secondary antibodies (Cy-3 goat anti-rabbit IgG; AF488 goat anti-rat IgG) were applied. Negative controls used only secondary antibody. Real time PCR was used to compare the levels of the chemokines MCP-1 and RANTES in adult and neonatal liver.

Results:

After IP infection, both adults and neonates showed similar influx of neutrophils to the sites of infection within the liver and spleen. At 24 hours post-infection with Listeria, adult liver and spleen showed increased staining for F4/80 and class II, which increased further and became confluent surrounding microabscesses at 48 and 72 hours. In contrast, the Listeria infected neonatal mouse showed some increase in F4/80 around microabscesses but no apparent increase in staining for MHC class II. The neonates showed greater staining for Listeria at each time point. MCP-1 and RANTES levels were higher in infected neonatal liver compared to adults.

Conclusion:

In the neonatal mouse, the innate immune response in the liver and spleen was characterized by a deficiency of activated macrophages. This deficiency was not correlated with hepatic expression of 2 macrophage chemokines.

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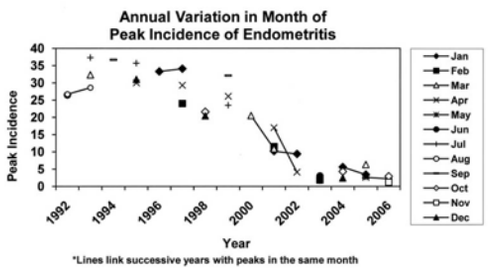
Is There a Seasonal Pattern in the Incidence of Post-Cesarean Endometritis? Tamula M Patterson, Alan TN Tita, William W Andrews. *Obstetrics and Gynecology, The University of Alabama at Birmingham, Birmingham, AL, USA.*

Objective: Several theories, including one suggesting a peak in July coincident with resident turnover, postulate seasonality in post-cesarean infections. We assessed whether there is seasonal variation in endometritis.

Methods: A retrospective cohort study of post-cesarean endometritis at our University-based institution using our obstetric computerized database to compare annual variation in monthly incidence patterns from 1992 to 2006. Prior to establishing an average aggregate seasonal pattern for all years, years

were assessed separately for a recurrent pattern of peaks and nadirs in incidence. Peak incidence (or nadir) was defined as any monthly incidence that differed from the mean incidence for the year by over 25%.

Results: A total of 22.4% (10,966) of 48,913 deliveries from 1992 to 2006 were by cesarean. Annual cesarean rates increased by an absolute rate of over 10%; while, post-cesarean endometritis rates decreased from 23% to 2%. Monthly incidence of post-cesarean endometritis did not reveal a consistent recurrent pattern of peaks (figure1) or nadirs. The month of July accounted for only 4 out of a total of 40 peaks for all years. The adjacent months of June and August accounted for only 3 each. The month with the highest number of peaks was April with only 7. These findings contraindicated the establishment of an average aggregate monthly pattern for all years.



Conclusion: The incidence of post-cesarean endometritis did not follow a seasonal pattern.

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***Haemophilus influenzae* Abscess: Inclusion in the Differential Diagnosis of a Large Pelvic Mass?** Dana R Ambler, Michael P Diamond, John Malone, Jr. *Obstetrics and Gynecology, Wayne State University/Detroit Medical Center, Detroit, MI, USA.*

Objective: This report describes an unusual case in which an abscess containing *H. influenzae* incorporates the entire uterine cavity without overt signs of infection.

Design: Case Report.

Setting: University Medical Center

Patient(s): A 39-year-old Caucasian female presented with right lower quadrant abdominal pain and a large abdominal pelvic mass in the absence of fever and elevated white blood count (WBC).

Intervention(s): Exploratory Laparotomy and Total Abdominal Hysterectomy.

Main Outcome Measure: Relief of symptoms at six-week follow-up.

Result(s): Evaluation with CAT-scan and ultrasound revealed a 20x14x10cm cystic mass arising from the uterus. A huge, fluid-distended endometrial cavity, uterine fibroid degeneration, or cystic degeneration of a uterine sarcoma were main considerations. Tumor markers were negative. Intraoperative findings included a large, 20cm uterine abscess that completely overtook the uterine cavity, displacing it posteriorly. The abscess was densely adhered to the sigmoid colon. The mass was ruptured during the surgical procedure when mobilizing it off the colon and cultures were taken. The microbiological culture illustrated *H. influenzae*. Pathological diagnosis confirmed an intramyometrial abscess, originating from the wall of the uterus occupying the entire uterine cavity, lined with granulation tissue, foamy macrophages, and chronic inflammation.

Conclusion(s): Intramyometrial abscesses can masquerade as degenerating fibroids and even with microorganisms can exist without signs or symptoms of infection.

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Impact of Positive Serologic Screen for CMV on Pregnancy Outcomes. Alan T Tita,[1] Alice R Goepfert,[1] Victoria Chapman,[1] William W Andrews,[1] Robert F Pass.[2] *[1]Obstetrics and Gynecology, MFM Division, University of Alabama at Birmingham, Birmingham, AL, USA; [2]Pediatrics, University of Alabama at Birmingham, Birmingham, AL.*

Objective: The significance of maternal cytomegalovirus (CMV) infection (positive sero-status) for maternal and fetal outcomes (other than related to congenital CMV infection) is uncertain and there is a paucity of relevant studies. We examined the impact on pregnancy-related hypertension, preterm birth, perinatal mortality and other pregnancy outcomes. **Design:** Cross sectional study of pregnancy outcomes among a group of new mothers between 13 and 54 years of age who delivered between 6/1999 and 1/2006, and were screened for Ig G antibody to CMV by enzyme immunoassay as part of a vaccine trial. CMV status was defined by antibody level (AU/ml) as positive (≥ 15.0) or negative (< 10.0); women with intermediate levels (equivocal status) were excluded. **Results:** Among 9802 women with pregnancy outcomes (mean gestational age = 39 weeks, range 24-44 weeks), 74.3% (n=7166) were positive for CMV. Pregnancy-related hypertension (Preeclampsia, Eclampsia, Gestational hypertension) occurred in 9.4 % of positive vs. 9.3% of negative women; RR (95%CI) = 1.0 (0.9, 1.2). The subgroups of preeclampsia or eclampsia (5.3% vs. 5.4%) and gestational hypertension (4.1% vs. 4.0%) were also not associated with CMV status. Premature birth <37 weeks [13.2% vs. 14.6%; RR = 0.9 (0.8, 1.0)], low birth weight <2500g [11.2% vs. 10.5%; RR = 1.1 (0.9, 1.2)] and perinatal mortality [2.2 vs. 1.6 per 1000; RR = 1.4 (0.5, 4.1) were also not impacted by CMV status as were the prevalence of small for gestational age, large for gestational age, gestational diabetes and cesarean delivery. Findings were unchanged after stratifying by race. Among 169 CMV-negative mothers who had repeat tests during subsequent deliveries, 19 (11.2%) became positive. This recent CMV infection, albeit limited by sample size, was not associated with pregnancy-related hypertension [5.3% vs. 9.3%; RR = 0.6 (0.1, 4.1)], premature birth [5.6% vs. 18.5%; RR = 0.3 (0.04, 2.1)] or other pregnancy outcomes. **Conclusion:** Positive CMV serostatus does not appear to adversely affect maternal and perinatal outcomes unrelated to congenital CMV.

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Uric Acid Concentrations Are Associated with Insulin Resistance in Normotensive Pregnant Women. S Katherine Laughon,[1] Janet M Catov,[1,2] James M Roberts.[1,2] *[1]Obstetrics, Gynecology and Reproductive Services, University of Pittsburgh Magee-Womens Hospital, Pittsburgh, PA, USA; [2]Magee-Womens Research Institute, University of Pittsburgh Magee-Womens Hospital, Pittsburgh, PA, USA.*

Background: In the nonpregnant population, serum uric acid concentrations increase with elevated insulin resistance perhaps secondary to reduced renal excretion of uric acid. This relationship has not been well established in pregnancy. One small study reported elevated uric acid associated with insulin resistance only in pregnant patients with hypertensive disease.

Objective: To investigate whether uric acid concentrations are increased in pregnant women with insulin resistance.

Study design: We used banked plasma and serum samples drawn at 18-21 weeks gestation (mean 20.4 weeks (± 2.0) from fasting subjects enrolled in the Pregnancy Exposures and Preeclampsia Prevention Study. Samples were available on 272 nulliparous women without pre-pregnancy diabetes. Pregnancies with IUGR were excluded. “Hypertensive women” were those with chronic hypertension (n=4), or who developed gestational hypertension (n=24) or preeclampsia (n=10) in later pregnancy. Uric acid and glucose were measured using colorimetric assays, insulin was measured by ELISA, and insulin resistance was estimated using the homeostasis model assessment (HOMA).

Results: Uric acid was correlated with HOMA (Spearman r=0.31, p<.0005). This association was present in both overweight and normal weight women, but HOMA increased more per 1 mg/dl increase in uric acid among overweight women (BMI >25 kg/m2), (1.46; 95%CI:1.17,1.82 and 1.20; 95%CI:1.03,1.40 respectively). Mean uric acid levels did not differ in “hypertensive women” versus normotensive women (3.74mg/dL ± 0.75 vs. 3.51mg/dL ± 0.86; p= 0.12. In 225 normotensive pregnant patients, insulin resistance increased (HOMA 1.34 (95%CI; 1.17,1.53) as uric acid increased, adjusted for maternal age, body mass index, and gestational age at sampling. In 38 pregnant patients with “hypertensive disease,” the increase was similar but was not significant (HOMA 1.31, 95%CI: 0.83, 2.07).

Conclusion: Even among normotensive women, increasing uric acid concentrations were associated with insulin resistance in mid-pregnancy. The combined effects of these conditions on pregnancy outcomes warrant further study.

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Do Risk Factors and Severity of Preeclampsia Differ by Maternal Race? Sindhu K Srinivas, Andrea G Edlow, Jamie Bastek, Christina M Andrela, Michal A Elovitz. *Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA.*

OBJECTIVE Current assumptions about preeclampsia (PRE) include an association between black race and an increased risk of PRE. The validity of these assumptions has not been well studied. We assessed whether traditional risk factors for PRE and severity of disease differ by race.

STUDY DESIGN Cases are prospectively identified with PRE using ACOG criteria. Controls are women delivering at term. Maternal race, height and pre-existing hypertension (CHTN) were assessed by patient report at enrollment.

Chi-square analyses were used to compare associations between race (Black (BL) vs non-Black (nBL)) and dichotomous characteristics. Student´s t-test was used to compare continuous variables.

RESULTS 1,030 patients were evaluated (439 cases and 591 controls). 85% and 15% of cases and 74% and 26% of the controls were BL and nBL respectively. The baseline prevalence of CHTN in BL and nBL controls was 6.15% and 2.65% (p=0.09). When comparing BL and nBL cases, BL women had a higher mean systolic blood pressure and screening BMI. BL women also had a trend toward being discharged on post partum blood pressure medicine when compared to nBL women. There was no difference in CHTN, diabetes, severity of disease, IUGR, or delivery <34 wks between the two groups (Table).

CONCLUSION Traditional medical conditions (CHTN and Diabetes) are associated with PRE, regardless of race. Further, maternal race does not appear to have a strong relationship with severity of disease or IUGR in women with PRE. An increased prevalence in obesity in BL women may contribute to their increased severity of hypertension when preeclamptic. Future research should focus on biologic pathways underlying PRE regardless of race.

Characteristics of cases by race

	BL%(N=372)	nBL%(N=67)	P Value
Age (yrs)	25.8+/-6.6	32.4+/-6.3	<0.001
Mean screen BMI	30.5+/-8.1	27.48+/-6.8	0.007
CHTN	15.6(58)	14.9(10)	0.89
DM	8.3(30)	7.4(5)	0.75
Diagnosis			0.22
GHTN	18(67)	17.9(12)	
Mild PRE	20.5(76)	13.4(9)	
Severe PRE	61.5(228)	69.7(46)	
IUGR<10%	27.7(103)	31(21)	0.54
Del <34 weeks	21.7(81)	23.8(16)	0.7
Mean creatinine	0.8+/-0.3	0.8+/-0.2	0.61
Any lab abnormality	35.3(131)	46.2(31)	0.08
Mean Systolic BP	174.2+/-17	168+/-16	0.01
IV BP medication	27.2(100)	16.4(11)	0.06
Discharge on BP medication	21.6(80)	13.4(9)	0.12

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Leading Causes of Intra-Uterine Fetal Demise in an Autopsy Case Series. Joan E Moss,[1] Hal K Hawkins,[2] Monica Longo,[1] George R Saade,[1] Judith F Aronson,[2] Elena Sbrana.[1] *[1]Dept. of Obstetrics & Gynecology, The University of Texas Medical Branch, Galveston, TX, USA; [2]Dept. of Pathology, The University of Texas Medical Branch, Galveston, TX, USA.*

OBJECTIVE:

To determine the leading causes of death in a case series of stillborn infants examined in a large hospital autopsy service, and to describe the most common post-mortem observations.

STUDY DESIGN:

One hundred and sixty one stillborn infants were examined. Gross pathology observations were recorded at autopsy and during the placental exam, and tissue sections were collected and examined microscopically. Immediate and underlying cause of death (COD) were recorded, along with contributory COD, concomitant/significant COD, and incidental findings. Statistical analysis was conducted using the software SPSS v.11.5.

RESULTS:

The immediate anatomic COD could be determined in 33.5% of all infants examined. In over 50% of these cases, COD was attributable to placental or umbilical cord findings affecting the maternal-fetal blood supply. The most prevalent among these findings were placental lesions (maternal floor infarction, placental abruption, fetal thrombotic vasculopathy), umbilical cord lesions (entanglement, true knot, compression, excessive length/twisting), and infectious/inflammatory processes (chorioamnionitis, chronic villitis).

Fetal anomalies incompatible with survival accounted for 38.9% of the immediate COD. In 11.1% of the cases, the immediate COD was due to vertical transmission of an infectious disease, with Candida and Parvovirus as the most prevalent. For the majority of stillbirths (66.5%), the immediate COD remained undetermined despite identification of several potential contributory factors. Abnormalities of development were observed in 34.2% of the infants examined. In 38.2% of the above cases the anomalies observed were incompatible with life. Karyotype analysis was done on 14.9% of all stillborns; results were obtained on 11.2% of cases, 5.6% of whom had a chromosome abnormality. Two cases of anencephalic fetuses presented with a combination of congenital abnormalities never previously described in the medical literature.

CONCLUSIONS:

Postmortem and placental examinations provide important information on the causes of fetal demise. Fetal anomalies incompatible with life, hypoperfusion, and infectious diseases represented the most common immediate cause of death. However, in over 66% of cases examined, the immediate cause leading to fetal death remained unclear.

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The Impact of Changing Practice in Obstetrics: Does Antenatal Testing for Advanced Maternal Age Increase Labor Induction and Cesarean Section Rates? Shilpi Mehta, Sindhu K Srinivas, Michal A Elovitz, Emmanuelle Pare. *OBGYN, University of Pennsylvania, Philadelphia, PA, USA.*

OBJECTIVE: Advanced maternal age (AMA) is associated with increased risk of intrauterine fetal demise (IUFD). Antenatal testing (AT) is widely used in clinical practice to prevent IUFD due to uteroplacental insufficiency and has been suggested to reduce the risk of IUFD in AMA women. We sought to assess the impact of AT on obstetrical interventions and compliance with new practice recommendations.

METHODS: Retrospective cohort of AMA women (40 and older at their due date) who delivered at or after 32 weeks. Non-exposed women delivered from July 2003 to December 2004 (when AT for AMA was not routinely recommended); exposed women delivered from July 2005 to December 2006 (after AT for AMA was introduced at our institution). Subjects were identified through the perinatal database; records were abstracted for demographics, medical history, and labor/delivery variables. Outcomes included rates of AT and induction of labor (IOL) and mode of delivery. Associations between AT for AMA and outcomes were tested using t test and chi square. Assuming a baseline rate of IOL of 20%, we had 80% power to detect an increase to 35% after the introduction of AT.

RESULTS: 276 women met the inclusion criteria: 147 delivered before the introduction of AT (non-exposed=before AT) and 129 delivered after the introduction of AT (exposed=after AT). Baseline clinical characteristics were similar in both groups. As anticipated, AT was more common in the after AT group than in the before AT group (92% vs 27%; p<0.0001). 86 women were not eligible for or declined trial of labor, thus not “at risk” for IOL and not included in all analyses. OB intervention rates were increased after AT compared to before AT (Table 1). The corrected IUFD rates were similar in both groups (8/1000 vs 7/1000).

CONCLUSION: At an academic center, compliance with new practice recommendations was excellent. Introducing AT testing for a new indication seemed to increase IOL and CS rates. These findings should be considered when assessing the risks:benefits ratio of antenatal testing.

Table 1: Rates of OB interventions

	Before AT	After AT	RR (95%CI)	p-value
IOL (women at risk)	36%	56%	1.56 (1.13-2.14)	0.006
CS (all women)	39%	53%	1.36 (1.05-1.76)	0.02
CS (women at risk for IOL)	16%	27%	1.71 (0.98-2.98)	0.06
Delivery for abnormal AT (all)	7%	15%	1.96 (0.97-3.98)	0.05

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Risk of Cesarean after Medically Indicated or Elective Induction. Angela Shay, Sonal Shah, Kimberly D Gregory. *Obstetrics & Gynecology, Cedar Sinai Medical Center, Los Angeles, CA, USA.*

Objective: To determine the percent contribution of “medically indicated” and “elective” inductions to the cesarean rate, and to compare rates by type of practice.

Method: We used an electronic database to identify inductions. Study population: Term singleton deliveries (39 0/7 weeks to 40 6/7; June 2004-December 2006). We stratified inductions into medically indicated—ACOG criteria (Practice Bulletin #10,1999) and elective—no medical indication identified. We calculated the cesarean rate stratified by indication for induction, indication for cesarean, and type of physician practice (solo only, solo with cross coverage, group & faculty practice). Results: The mean age was 31.7 (±5.8) for medically indicated and 32.5 (±5.6) for elective inductions (p<0.0001). Type of induction varied by race/ethnicity. Caucasians and African Americans were more likely to undergo elective inductions (p<0.0001). There were 16661 deliveries, and 5273 cesareans (CS rate=31.6%). There were 3294 inductions: 1501 medically indicated, 1793 elective. CS rate for study patients was 20.9% (687/3294); or 13.1% (687/5273) of all cesareans. The CS rate was 24.3% (medically indicated group) and 18.0% (elective group) (RR 1.4, 95% CI 1.2-1.6). We stratified indications for cesarean into the following: failed induction (cervix <4 cm dilated), arrest of dilation, arrest of descent, failed operative delivery, fetal intolerance of labor (FIL), and “other” reasons (e.g., pre-eclampsia, abruption, chorioamnionitis, malpresentation). Both FIL and

"other" reasons were more likely to occur among the medically indicated group (RR 1.7, CI 1.2-2.3 FIL and RR 3.1, CI 1.8-5.2 "other"). Physicians in solo practice had higher rates of elective inductions (p<0.0001), but there was no association between cesarean and practice type.
Conclusions: Inductions accounted for 13.1% of cesareans. These results suggest an increased risk for CS for patients undergoing medically indicated inductions at our institution. There was no association between cesarean and type of practice, whether solo or group, suggesting institutional clinical policies may be more important than practice type in determining delivery outcome after induction. Further research is needed to understand how age, race/ethnicity, or other unmeasured patient factors may impact these findings. Given rising rates of both cesarean delivery and inductions, this information may be pertinent to women considering elective induction prior to 41 weeks.

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Is Tissue Factor Responsible for the Consumption Coagulopathy Associated with Fetal Death? Offer Erez,[1] Francesca Gotsch,[1] Juan Pedro Kusanovic,[1] Shali Mazaki-Tovi,[2] Edi Vaisbuch,[1] Chong Jai Kim,[3] Tinnakorn Chaiworapongsa,[1] Deborah Hoppensteadt,[4] Jawed Fareed,[4] Nandor G Than,[1] Chia-Ling Nhan-Chang,[2] Moshe Mazor,[5] Sonia S Hassan,[2] Pooja Mittal,[2] Roberto Romero.[1] *[1]Perinatology Research Branch, NICHD, NIH, DHHS, Bethesda, MD, USA; [2]Dept of OB/GYN, Wayne State University, Detroit, MI, USA; [3]Dept of Pathology, Wayne State University, Detroit, MI, USA; [4]Dept of Pathology, Loyola University Health System, Maywood, IL, USA; [5]Dept of OB/GYN, Soroka Medical Center, Beer Sheva University of the Negev, Beer-Sheva, Israel.*
Objective: Fetal demise can lead to a consumptive coagulopathy ("*fetal death syndrome*") traditionally attributed to the release of "*tissue thromboplastin*", now known as "*tissue factor*" (TF). TF is the most potent activator of coagulation. Despite the appeal and acceptance of this proposed pathophysiology, there is no evidence supporting this view. This study was undertaken to determine if fetal death prior to development of fetal death syndrome is associated with changes in maternal plasma concentration of CD40L (a marker of platelet activation), TF and its soluble inhibitor (TFPI).
Methods: A cross-sectional study included the following groups: 1) women with normal pregnancy (n=71) and 2) patients with fetal demise without disseminated intravascular coagulation (n=50). Plasma concentrations of sCD40L, TF and TFPI were measured by ELISA. Standard coagulation tests were performed. Non-parametric statistics were used for analysis.
Results: 1) Patients with fetal demise had a higher median maternal plasma sCD40L concentration than women with normal pregnancy (median 1213.3 pg/ml, range 118-3818 vs. median 369.5 pg/ml, range 63.5-1848.7, p<0.001); 2) there was no significant difference between the groups in the median maternal plasma TF concentration and 3) in contrast, the median maternal plasma TFPI concentration was significantly lower in patients with fetal demise than in women with normal pregnancy (median 45.6 ng/ml, range 13.6-115.2 vs. median 66.7 ng/ml, range 37.4-86.5, p<0.001).
Conclusions: 1) A change in the plasma concentration of TF was not demonstrated; 2) a change in the ratio of TF/TF inhibitor pathway may predispose to thrombin generation and activation of the coagulation cascade; 3) however, maternal platelet activation is present in patients with a fetal demise without fetal death syndrome and 4) the role of TF in fetal death syndrome remains to be proven.

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Lipoic Acid Inhibits Matrix Metalloproteinase 9 Production, Activity and Prostaglandin E_2 Secretion by Cultured Amnion Epithelial and Mesenchymal Cells. R Moore, J Novak, D Kumar, J Moore. *Case Western Reserve University, Cleveland, OH, USA.*
Introduction: Cytokines, free radicals, matrix metalloproteinases (MMP) and prostaglandins (PG) have been implicated in processes of fetal membrane rupture and labor. Dietary anti-oxidant supplementation has been suggested as a possible therapy for high risk patients, however, clincal evidence supporting the efficacy of agents such as vitamin C or N-acetylcysteine remains controversial. In fact, we have previously shown that vitamin C increases matrix metalloproteinase (MMP) 9 activity in isolated fetal membrane fragments and fails to inhibit Tumor Necrosis Factor (TNF) induced fetal membrane weakening *in vitro*. In this study, we examine the effect of the naturally occurring anti-oxidant, α-lipoic acid, on TNF induced MMP9 activity/protein and PGE_2 secretion in isolated amnion epithelial and mesenchymal cells.
Methods: Amnion epithelial and mesenchymal cells were pre-treated with increasing doses of α-lipoic acid (0-1mM/6h), then with increasing doses of TNF (0-50ng/ml/24h). Medium and cells were analyzed by gelatin zymography/western blotting for MMP9/MMP2 activities/protein. PGE_2 output was determined by immunoassay.
Results: TNF induced a dose dependent increase in MMP9 production, secretion and activity in amnion epithelial cells. TNF (50ng/ml) induced an 18 fold increase in cellular active MMP9 production and 3 fold increase in secreted MMP9 enzyme activity by amnion epithelial cells. These increases were reduced 62-100% following 6h pre-treatment with 0.5-1.0mM α-lipoic acid. MMP2 protein/activity and PGE_2 secretion by amnion epithelial cells were barely detectable and unaffected by TNF and/or α-lipoic acid treatment.
In striking contrast, mesenchymal cells exhibited little basal or TNF induced MMP9 protein/activity. MMP2 protein/activity in mesenchymal cells were unaffected by either TNF and/or α-lipoic acid. However, TNF treated mesemchymal cells exhibited a dose dependent increase in PGE_2 production (13 fold increase/50ng/ml TNF/24h) that was inhibited by 70%-100% following 0.1- 1.0mM α-lipoic acid pre-treatment.
Conclusions: α-Lipoic acid inhibits MMP9 production/activity and PGE_2 secretion by amnion epithelial and mesenchymal cells. Future studies may determine whether this naturally occurring anti-oxidant might be useful in the prevention of membrane weakening and premature membrane rupture.
Supported by NIH HD048476 to JJM.

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A Positive Feed-Forward Regulatory Loop Maintains Cervical Competency during Pregnancy. Xiang-Hong Li, Doan Y Dao, Ruth A Word. *Ob/Gyn, UT Southwestern, Dallas, TX, USA.*
Objective: Nearly fifty years after the discovery of Microphthalmia-associated transcription factor (MiTF), its gene was identified as a specialized transcription factor that dictates cell-specific differentiation. Unique MiTF isoforms are generated from alternative promoter usage. An isoform of MiTF (MiTF-CX) is down-regulated in cervical stromal cells of the ripened cervix. Further, MiTF-CX inhibits IL-8 gene expression and thereby suppresses signaling of the final pathway in cervical ripening. Since MiTF binds to canonical Eboxes (CANNTG) in promoter regions of target genes, we sought to determine if MiTF regulated its own promoter through Ebox motifs. **Methods:** The 2 kb genomic DNA sequence upstream of the MITF-CX transcription start site was cloned into pGL3 luciferase reporter vectors which were co-transfected with wild type or mutMiTF-CX (impaired DNA binding) into cervical stromal cells or HEK293 cells. At 48 h, promoter activity was determined and normalized for transfection efficiency. **Results:** Gel-shift assays conducted with oligonucleotides corresponding to 11 Eboxes in the MiTF-CX promoter revealed two strong binding sites (Eboxes $3^{-983\text{ to }-977}$ and $9^{-329\text{ to }-324}$). Specific binding was established using oligonucleotides with or without mutated Ebox, antibody supershift experiments, competition with cold probe, and absence of binding to mutMiTF-CX. Binding specificities were confirmed in nuclear extracts from cells that overexpressed MiTF-CX, but not control or mutMiTF-CX. Reporter gene studies indicated that MiTF-CX, but not MiTF-M, increased MiTF-CX promoter activity 4- to 5-fold. Whereas mutations in Ebox 3 or 9 resulted in significant decreases in MiTF-stimulated promoter activity, MiTF-induced increases in promoter activity were abolished by mutations in both Eboxes. **Conclusions**: Collectively these experiments indicate that MiTF-CX is a vital regulator of its own promoter activity and acts in a positive feed-forward loop through two specific binding sites in its promoter. Moreover, isoform-specific amino acids are important to mediate MiTF-induced MiTF-CX promoter activity. Decreasing MiTF protein or mutating its promoter would interrupt this loop resulting in rapid reduction of MiTF synthesis. Since MiTF-CX suppresses IL-8 gene expression in cervical stromal cells, we suggest that preservation of MiTF-CX-induced MiTF gene expression is an important mechanism to maintain the "brake" on cervical ripening and ensure cervical competency during pregnancy.

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The Role of CD44 in Cervical Remodeling. Denisse Sanchez, Brenda Timmons, Mala Mahendroo. *Obstetrics and Gynecology, UT Southwestern Medical Center, Dallas, TX, USA.*
OBJECTIVE: Prior to the onset of parturition, the uterine cervix undergoes a remodeling process from a closed, rigid structure, to one that is soft and dilatable. Many changes occur, including increases in hyaluronan (HA), a glycosaminoglycan that facilitates loosening of the collagen matrix. In the postpartum period, the concentration of HA is reduced to that of the nonpregnant state. CD44, a transmembrane glycoprotein expressed in hematopoietic and epithelial cells, is a receptor for HA. CD44 expression by immune cells is

important in extravasation of leukocytes into tissue. CD44 may also be required for HA catabolism through the action of hyaluronidases 1 and 2. In the cervix, CD44 is expressed in the endo- cervical epithelia as well as in immune cells localized in the stromal matrix. To study the importance of CD44 in cervical remodeling, mice with a null mutation for CD44 ($CD44^{-/-}$) were evaluated during pregnancy, parturition and postpartum.

METHODS: Changes in HA amount and size distribution were assessed using HA molecular weight gels and Fluorophore Assisted Carbohydrate Electrophoresis. To identify defects in cervical remodeling in the $CD44^{-/-}$ mice, differences in expression for genes regulated in the cervix were studied by quantitative real time PCR . To determine whether CD44 plays a role in the recruitment of immune cells during cervical ripening and postpartum repair, immunohistochemistry with antibodies against leukocytes was done.

RESULTS: In the postpartum period, there is a higher ratio of high molecular weight HA relative to low molecular weight HA in the $CD44^{-/-}$ mice. This suggests that postpartum breakdown of HA in $CD44^{-/-}$ cervices is delayed. As compared to WT cervix, there was a significant increase in hyaluronidase 2 (Hyal-2) mRNA in the postpartum period. The distribution and relative numbers of immune cells in the $CD44^{-/-}$ cervix was similar to WT.

CONCLUSION: These studies provide evidence that CD44 may play a role in remodeling of the postpartum cervix back to the nonpregnant state. Our current data suggests that the catabolism of HA after birth is delayed in the mutant mice and upregulation of Hyal2 may compensate to allow HA removal. Furthermore, the activity of Hyal-2 may be dependent on CD44. Little difference in the recruitment of immune cells between CD44 and WT animals suggest that CD44 expression is not required for this process. These experiments provide a greater understanding for the role of CD44 and HA in cervical remodeling.

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In Vitro Relaxin H2 Strengthens Fetal Membranes? S Yousfi,[1] J Novak,[1] R Moore,[1] D Kumar,[1] M Perez,[1] B Mercer,[2] J Moore,[1,2] G Bryant-Greenwood.[3] *[1]Pediatrics; [2]Reproductive Biology, Case Western Reserve University, Cleveland, OH; [3]Devel & Reprod Biology, Univ Hawaii, Honolulu, HI.*

Background: Relaxin is a systemic hormone produced by the corpus luteum, decidua and placental syncytiotrophoblast. In vitro Relaxin (H2) has been reported to increase extracellular matrix (ECM) remodeling and, specifically, MMP activity in all the tissues where it has been utilized. A single study of the effect of Relaxin on fetal membrane (FM) biophysical properties showed a biphasic pattern with low dose causing mild weakening and higher doses showing no effect. We have demonstrated that towards end gestation, FM undergo a programmed, biochemically mediated weakening process with concomitant ECM remodeling and cellular apoptosis. In addition, we have reported that in vitro incubation of FM with TNF and IL-1b can reproduce the entire weakening and remodeling process. Evidence is less clear for other potential biochemical mediators. We hypothesized that Relaxin would weaken human FM by increasing ECM remodeling.

Objective: To investigate the effects of In Vitro Relaxin (H2) on FM biophysical properties.

Method: Full thickness FM fragments were incubated with or without increasing (0-100 ng/ml) doses of Human Relaxin (H2). After 1-3 days of incubation, FM fragments were strength tested using our previously published ball-burst methodology. Fragments were also tested for biochemical markers of remodeling: gelatinase activity and MMP protein. **Results:** Human Relaxin (H2) induced a dose dependent increase in FM break strength. Although the Relaxin (H2) induced increase in break strength was seen at 2 days, it was most consistent at 3 days. At the highest dose (100 ng/ml) utilized, a consistent 20% increase in break strength was observed ($P< 0.01$) with concomitant decreases in MMP 9 gelatinase activity and protein abundance. This effect was also observed at lower doses of Relaxin (H2) but to a lesser degree and inconsistently.

Conclusion: Contrary to our expectation, Relaxin, paradoxicaly increased fetal membrane strength at high doses. Mechanism(s) through which Relaxin causes this effect are unknown. Supported by # HD048476.

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Duration of Rupture of Fetal Membranes and Labor Are Both Associated with Spontaneous Separation of Fetal Membrane (Amnion & Chorio-Decidua) Components. D Kumar,[1] P Shaniuk,[1] J Smith,[1] K Bryant,[1] R Moore,[1] J Novak,[1] B Mercer,[2] JJ Moore.[1,2] *[1]Pediatrics; [2]Reproductive Biology, Case Western Reserve University, MHMC, Cleveland, OH.*

BACKGROUND: During in vitro experiments we have shown that separation of amnion from choriodecidua occurs as an integral part of the process of fetal membrane (FM) rupture. Although spontaneous amnion and choriodecidual separation is seen in FM after both SVD and elective C/S deliveries, its etiology is uncertain. Biochemical degradation at the amnion-choriodecidua interface may be a key contributing factor. Our previous biomechanical studies have demonstrated that separated FM require less physical Work to Rupture than intact membranes. The purpose of this study was to determine whether FM separation was associated with clinical differences in the birth process.

HYPOTHESIS: During term, normal labor, spontaneous separation of FM is associated with differences in the clinical parameters of labor and delivery.

STUDY DESIGN: FM from consecutive term deliveries were cut off the placental disk. Separated areas of FM were cut from the intact areas. Both were weighed and their weight ratios determined. Maternal medical, pregnancy, and delivery data were collected and analyzed.

RESULTS: 221 Term FM had the following characteristics: maternal age 26±6.4 yr, gravida 3.1±2.2, gestation 39±1.1 wks, Elec. CS 10.0%, duration of ROM 403±417 min, duration of contractions 642±440 min, African American 45%. 40% of the FM had <10 % separation; 11% had more than 95 % separation. SROM FM with >10% separation (vs. <10%) had significantly shorter duration of ROM ($p=0.03$) and Admission to birth ($p=0.05$) times. SROM FM with >95% separation (vs. <10%) had even shorter ROM ($p=0.006$), Duration of Contractions ($p=0.002$) and Admission to Birth ($p=0.0003$). The >95% group (vs. <10%) was further along, gestationally ($p=0.05$). SROM FM (vs. AROM) had shorter Admission to birth ($p=0.008$), but longer ROM to birth ($p<0.0001$) times. Absence of epidural ($p=0.001$), SROM mode of rupture ($p=0.04$), SVD (vs. Elec. C/S) ($p=0.06$), and the presence of meconium ($p=0.002$), were all associated with increased FM separation.

CONCLUSION: Spontaneous separation of fetal membranes is nearly universal and is associated with increased gestation, spontaneous rupture of membranes, shorter duration of contractions, and SVD.

SPECULATION: We speculate that programmed biochemical changes initiate FM separation which then facilitates ROM and childbirth.

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Whole Genome Array and si-RNA Investigation of the Function of NFKB in Human Amnion Epithelial Cells. Sheri E Lim,[1] Shirin Khanjani,[1] Yun S Lee,[1] TG Teoh,[1,2] Philip R Bennett.[1] *[1]Institute of Reproductive and Developmental Biology, Imperial College, London, United Kingdom; [2]Maternal & Fetal Medicine, St. Mary's Hospital, London, United Kingdom.*

Introduction: Labour is associated with activation of NFkappaB in the amnion. NFkappaB increases prostaglandin synthesis through the upregulation of cyclooxygenase-2 (COX-2), which is essential to the labour process. COX-2 mRNA expression increases with gestation in the amnion. Primary amnion epithelial cells cultivated from tissue collected prior to the onset of labour display a spectrum of NFkappaB activation, similar to the spectrum of COX-2 expression presumably relating to the nearness of labour. Our aim was to investigate the full range of genes under NFkappaB control in amnion epithelial cells by using whole genome arrays.

Methods: Amnion from 20 women undergoing elective caesarean section was collected and primary cell cultures established. Total RNA and protein were extracted from each culture. Nuclear localization of NFkappaB is required for its activation. Western analysis of nuclear p65 was therefore performed to identify the samples displaying the lowest and highest NFkappaB activity. The corresponding RNA samples displaying the three lowest and three highest nuclear p65 protein concentrations were used for whole genome analysis using Affymetrix U133 arrays.

Results and Conclusions: We identified 919 significantly regulated genes. The gene with the highest fold change was COX-2 (x44.4) followed by oxytocin receptor (x24.1), Ch10ORF (x19.6), integrinA2 (x17.7), and connective tissue growth factor (x14.9). Other significant genes included Interleukin-8 (IL-8) (x6.3). Pathway analysis revealed the majority of other NFkappaB associated genes were involved in cell signaling, turnover and proliferation. We used real-time PCR (RTQ-PCR) to validate COX-2 and IL-8 expression. To prove that COX-2 is directly regulated by NFkappaB, primary amnion epithelial cells were then transiently transfected with NFkappaB p65 siGENOME SMART pool and siCONTROL non-targeting siRNA Pool. Western blot analysis confirmed knockdown of NFkappaB p65 associated with inhibition of COX-2 demonstrating that NFkappaB is essential for COX-2 expression.

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Characterization of the Sub-Failure Structure-Strength Behavior of the Fetal Membrane. Erinn M Joyce,[1] Michael S Sacks,[1] John J Moore.[2] *[1]Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA; [2]Departments of Pediatrics and Reproductive Biology, Case Western Reserve University, Cleveland, OH, USA.*

Objective: Premature birth is a major public problem accounting for over 13,000 deaths and 30,000 surviving infants with life-long morbidity yearly. In order to develop a rational basis for treatment and prevention of premature fetal membrane (FM) failure, we first need to understand the sub-failure FM structural and mechanical behavior at near full term.

Methods: We utilized planar biaxial mechanical testing, which approximates the physiologic loading state, for mechanical evaluation of the FM, and a structural constitutive model approach was used to offer insight into the structure-strength of the FM by integrating information on tissue composition and structure. Small angle light scattering (SALS) was used to nondestructively quantify the collagen fiber architecture of both intact and separated FM layers.

Results: In the stress free state, the gross collagen fiber architecture of the FM and separated layers were not homogenously align but exhibited small regions of fiber alignment. The amnion layer displayed the greatest alignment. The model fit the equi-biaxial strain data well ($r^2 = 0.99$) and indicated that FM collagen fibers were rapidly recruited and straightened well below failure stress levels. Collagen fibers were gradually recruited followed by a drastic increase in fiber recruitment.

Conclusion: This study provided the first data on the effective collagen fiber stiffness in the intact FM under physiologic biaxial loading, which was related to quantitative collagen fiber architectural measures. Modeling results indicated that the collagen fibers became fully loaded and straighten well below physiological loading levels. Failure did not occur during physiological loading, indicating that fibers do not begin to fail until all collagen fibers are fully straightened and bearing load. This result suggested modest structural reserve in the FM collagen architecture, and *may be an important aspect of its failure properties*. Previously, we demonstrated that a physically "weak zone" exists overlying the cervix in the FM, evident of collagen remodeling and cellular apoptosis. We are currently extending the present study to include the "weak zone" tissues, allowing us to elucidate the micro-mechanical mechanisms that facilitate failure in this newly identified FM zone. Supported by NIH 48476.

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Transcriptional Regulation of Hyaluronan Synthesis in the Cervix. Anjana Tiwari, Mala Mahendroo. *OB/GYN, UT Southwestern Medical Center, Dallas, TX, USA.*

OBJECTIVE: The extracellular matrix of the cervix undergoes extensive remodeling during parturition. Hyaluronan (HA) is a major constituent of the extracellular matrix of the term pregnant cervix. The onset of labor is preceded by an increase in HA and after delivery, the concentration of cervical HA gradually decreases to that of the non-pregnant state. These dramatic changes suggest that HA plays an important role during parturition. Hyaluronan synthase 2 (HAS2) is one of three known HA synthases and the most abundant isoform in the pregnant cervix. Transcripts for HAS2 are regulated by two alternative promoters, one upstream of the first coding exon (proximal promoter) and another upstream on an untranslated exon 1 (distal promoter). The focus of the current study is to further our understanding of the transcriptional regulation of HAS2 during cervical ripening.

METHODS: RNA blotting was carried out using transcript specific probes corresponding to the distal and proximal promoter of the mouse HAS2 gene. The regulation of HAS2 was evaluated in a cervical epithelial cancer cell line (Caski cells) which we have previously shown to express endogenous HAS2. Regulation of HAS2 expression by epidermal growth factor was assessed in the Caski cells by western blotting and quantitative real time PCR assessment of transcripts.

RESULTS: HAS2 mRNAs in the nonpregnant (NP) and pregnant cervix are transcribed from the distal promoter upstream of exon 1. Two transcripts of approximately 4.1kb and 2.8kb were detected that arise from use of 2 polyadenylation sequences. As compared to NP, the expression of HAS2 is increased 4, 23, and 9 fold on gestation days 15, 18 and shortly postpartum respectively. HAS2 mRNA expression is increased upon treatment of Caski cells with epidermal growth factor (30ng/ml) and is suppressed in cells treated with AG1478 (20μM), an inhibitor of EGF receptor phosphorylation. Maximal stimulation was observed at 4 and 8 hours of treatment.

CONCLUSION: HAS2 is the major HA synthase expressed during cervical ripening and the majority of transcripts are driven by the distal promoter in the HAS2 gene. In vitro studies using Caski cells suggest HAS2 is regulated in part by the EGF signaling pathway resulting in a several fold increase in HAS2 expression. These results provide an understanding of HAS2 gene regulation at the time of cervical ripening which will ultimately enhance our understanding of the molecular mechanisms important to cervical ripening.

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Cigarette Smoke Extract Causes Cell Death in Cultured Human Chorion and Decidua Cells. Chad A Grotegut, Bernard J Canzoneri, Liping Feng, Phil Heine, Amy P Murtha. *Obstetrics and Gynecology, Duke University, Durham, NC, USA.*

Objective:

Preterm premature rupture of the fetal membranes accounts for approximately 30% of all preterm deliveries. Cigarette smoking independently carries a four-fold increase risk for PPROM. Our laboratory has previously demonstrated that the chorion layer undergoes apoptosis in women with PPROM. This study was conducted to determine if extract of cigarette smoke causes cell death in specific cells of the fetal membrane.

Methods:

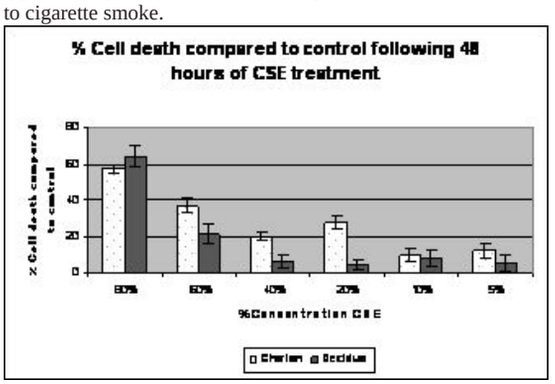
Fetal membranes were collected at the time of elective cesarean section from women without labor and at term. The chorion and decidua layers were separated and purified on a gradient spin column and then plated near confluence. Cigarette smoke extract (CSE) was collected in cell media and used to treat chorion and decidua cells in culture at concentrations ranging from 1% to 100% in 96-well plates. Cell viability was determined at 24, 48 and 72 hours following treatment with a non-radioactive cell viability assay. Data were analyzed using paired t test (Analyse-It, Leeds, UK).

Results:

Chorion and decidua cells underwent cell death when exposed to CSE in a dose dependent fashion. Increasing concentrations of CSE resulted in increased cell death at 48 hours in both cell types (Figure 1). At 48 hours, chorion exhibited greater percent cell death compared to decidua at concentrations of 20, 40 and 60% CSE (p=0.003, 0.019, and 0.069, respectively). For any given concentration of CSE, the degree of cell death increased with increasing length of exposure (36, 48 and 72 hours) for each cell type. Chorion cells routinely exhibited greater percentage of cell death following treatment with CSE at concentrations ranging from 20-60% compared to decidua cells.

Conclusions:

Human chorion and decidua cells in primary cell culture exhibit a dose-response and time dependent cell death in the presence of CSE. Human chorion cells show greater sensitivity to cell death when compared to decidua cells. Further studies are needed to determine the mechanisms through which these cell types undergo cell death and the implications for the differential sensitivity to cigarette smoke.



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Changes of Lipid Peroxidation and Protein Carbonyls Formation in the Amniotic Fluid of Women with Preterm Premature Rupture of Membranes. Yoon Ha Kim,[1] Tae-Bok Song,[1] Cheol Hong Kim,[1] Jong Woon Kim,[1] Moon Kyoung Cho,[1] Sung Yeul Yang,[2] Bong Whan Ahn.[2] *[1]Obstetrics & Gynecology, Chonnam National University Medical School, Gwangju, Korea; [2]Biochemistry, Chonnam National University Medical School, Gwangju, Korea.*

Objective: To investigate the lipid peroxide levels and protein carbonyls levels in the amniotic fluid of pregnant women with preterm premature rupture of membranes (PPROM).

Materials and methods: The lipid peroxide levels in the amniotic fluid of normal pregnancy (n=20) and pregnant women with PPROM (n=20) were

measured by thiobarbituric acid reaction. The protein carbonyl contents in the amniotic fluid of normal pregnancy (n=20) and pregnant women with PPROM (n=20) were determined by the 2,4-dinitrophenylhydrazine method. After amniotic fluid of them were mixed and incubated up to 5 hours with 0.2ml of 1mM moxalactam, cefodizime, amoxacillin, erythromycin, the lipid peroxide levels and protein carbonyl contents in them were measured.
Results: 1. The lipid peroxide levels in the amniotic fluid of pregnant women with PPROM was significantly higher than that of normal pregnancy (9.74±0.48 vs. 7.20±0.38 nmol/mg protein, p<0.01). 2. The protein carbonyl levels in the amniotic fluid of pregnant women with PPROM was significantly higher than that of normal pregnancy (13.0±0.33 vs. 11.27±0.17 nmol/mg protein p<0.01). 3. The lipid peroxide levels and protein carbonyls formation by moxalactam in the amniotic fluid of pregnant women with PPROM was significantly higher than basal level (12.08 ± 0.81 vs. 9.74±0.48 nmol/mg protein, 20.08 ± 0.66 vs. 13.0±0.33 nmol/mg protein, p<0.01). 4. The lipid peroxide levels and protein carbonyls formation by cefodizime in the amniotic fluid of pregnant women with PPROM was significantly lower than basal level (5.04 ± 0.33 vs. 9.74±0.48 nmol/mg protein, 9.76 ± 0.35 vs. 13.0±0.33 nmol/mg protein, p<0.01). 5. There were no significant differences in the levels of lipid peroxide and protein carbonyls by amoxacillin and erythromycin in the amniotic fluid of pregnant women with PPROM between antibiotics-induced and basal levels.
Conclusion: The lipid peroxidation and the protein carbonyls formation were increased in the amniotic fluid of pregnant women with PPROM. Antibiotics-induced lipid peroxide and protein carbonyl levels were changed in the amniotic fluid of pregnant women with PPROM. Further studies on our results may be beneficial in the selection of antibiotics for pregnant women with PPROM.

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Regulation of Matrix Metalloproteinase-2 and -9 in Term Decidual Cells: Implications for Chorioamnionitis-Induced Preterm Delivery. Ceyda Oner, Frederick Schatz, Yesim Uz, Umit A Kayisli, Lynn Buchwalder, Mizanur Rahman, Aydin Arici, Charles J Lockwood. *Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Background: Chorioamnionitis (CAM) is a major antecedent of preterm delivery (PTD) associated with elevated amniotic fluid TNFα and IL1B. We hypothesized that these cytokines enhance the term decidual cell (DC) expression of the matrix metalloproteinases (MMP) 2 and 9, which can then promote PTD by degrading the extracellular matrix of the decidua, fetal membranes, and cervix.
Methods: Immunostaining for MMP-2, MMP-9, and vimentin (a DC marker) was performed on CAM-complicated (n=5) and gestational age-matched control decidua (n=5), and staining intensities were evaluated by HSCORE. Confluent, leukocyte-free term DCs were primed with 10-8 M estradiol (E2) or E2 + 10-7 M medroxyprogesterone acetate (MPA), and then switched to a defined medium with E2 +/- MPA with or without 1 ng/mL of IL1B or TNFα. Secreted MMP-2 and MMP-9 levels were measured by ELISA (n=8), and quantitative RT-PCR assessed MMP-2 and MMP-9 mRNA levels (n=4).
Results: Tissue staining revealed that MMP-2 and MMP-9 levels in CAM-complicated decidua (HSCORE mean±SEM: 203±14 and 198±12, respectively) were significantly higher than in control decidua (136±6, and 114±6 respectively; p<0.05). In cultured term DCs incubated with E2, TNFα and IL1B significantly increased secreted levels of MMP-9 compared to E2 alone (pg/mL/μg protein: 162.3±110.1 and 20.4±6.5, respectively, vs. 0.5±0.2; p<0.05). In parallel incubations with E2+MPA, basal MMP-9 output was lowered by 50%, and TNFα- and IL1B- elicited MMP9 levels were blunted by 70% and 40%, respectively. RT-PCR confirmed that TNFα and IL1B increased MMP9 mRNA levels (p<0.05), although mRNA levels in E2+MPA incubations were not different from those of E2 alone. MMP2 levels in all treatments were similar.
Conclusions: MMP-2 and MMP-9 are elevated in CAM decidua compared to controls. Our in vitro results suggest that MMP-9 expression is enhanced by the high levels of IL1B and TNFα associated with CAM, and that MPA may be able to blunt this effect. We have previously found a similar regulatory mechanism of MMP-1 and MMP-3 and their over-expression in CAM-complicated tissues. Synergy among these MMPs may represent a potent pathogenic mechanism of CAM that can be targeted through the therapeutic use of progestins in preventing CAM-induced PTD.

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Localization and Expression of Monocyte Chemotactic Protein (MCP) 1 in Nonpregnant, First Trimester and Term Pregnant Human Cervix. Domerudee Preechapornprasert,[1] Patama Promsonthi,[1] Wasun Chantratita,[2] Mana Rochanawutanon,[2] Patcharee Karnsombut,[2] Chutatip Srichunrusami,[2] Stephen J Lye,[3] Boonsri Chanrachakul.[1] *[1]Obstetrics and Gynecology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [2]Pathology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [3]Obstetrics and Gynecology, Samuel Lunenfeld Research Institute, Toronto, Canada.*
Objective: Cervical ripening is an inflammatory process involving chemokines, cytokines and various mediators. Recent study has shown that the level of monocyte chemotactic protein (MCP) 1, a chemokine, increases in amniotic fluid during spontaneous labor. The aim of this study was to examine the localization and expression of MCP 1 in human cervix before pregnancy, during pregnancy and after the onset of labor.
Methods: This study was approved by the Local Ethics Committee and written informed consent was obtained from each participant. Cervical biopsies were taken from 4 groups of women; nonpregnant women, first trimester pregnant women, term pregnant women with and without labor. Tissue samples were fixed in 10% formal saline for paraffin section. Immunohistochemistry (n = 5 each) was performed by avidin biotin complex (ABC) technique using monoclonal antibody specific to human MCP 1. The MCP 1 messenger(m) RNA was identified by reverse transcription-polymerase chain reaction using gene specific primer against MCP 1 and MCP 1 receptor (n = 10 each).
Results: Immunohistochemistry demonstrated MCP 1 in cervical tissues from all four groups of women. MCP 1 was localized on plasma membrane and cytoplasm of both squamous epithelial and columnar cell lining of endocervical gland. MCP 1 and MCP 1 receptor mRNA were identified in nonpregnant, first trimester and term with and without labor human cervix.
Conclusion: MCP 1 and MCP 1 receptor were located in cervical tissues of nonpregnant and pregnant women at different gestation both before and after the onset of labor.Ongoing studies are investigating the role of this chemokine during pregnancy and labor.

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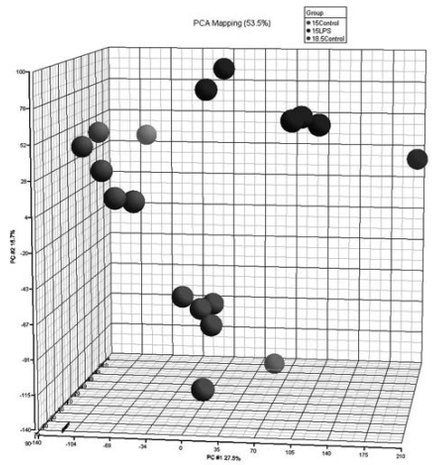
Preterm and Term Cervical Ripening: Similar or Divergent Molecular Mechanisms? Juan M Gonzalez, Hua Xu, Michal A Elovitz. *OBGYN: CRRWH, Univ. of Pennsylvania, Philadelphia, PA, USA.*
Introduction: While premature cervical ripening is implicated in preterm birth (PTB), the mechanisms of cervical ripening remain poorly understood. Whether preterm cervical ripening is just an aberrant regulation in timing or whether divergent mechanisms and pathways are involved in preterm versus term cervical ripening remains to be elucidated.
Methods: Cervical tissue was collected from 3 groups of CD-1 mice. **Group 1**: mouse model of PTB that utilizes intrauterine infusion of lipopolysaccharide (LPS). **Group 2**: E15 dams representing preterm controls. **Group 3:** E18.5-19 dams selected from a timed pregnant batch where half of the dams had delivered representing term cervical ripening. N=6 dams/treatment group. 18 separate RNA samples were used for microarray analysis (MA). Significance Analysis for MA and Partek software was used for biostatistical analysis. Pathway analysis was performed using DAVID. Quantitative PCR was performed to confirm the most differentially regulated genes.
Results: Using a cut-off of 64-fold change with P value of <0.0001, 368 genes in the cervix were differently regulated between the 3 groups. Principal component analysis revealed three distinct groups (SEE GRAPH). Functional annotation clustering demonstrated the following pathways: 1) In preterm cervical ripening (E15 LPS vs E15): immune and inflammation response, defense response 2) in term cervical ripening compared to preterm controls: negative regulation of cellular process and biological process, ECM, cell-cell communication. QPRC confirmed the highly significant differences found in MA (SEE TABLE).
Conclusions: The molecular mechanisms and pathways governing preterm and term cervical ripening are distinctly different. Elucidating these unique pathways can lead to improved therapeutics for prevention of PTB as well as for postdate pregnancies.



QPCR Confirmation of Genes Involved in Cervical Ripening (fold difference in MRNA expression)

Gene	Preterm E15 LPS/E15	Term E 15 LPS/E 18.5
IL-6	71	-1.3
TNF	17.8	-5.3
chemokine ligand 10	162.7	-3.8
Pentraxin related gene	64.4	-1.96
S100 calcium binding protein A8 (calgranulin A)	52.6	1.4
S100 calcium binding protein A9 (calgranulin B)	102.9	-1.1
desmoglein 1 alpha	-1.4	26
desmoglein 1 beta	-1.6	19.8
Keratin 16	121	376

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Cervical Ripening at Term Involves Activation of Apoptotic Enzymes. Maria KB Sennstrom,[1] Valentina Ciani,[2] Gunvor E Ekman.[1] *[1]Obstetrics and Gynecology, Women and Child Health, Karolinska Institute, Stockholm, Sweden; [2]Obstetric and Gynecology, University of Siena, Siena, Italy.*

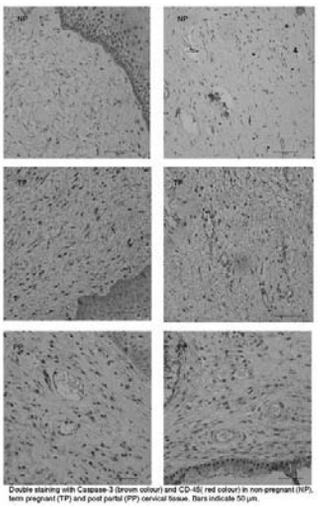
Aim: to investigate if the human cervical ripening at term involves programmed cell death.

During the final cervical ripening the extracellular matrix dominated cervix undergoes an extensive remodelling of the tissue. Inflammatory mediators such as the cytokines increase in ripening cervical tissue at term. Programmed cell death, apoptosis, has been suggested as important in this process. Apoptosis can be induced by inflammatory mediators such as cytokines. We also looked upon the distribution of inflammatory cells in cervical tissue.

Materials and Methods: Cervical biopsies from pregnant women at term and post partum women with fully ripened cervix were studied. Biopsies from non-pregnant women served as controls. Immunohistochemical analysis of the apoptotic enzyme Caspase-3 and the inflammatory cell marker CD 45 was performed on paraffin embedded sections of cervical tissue. Double staining was performed. Mann-Whitney U-test was used for statistical analysis.

Results: There was a significantly higher frequency of Caspase-3 staining in the post partal sections from ripened cervical tissue compared to tissue from term pregnant (p=0.002) with unripe cervix and from non-pregnant patients (p=0.00067). The inflammatory cells staining for CD 45 increased in post partal and term pregnant sections compared to non-pregnant (p=0.016). There was a higher frequency of Caspase-3 positive cells in the post partal tissue than of CD45 positive cells (p=0.00011). The localization of CD-45 positive staining was highest in the epithelia and basal lamina while Caspase-3 staining was most pronounced in stromal tissue and around vessels.

Conclusion: Our data show apoptotic activity in stromal tissue in fully ripened human cervix at term of pregnancy, suggesting that apoptotic mechanisms are involved in the extracellular matrix remodelling at term. The apoptotic activity is not co-localized with inflammatory cells suggesting non-infectous inflammation with apoptosis as important for cervical ripening at term.



Double staining with Caspase-3 (brown colour) and CD-45 (red colour) in non-pregnant (NP), term pregnant (TP) and post partal (PP) cervical tissue. Bars indicate 50 µm.

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Measurement of the Stress Relaxation Response to Tension in the Pregnant Rat Cervix. Julie M Johnson,[1] Pradeep Guduru,[2] Kristin Myers,[3] Simona Socrate,[3] Michael House,[4] Huiling Ji,[1] Vit Long,[1] Edward K Chien.[1] *[1]Department of Ob/Gyn, Division of MFM, Brown University, Women & Infants Hospital of Rhode Island, Providence, RI, USA; [2]Division of Engineering, Brown University, Providence, RI, USA; [3]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA; [4]Dept. of Ob/Gyn, Division of MFM, Tufts- New England Medical Center, Boston, MA, USA.*

Objective: Cervical biomechanical responses are important for accommodating the increased stress induced by an enlarging uterus. A mechanical testing system was modified to evaluate the stress relaxation response in the pregnant cervix.

Methods: Tissue harvested from the non pregnant and timed pregnant (days 12, 16, 20, 21, and 22) Sprague-Dawley rats underwent tensile testing using an Instron 5800 Material testing system. The testing regimen consisted of tissue extension to near maximal strain over 60 seconds followed by a period of constant strain for 60 minutes, then return to rest over 60 seconds. This cycle was repeated 2 additional times with a 60 minute rest period between cycles. Strain and force measurements were recorded at 1 second intervals. 4-6 animals were used for each time point. In addition, stress and strain at the yield point was also determined for each gestational time point.

Results: The pregnant and non pregnant samples exhibit marked differences in response in both stress and strain. The timed pregnant tissue demonstrated progressively increased compliance and lower nominal stress compared to non pregnant tissue. Peak nominal stress declined with each successive cycle. This was demonstrated in the peak nominal stress and strain values at the yield point.

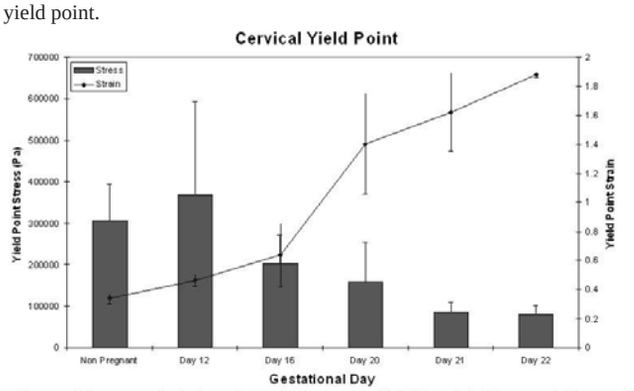

Figure. Stress and strain values at the cervical yield point. Bars and diamonds represent means and error bars standard deviation. Values are based on 3-6 animals for each time point. PA-pascals.

Conclusion: The cervix becomes more distensible (compliant) but less resistant to force with increasing gestational age.

Funding: Salomon Research Award Brown University & Women and Infants Hospital Research Fund; PHS NIH-NCRR P20 RR018728 & P20 RR017695.

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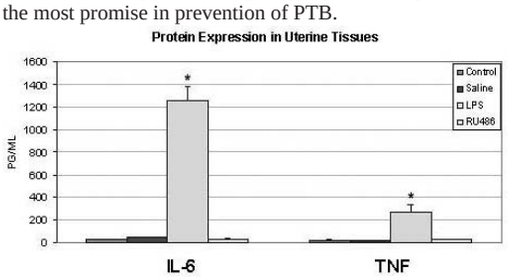
A Cytokine Response in the Uterus Is Not Necessary for Preterm Birth. Juan Gonzalez, Hua Xu, Ella Ofori, Michal A Elovitz. *OBGYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

Introduction: A growing body of evidence supports that inflammatory processes are implicated in spontaneous preterm birth (PTB). Using an inflammatory and non-inflammatory mouse model of PTB, we sought to determine if activation of these inflammatory pathways are essential for PTB and/or cervical ripening to occur.

Methods: Timed pregnant CD-1 mice were used in these two models of PTB: 1) a model of intrauterine inflammation where lipopolysaccharide (LPS) is injected into the uterine horn (n=6); controls for this model received intrauterine saline (n=6) and 2) a non-infectious model of PTB using RU486 SQ (150ugrams/dam) (n=5); controls for this model received no intervention (n=6) were used for these studies. For both models, 6 hours later uterine and cervical tissues were harvested. The tissues were processed for protein and RNA studies. ELISAs were performed to assess IL-6 and TNF-alpha in the uterine tissue. MRNA expression of IFN γ, IL-6, IL-1β, IL-10, TNF-alpha were assessed in cervical tissue from both models by quantitative PCR.

Results: In uterine tissues, both IL-6 and TNF were significantly elevated in the LPS-induced PTB when compared to control, saline, and non-infectious-induced preterm birth (P<0.001) (FIGURE 1). In cervical tissue, an increase in IL-6, IL-1beta and TNF mRNAwas observed in both models, while IFN-gamma and IL-10 were only increased in the LPS model. (FIGURE 2).

Conclusions: Up-regulation of pro-inflammatory cytokines in the uterus do not appear to be essential for PTB. Cytokine expression in the cervix is greater in an inflammatory model of PTB but is also present in a non-infectious model. These studies suggest that targeting a cytokine response in the cervix may hold the most promise in prevention of PTB.



Fold Change in Cytokines in Cervical Tissues		
	LPS/Saline	RU486/Controls
IFN-γ	10.3*	0.63
IL-6	68*	6.6*
IL-1β	15.7*	5.9*
IL-10	24*	1.8
TNF-α	12*	3.1*
*P value <0.05		

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Fetal Macrophages Increase in Mouse Amniotic Fluid during Late Gestation and Manifest Characteristics of Pulmonary Origin. Alina Montalbano, Carole R Mendelson. *Biochemistry and Obstetrics-Gynecology, UT Southwestern Medical Center, Dallas, TX, USA.*

The initiation of labor at term and preterm is associated with an inflammatory response, with increased interleukins in amniotic fluid (AF) and infiltration of the myometrium by neutrophils and macrophages (MΦ). Whereas, in preterm labor, intra-amniotic infection may provide the stimulus for increased AF interleukins and inflammatory cell migration, the stimulus for these events at term has remained uncertain. In studies using pregnant mice, we observed that the MΦ that invade the maternal uterus near term arise from the fetus. Furthermore, we obtained compelling evidence that surfactant protein-A (SP-A), a developmentally regulated C-type lectin secreted by the fetal lung into AF near term, activates AF MΦ, which migrate to the uterus where they promote an inflammatory response culminating in labor. We propose that interactions of MΦ surface receptors with SP-A, at term, or bacterial lipopolysaccharide at preterm, initiate changes in MΦ phenotypic properties, resulting in the enhanced expression of genes that promote their migration to the uterus. The objectives of the present study were to analyze the numbers and phenotypic properties of mouse AF MΦ during late gestation and to identify their putative tissue source(s) of origin. To assess changes in the number of MΦ in AF during late gestation, AF cells were isolated and stained for the MΦ marker F4/80. The density of adherent F4/80$^+$ cells greatly increased in equivalent volumes of AF between 15.5 and 18.5 days postcoitum (dpc) (19.5 dpc = term). Interestingly, the F4/80$^+$ cells at 18.5 dpc were highly similar in morphology to those present in 18.5 dpc fetal lung, but distinctly different from those in fetal liver, suggesting their pulmonary origin. The AF MΦ were foam cell-like, suggesting the presence of lipid inclusions, a property shared by adult alveolar MΦ. To further analyze gestational changes in the MΦ population(s) in mouse AF, we used flow cytometric analysis. In our initial studies, cells isolated from AF were stained for F4/80 and for CD45, a pan-leukocyte marker. We observed that AF from 15.5 and 18.5 dpc mice contained two sub-populations of CD45$^+$F4/80$^+$ cells. Studies are in progress to analyze these AF MΦ populations for expression of cell surface antigens indicative of their maturity, activation state and chemotactic properties in association with the developmental induction of SP-A synthesis and secretion by the fetal lung.

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Progesterone Receptors (PRs) and Inflammation-Related Gene Products in the Human Cervix during Pregnancy, Cervical Ripening, and Parturition. April Bleich, Patrick Keller, R Ann Word. *Ob-Gyn, UT Southwestern, Dallas, TX, USA.*

The role of PRs, proinflammatory cytokines, cell adhesion molecules, COX-2, and toll-like receptors in mediating cervical ripening prior to labor is not clear. The objective of this study was to quantify PR isoforms and determine the relative expression of certain inflammation-related genes in cervical stroma from nonpregnant and pregnant women in early gestation (EG), term before and after cervical ripening, and during labor. **Methods:** Standard curves of PR-B, -A, PRA+B and qPCR were used to quantify total and PR-B in cervical stroma from 9 nonpregnant (proliferative, n = 4; progestin treatment, n = 5) and 35 pregnant women undergoing hysterectomy (EG, n = 7; term before ripening, n = 10; after ripening, n = 7; in labor, n = 11). Cervical status was determined by modified Bishop scoring. **Results**: Total PR expression was maximal in nonpregnant women in the proliferative phase (47.6 ± 8.7 pg/ug cDNA) and decreased 80% by progestins (8.6 ± 2.7 pg/ug cDNA). This level was maintained in stroma from pregnant women before labor (6.6 ± 1.3, EG.; 9.9 ± 1.8 before ripening; 12.5 ± 2.9 after ripening pg/ug cDNA). In contrast, total PR was decreased significantly in the dilated cervix (2.4 ± 0.6 pg/ug, $P < 0.05$). Interestingly, whereas PR-B was 30 ± 2% that of total PR in the nonpregnant cervix, PR-B mRNA levels were 52 ± 4% to 70 ± 6% in cervical tissues from all pregnant women and did not vary with labor status. Using immunoblot analysis and PR-specific antibodies (PgR1294), PR-B immunoreactivity was 50% that of total PR in all samples from pregnant women, and both PR-A and -B were downregulated significantly in the dilated cervix (from 44 ± 9 to 19 ± 8 units/atub). Decreased expression of PR in the dilated cervix was accompanied by significant increases in IL-8, MCP-1, TLR-2, CD62L, COX-2, and S100A9 mRNA (all $P < 0.05$, ANOVA) but not CD11b or TLR-4. PGDH mRNA was decreased significantly in the dilated cervix. With the exception of COX-2, expression of these genes was similar before labor regardless of cervical ripening. **Conclusions:** Both PR and PR-B are decreased proportionately in the dilated cervix, but not during cervical ripening. Further, a number of inflammatory gene products are increased dramatically in the cervix during labor, but not before. Taken together, the results suggest that cervical ripening is distinct from cervical dilation and involves upregulation of COX-2 but not IL-8, MCP-1, or toll-like receptors.

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Association between Interleukin-6 (IL-6) and IL-6 Receptor (IL6-R) Haplotypes and Amniotic Fluid IL-6 Concentrations in Preterm Birth. Digna R Velez,[2] Ramkumar Menon,[1] Stephen J Fortunato,[1] Scott M Williams.[2]
[1]The Perinatal Research Center, Centennial Women's Hospital, Nashville, TN, USA; [2]Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA.

OBJECTIVE: To examine association of amniotic fluid Interleukin 6 (IL-6) concentration with IL-6 and its receptor IL6-R haplotypes in term and preterm Caucasians (C) and African Americans (AA) samples.

METHODS: In this study case (preterm birth -PTB [<36 weeks]) and control (term [>37weeks]) amniotic fluid (AF) IL-6 concentrations were analyzed for association with haplotypes of the IL-6 and IL6R genes in AA and C separately. In IL-6, eight, and in IL-6R, 22 single nucleotide polymorphisms (SNPs) were examined. AA and C maternal and fetal genotypes were assessed (AA:35 maternal:cases-57 controls-34 fetal: cases-54 fetal controls-; C: maternal cases-88, controls-40, fetal:cases-86; controls=36). Haplotype associations were performed by using a sliding window with outcome IL-6 concentration. Analyses were performed separately on maternal and fetal DNA.

RESULTS: The strongest haplotype associations were observed in IL-6R rather than in IL-6. In C fetal DNA IL-6R haplotypes defined by markers 16311-21909 bp from the transcription start site associated most strongly with AF IL-6 concentrations (global $p=1.6x10^{-3}$) and in AA maternal IL-6R haplotype markers at 16311-21909-22214-26274 (global $p=2.30x10^{-3}$ associated with IL-6 concentrations. In the C fetal cases the 16311-21909 haplotype with the highest concentration was A-G (log(cytokine) = 3.67 pg/ml). In AA maternal samples the highest concentration was observed for haplotype T-T-G-C at 16311-21909-22214-26274.This was seen in both cases and controls at (case log (cytokine) = 3.84; control log(cytokine) = 3.49 pg/ml). Significant associations from haplotype analyses converged on three regions of the IL-6R in both races. No strong differences were observed between the haplotypes of cases with and without microbial invasion of the amniotic cavity (MIAC), with the exception of AA fetal samples that showed two overlapping haplotypes in IL-6 that associated in cases with MIAC but not in cases without MIAC (-661 and -636; -636 and -237)(both with $p < 1x10^{-3}$)

CONCLUSION: Differences in the AF IL-6 concentration in PTB do not result from single SNP effect on IL-6 but are a result of complex relationships between IL-6 and IL-6R haplotypes. These associations exhibit racial disparity.

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The Role of TNF-α in Parturition. Helen Alexander,[1] Amanda Tattersall,[1] Mark Tattersall,[1] Suren Sooranna,[1] Peta Grigsby,[2] Leslie Myatt,[2] Mark Johnson.[1]
[1]Obstetrics & Gynaecology, Imperial College, London, United Kingdom; [2]Obstetrics & Gynaecology, University of Cincinnati College of Medcine, Cincinnati, OH, USA.

Introduction: Tumour necrosis factor-alpha (TNF-α) is thought to play a role in inflammation-induced preterm labour since the decidua produces TNF-α in response to bacterial products and amniotic fluid TNF-α concentrations are increased in the presence of intra-amniotic infection. The aims of this study were to (i) investigate the expression of myometrial TNF-α and its receptors in relation to the onset of preterm and term labour; (ii) to identify which intracellular pathways are activated by TNF-α; and to investigate the effect of TNF-α alone and in combination with IL-1β or IL-8 on gene expression in uterine myocytes.

Methods: Biopsies of human myometrium were taken at caesarean section from women before and after the onset of preterm and term labour and analysed for TNF-α and its receptor mRNA expression. A further 6 samples were obtained before the onset of labour (n=6) from which myocytes were isolated and cultured in 6-well plates. When cells were 85-95% confluent either TNF-α at a concentration of 1ng/ml was added to the cells for 7, 15, 30, 60 and 120min and the cells analysed by western blotting or TNF-α at concentrations of 0, 0.1 and 1ng/ml was added either alone or in combination with similar concentrations of IL-1β or IL-8 to cells for 6 hours and mRNA was extracted and converted to cDNA to determine IL-8 and GAPDH gene expression by qPCR.

Results: There was no change in TNF-α mRNA expression in relation to the onset of labour, but the expression of TNFR1 and TNFR2 mRNA levels were significantly increased with gestation and further increased with the onset of labour. Incubation of uterine myocytes with TNF-α (1ng/ml) activated all three MAPK substypes: ERK, JNK, and p38, activation peaked between 7-15 minutes. Preliminary data suggest that TNF-α induces IL-8 mRNA expression but exposure to IL-1β or IL-8 itself did not enhance this response.

Conclusions: Although there is no significant increase in TNF-α concentration from baseline during labour we have shown TNF receptor mRNA levels do increase with labour at term. Exposure of isolated uterine myocytes to TNF-α causes activation of all MAPK subtypes and an increase in IL-8 mRNA expression. This enhanced MAPK-dependent IL-8 expression at term may be mediated via increased myometrial sensitivity to TNF-α through increased TNF receptor expression.

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Leukocytes in the Cervix and Peripheral Blood during Cervical Remodeling. Brenda C Timmons,[1] Anna-Marie Fairhurst,[2] Mala S Mahendroo.[1]
[1]Obstetrics and Gynecology, UT Southwestern Medical Center, Dallas, TX, USA; [2]Immunology, UT Southwestern Medical Center, Dallas, TX, USA.

OBJECTIVE: The molecular mechanisms involved in cervical ripening are not well understood. Immunohistochemical studies from our lab report a recruitment of inflammatory cells to the cervical stroma one day before birth in the mouse using a neutrophil/monocyte marker (Neutrophil 7/4). In this study, we sought to identify and quantitate inflammatory cells migrating into the mouse cervix and to determine if this recruitment was affected by changes in progesterone levels. Peripheral blood was also evaluated to see if changes in the cervix was paralleled in blood.

METHODS: Flow cytometric analysis was performed using cervical cells and peripheral blood obtained before and during cervical ripening along with 2-4h postpartum. Dispersion of cervical cells was optimized. These cells were stained with a panel of fluorescent conjugated antibodies directed against leukocyte antigens and analyzed on an LSRII flow cytometer. Cells were also sorted and stained to visualize cell morphologies. To determine the effect of progesterone on the migration of leukocytes, gestation d15 mice were treated for 13h with a progesterone receptor (PR) antagonist prior to tissue collection.

RESULTS: Neutrophils do not appear to increase in the cervix until after birth. Monocyte (Mo) numbers do increase during cervical ripening (late day 18, d18.75) and remain high through postpartum (PP). Macrophages (MØ) are present prior to cervical ripening and steady state levels are maintained during labor and PP. PR antagonist treatment on d15 resulted in a premature increase in Mo but not neutrophils or MØ. In contrast to the cervix, Mo and neutrophil numbers do not significantly increase in the peripheral blood until PP. Results from lymphocyte studies suggest a low level of B and T cells in the cervix. In the peripheral blood, B cells remain consistent through parturition and the T cells decrease by d18.75 and continue to decrease PP.

CONCLUSION: Tissue Mo are increased in the cervix during ripening. This recruitment is dependant on loss of PR function. In contrast, neutrophils are increased in the PP cervix while MØ numbers appear constant. Timing of changes in Mo and neutrophil numbers in the peripheral blood differed from that observed in the cervix suggesting quantitation of these cell types in blood is not reflective of what is occurring in the cervix during ripening, dilation and PP repair.

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Novel Interactions between NF-κB and Other Labour-Associated Transcription Factors Identified by a TF-TF Array. Shirin Khanjani, Yun S Lee, Suren R Sooranna, Mark R Johnson, Phillip R Bennett. *IRDB, Imperial College, London, United Kingdom.*

Introduction:

External stimuli lead to changes in cellular gene expression through activation of inducible transcription factors. NF-κB is a ubiquitous transcription factor classically associated with inflammation, which is activated in response to infection and proinflammatory cytokines such as those prevalent during the labour. The TF-TF Interaction Array uses a novel technology for detecting interactions between transcription factors based on binding of TFs to their own consensus DNA binding sequence.

Materials and methods:

Primary myometrial cells were grown until 90-95% confluent and stimulated with 1ng/ml IL-1β prior to nuclear protein extraction. The nuclear extracts were incubated with the provided set of biotin-labeled, double-stranded oligonucleotide probes, which represent a known library of cis-elements. During the incubation step, these TF probes bind to their specific TFs in the nuclear extract. Next, immunoprecipitation was performed using an antibody against NF-κBp65, which pulled out NF-κBp65 and any TFs bound to it, bound to corresponding cis-elements. Normal IgG was used in a parallel experiment to represent a negative control. Free cis-elements and nonspecific binding proteins were washed away and the cis-elements were finally eluted and hybridized to the Array membrane, which is spotted with different TF consensus sequences.

Results:

Table 1 shows the different TFs interacting with NF-κBp65 based on the degree of binding stimulated by IL-1β compared to no-IL-1β control.

Conclusion:

These data show that IL-1β stimulation causes NF-κB to bind to a wide variety of other treansciption factors. Of particular interest in the area of parturition is binding to the other pro-inlammatory TFs such as C/EBP and AP-1, which are known to regulate labour-associated genes in synergy with NF-κB. The lack of association between NF-κB and PR without IL-1β stimulation supports the concept that with the onset of labour inflammation leads to functional progesterone withdrawal, rather than PR acting to inhibit inflammation.

Table 1

High	AP-1, C/EBP, CBF, CREB , c-Myb, E2F-1, Ets, Ets-1/PEA3,FAST-1, GAS/ISRE, HSE, MEF-1, MEF-2, Myc-Max, NF-1, NFATc, NF-E1, NF-E2, Pax-5, Pbx1, Pit 1, Stat3, Sp1
Medium	CDP, PRE, RAR(DR-5), RXR(DR-1), Stat6, TR(DR-4), USF-1, VDR(DR-3)
Low	AP-2, ARE, Brn-3, ERE, OCT-1, P53, SIE, Smad SBE, Smad ¾, Stst 5, MRE
No binding	HNF-4, EGR, GATA, IRF-1, PPAR, SRE, Stat1 p84/p91, TFIID, TR

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Role of Progesterone Withdrawal in Remodeling of the Cervix in a Nonpregnant Murine Model for Parturition. Alexandra A Burns, Jennifer L See, Thomas J Lechuga, Michael A Kirby, Steven M Yellon. *Physiology, Anatomy, Pediatrics, and the Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA.*

Objective: Pre-partum cervical ripening involves remodeling of collagen structure and inflammatory immune cell activity (*JSGI 10:323,2003; Reprod Biol Endo 3:39,2005*). Although parturition is associated with systemic or local progesterone withdrawal (*AJOG 196:289,2007*), effects of a decline in progesterone on cervical ripening is not known. The present study tested the hypothesis that progesterone withdrawal promotes collagen degradation, innervation, and immune cell trafficking in the cervix of nonpregnant mice. Methods: Adult virgin female C57Bl6 mice received capsules (sc) with oil vehicle (**V**) or estradiol (**E**) and progesterone (**P**) to simulate concentrations in pregnancy (*Hum Reprod 12:602,1997*). After 15 days, mice in the **V** and **E+P** groups were euthanized. The **P** capsule was removed from some mice on day 17 (**E-P**) and groups killed on days 18 and 19. Cervix sections were stained for collagen, nerve fibers, macrophages, or neutrophils (n=6/group/day; 3 sections/cervix; *Biol Reprod 61:879,1999; JSGI 12:578,2005*). Stained macrophages and neutrophils were counted (Image Pro-Plus 6, Media Cybernetics). Results: **E+P** treatment for 15 days promoted hypertrophy of the cervix compared to **V** controls, i.e., collagen content and structure diminished, cell nuclei density declined, and nerve fibers increased. Removal of **P** did not affect these endpoints. For immune cells, **E+P** for 15, 18, or 19 days decreased immune cell numbers. By contrast, **P** removal increased macrophages and neutrophils in the cervix on days 18 and 19 ($p<0.05$, **E-P** vs **E+P** groups, respectively). The census of resident immune cells in **E-P** groups at 24 and 48 h after **P** removal equaled that in the **V** group. Conclusions: Mimicking gonadal steroid concentrations in circulation during pregnancy promotes hypertrophy and suppresses immigration of immune cells in the cervix. In this non-pregnant murine model for parturition, *progesterone withdrawal recruits immune cells, but fails to promote further remodeling or hyperplasia of nerve fibers in the cervix.* The findings raise the possibility that ripening of the cervix requires not only recruitment, but also activation of immune cells. Whether proinflammatory activities by specific immune cells affect nerve fiber hypertrophy or neural activity, as part of the mechanism for ripening of the cervix, remains to be determined.

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Progesterone/Progestin Increases Cellular Respiration in Cells Expressing an Outer Membrane Mitochondrial Truncated Progesterone Receptor. Qunsheng Dai, Millie A Behera, Thomas M Price. *Reproductive Endocrinology and Fertility, Duke University, Durham, NC, USA.*

Background: Progesterone actions have been described in cell types lacking expression of the recognized nuclear progesterone receptors (PR) B and A. We have previously identified and characterized a truncated PR (PR-M), that localizes to the mitochondrion by multiple experimental techniques, including confocal imaging of a recombinant GFP fusion protein, western blot analysis after cellular fractionation of nuclear PR negative T47D-Y breast cancer cells and western blot analysis of purified human heart mitochondrial proteins. Initial studies with nuclear PR negative MCF-10A breast epithelial cells shown to express PR-M demonstrated an increase in mitochondrial membrane potential (MMP) with progesterone/progestin treatment. These studies led to the **hypothesis** that progesterone modulates cellular respiration via the mitochondrial receptor, PR-M. **Objectives:** The present studies sought to further localize PR-M to the outer, inner or matrix portion of the mitochondrion and to correlate the increase in MMP with total cellular ATP production. Additionally, the potency of progesterone and synthetic progestins on the change in MMP was evaluated**. Methods**: The location of PR-M was determined by western blot analysis after fractionation of human heart mitochondria with digitonin treatment and differential centrifugation. MMP was determined in MCF-10A breast epithelial cells and A673 rhabdomyosarcoma cells by the change in fluorescent emission of JC-1. Total ATP was determined by a bioluminescent assay. **Results:** Western blot analysis after mitochondrial fractionation showed PR-M localization exclusively in the outer membrane. A dose-dependent increase in MMP was seen in cell lines with 30-60 min treatment with progesterone and R5020 which were inhibited by a specific PR antagonist, RTI-6413-049b, and not affected by the translational inhibitor, cycloheximide. Similar changes in MMP were seen with the same concentration of progesterone, MPA and R5020. Progesterone/progestin treatment for 120 min led to an increase in total cellular ATP without a change in cell number. **Conclusions:** Progesterone/progestin treatment results in an increase in cellular respiration in cells expressing an outer mitochondrial membrane PR and known to lack nuclear PR expression. This may represent a mechanism whereby progesterone enhances cellular energy production to meet the demands of pregnancy.

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The Effect of NF-kappa B upon Expression of Prostaglandin E_2 Receptor EP-4. Mandeep SK Kandola, Shirin Khanjani, Phillip R Bennett. *Parturition Research Group, Institute or Reproductive & Developmental Biology, Imperial College, London, United Kingdom.*

Introduction

PGE_2 is a major product of the fetal membranes, decidua and myometrium and plays an important role in cervical ripening and myometrial contractions. There are four PGE_2 receptors, EP-1 and EP-3 mediate contractions whilst EP-2 and EP-4 mediate quiescence. EP-4 contains multiple consensus sequences for transcription factors known to be of importance in labour, in particular NFkappaB. We therefore performed experiments to determine the effect of activation and inhibition of NFkappaB upon EP-4 expression.

Methods

Myocytes plated in 6 well plates were treated with IL-1β (1ng/ml), to activate NFkappaB. Myometrial cells were also transiently transfected with NFkappaB p65 siGENOME SMART pool and siCONTROL non-targeting siRNA to knock down NFkappaB p65. RNA was extracted for amplifying EP-4 using quantitative RT-PCR with amplification of L19 as a control to normalise data.

Results

IL-1β caused an increase in expression of EP-4. Knock down of NFkappaB using si-RNA resulted in a further increase in EP-4.

Conclusions

This data suggests that although IL-1β stimulates expression of EP-4 it does so through an NFkappaB independent mechanism. The upregulation of EP-4 with siRNA knock down of p65 suggests that activation of NFkappaB would inhibit EP-4 expression consistent with the concept that activation of NFkappaB at term causes the myometrium to adopt a more contractile phenotype.

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Effects of Interleukin-6 (IL-6) Null Mutation on Molecular Regulation of Parturition in Mice. Sarah A Robertson,[1] Dean Zaragoza,[2] Inge Christiaens,[2] David M Olson.[2] *[1]Centre for Reproductive Health, University of Adelaide, Adelaide, South Australia, Australia; [2]Perinatal Research Centre, University of Alberta, Edmonton, AB, Canada.*

Introduction: A role for the pro-inflammatory cytokine IL-6 is suggested in preterm and term birth, independent of the presence of infection. We previously showed that mice with a null mutation in the IL-6 gene (KO) delivered one day later than wild type (WT) mice due at least in part to altered timing of uterine expression of prostaglandin (PG) $F_{2\alpha}$ receptor, PTGFR, mRNA. We also observed differences in mRNA expression of other uterine activation proteins (UAPs), PG H synthase (PGHS)-2, oxytocin receptor (OTR) and connexin-43 (CX-43), suggesting multiple physiological effects for IL-6 in term delivery.

Objective: To examine the effect of IL-6 deficiency on the peri-partal uterine

mRNA expression of the PGE_2 receptors (EP) 2, 3 and 4; the post-partum changes of all UAPs; and the relationship of these to serum progesterone (P_4) concentrations.

Methods: Gestational length was observed in pregnant C57BL/6 WT (N=61) and KO (N=39) mice. UAP mRNA levels were measured by real time RT-PCR in WT and KO dams sacrificed from d15 through delivery and up to 24h post-delivery. Serum P_4 was determined by RIA. Data were analyzed by one-way and two-way ANOVA using the Holm-Sidak test to differentiate treatment effects at $p<0.05$.

Results: Birth was delayed in the IL-6 KO mice (20.7±0.2d vs. 19.7±0.1d), and this affected the timing of peri-partal changes for all UAPs similarly. Both EP2 and EP4 (relaxatory receptors) were elevated at d18 in KO dams, but returned to low levels before delivery and were elevated at delivery (EP2) or afterwards (EP4). EP3 levels did not change. OTR increased several hours before delivery in all dams. CX-43 increased at delivery, then fell, while PGHS-2 increased at delivery and remained elevated afterwards. PTGFR mRNA increased 3-4-fold at delivery and a further 2-3-fold after delivery, suggesting loss of $PGF_{2\alpha}$ permitted enhanced PTGFR expression. P_4 serum concentrations fell pre-partum in both groups.

Conclusions: IL-6 KO alters the expression pattern of several pregnancy and parturition-related genes and may delay the pre-partum P_4 fall, suggesting a potential ovarian effect. A uterine role for IL-6 in regulating the timing of normal term parturition cannot be ruled out.

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Regulation of Trophoblast Innate Immune Responses by Vitamin D. Martin Hewison,[1] Rene F Chun,[1] Nancy Liu,[1] Chantelle Moffatt-Blue,[2] Amber Kaplan,[2] Ozlem Equils.[2] *[1]Endocrinology, Diabetes and Metabolism, Cedars-Sinai Medical Center-UCLA, Los Angeles, CA, USA; [2]Pediatrics, Cedars-Sinai Medical Center-UCLA, Los Angeles, CA, USA.*

Objective: Recent studies have highlighted the prevalence of vitamin D deficiency in pregnant women, particularly in those from ethnic groups with darker skin who require higher levels of UV light to make parental vitamin D. As the active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is a potent immunomodulator, we postulated that vitamin D deficiency may lead to dysregulated placental immunity. Both trophoblast and decidua express the enzyme 1α-hydroxylase (CYP27b1) which catalyzes synthesis of $1,25(OH)_2D_3$ from the inactive pro-hormone 25-hydroxyvitamin D_3 ($25OHD_3$). In view of the role of trophoblast as a barrier site protecting the fetus against infection, we investigated the impact of CYP27b1, $25OHD_3$ and $1,25(OH)_2D_3$ on innate immune responses in trophoblastic cells.

Methods: 3A trophoblast cell line was used to assess the effect of vitamin D on innate immune responses. The cells were treated for 24 hrs with various concentrations of $1,25(OH)_2D_3$ (1-100 nM) and antimicrobial cathelicidin expression was assessed by real time PCR. To assess whether expression of CYP27b1 was affected by pathogenic stimuli, 3A cells were treated with ligands for toll-like receptor (TLR) 1-9, CYP27b1 expression (real time PCR) and $25OHD_3$ utilization were assessed.

Results: 3A trophoblast cell line; which shows temperature-sensitive differentiation, revealed expression of CYP27b1 with higher levels of enzyme activity under conditions of syncytiotrophoblast development. Cells treated for 24 hrs with $1,25(OH)_2D_3$ showed dose-dependent induction of the antimicrobial defensin cathelicidin expression (3.5-17 fold induction), whilst cells treated pro-hormone $25OHD_3$ (100 nM) showed 2.5-fold induction of cathelicidin expression. Activation of TLR3 (poly I:C) and TLR4 (lipopolysaccharide) enhanced the expression of CYP27b1 (2- and 2.5-fold) and increased the sensitivity to $25OHD_3$ as a consequence.

Conclusion: These data show that autocrine synthesis of $1,25(OH)_2D_3$ from $25OHD_3$ can stimulate trophoblast immune responses in a similar fashion to macrophages. As $25OHD_3$ is the major circulating form of vitamin D, we hypothesize that trophoblast innate immunity may be significantly compromised under conditions of vitamin D deficiency.

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Secretory Leukocyte Protease Inhibitor Is Increased in Human Myometrium at Parturition. Suren R Sooranna,[1] Peta L Grigsby,[2] Phillip R Bennett,[1] Leslie Myatt,[2] Mark R Johnson.[1] *[1]Obstetrics & Gynaecology, Imperial College, London, United Kingdom; [2]Obstetrics & Gynecology, University of Cincinnati, Cincinnati, OH, USA.*

Introduction: Secretory leukocyte protease inhibitor (SLPI) is a potent 12-kDa protein inhibitor of neutrophil elastase and it is a mediator of mucosal immunity and an inhibitor of NFkB regulated inflammatory responses. However, its source, function and regulation within the uterus during pregnancy and at parturition are not well defined. It has previously been shown to be present in fetal membranes and cervical mucus and in amniotic fluid, where its levels is increased from second trimester to term and with a further increase at parturition. SLPI has also been shown to be responsive to progesterone in human epithelial cells. Our aim was to determine the effects of Il-1β on SLPI gene expression in human myometrium.

Methods: Primary human uterine myocytes were isolated from non labouring myometrium and cultured in 6 well plates and when cells were 70-80% confluent they were serum starved overnight and incubated with 1μM 6α-methyl-17α-hydroxy-progesterone acetate for 48h and with or without 1ng/mL Il-1β for a further 6 hours. At the end of incubations RNA was extracted and converted to cDNA. Paired upper and lower segment myometrial tissue was collected at caesarean section either before or after the onset of term or pre-term labour and frozen for extraction of RNA (n=6 for PTNL, PTL, TNL and L). Copy numbers of SLPI, GAPDH and beta-actin were measured by qPCR.

Results: 6 h incubation of uterine myocytes with 1ng/mL Il-1β caused a marked increase in SLPI by 201% (n=12; $p<0.015$). Incubation of uterine myocytes with 1μM progesterone for 48h also increased SLPI by 139% (n=12; $p<0.05$). Incubation with Il-1β for 6h in the presence of 48h with progesterone increased SLPI: GAPDH mRNA ratio from 10.50 ± 3.37 to 33.33 ± 10.72 (mean ± SEM; $p<0.004$). SLPI expression was similar in the upper and lower segment myometrium in preterm patients. In term myometrium the SLPI: beta-actin mRNA ratio was increased by 50- and 200- fold in term labour versus term non labour samples in the lower and upper segment respectively (mean ± SEM; $p<0.05$).

Conclusions: These data show SLPI is present in human myometrium and that it is increased by Il-1β. Progesterone also increases its expression. Its expression is increased in term labour where its anti-bacterial, anti-fungal and anti-viral properties could allow it to act as an endogenous block to infections.

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Pre-B Cell Colony Enhancing Factor (PBEF): Insights into Its Mechanisms of Action in Amniotic Epithelial Cells (AEC). Claire E Wright, Gillian D Bryant-Greenwood. *Pacific Biosciences Research Center, University of Hawaii, Honolulu, HI, USA.*

Objective: Pre-B cell colony-enhancing factor (PBEF) downstream MAPK signaling and transcription factor activation.

Introduction: PBEF is expressed in all layers of the human fetal membranes and the myometrium and is upregulated by labor, infection, NF-KB, AP-1 and stretching. PBEF has the ability to protect a variety of cells from apoptosis, however it also appears to act as an insulin mimetic via the insulin receptor (Fukuhara et al. Science 2005: 307;426-430). Its levels are elevated in obese patients, those with type 2 diabetes and in gestational diabetes. Because PBEF has poorly understood biological activities an investigation of MAPK signaling and transcription factor activation induced by PBEF has been undertaken in order to gain insights into its mechanisms of action.

Methods: Primary AEC were isolated from fetal membranes and treated with rhPBEF (100ng/ml) for 1h, lysed and the resultant proteins labeled with Cy3 or Cy5 (Amersham). There were used on a protein microarray containing 64 constituents of the MAPK signaling pathways (Sigma). Isolated AEC were transfected with luciferase constructs for NF-KB, AP-1, CRE, HSE and GRE response elements using the exgen500 reagent. The cells were treated with rhPBEF (0.1, 1.0, 10, 100 ng/ml) 4 hrs, lysed and 50ul of sample was analyzed for luciferase activity with Dual-luciferase reporter assay system (Promega). Co-transfection with GFP and SV40 luciferase constructs to control for transfection efficiency and cell number (respectively) was also performed for each experiment.

Results: PBEF significantly upregulated 20 MAPK signaling components including 9 functioning as part of the ERK pathways and 5 belonging to p38 signaling. It also activated NF-KB and cAMP response elements. However, it significantly down regulated 3 signaling molecules belonging to the JNK pathway that resulted in decreased c-Jun phosphorylation.

Conclusions: PBEF signaling in AEC is consistent with its anti-apoptotic ability and its upregulation of the pro-inflammatory cytokines. Although some MAPK components responsive to PBEF could be associated with insulin receptor signaling, some may not. Therefore, it appears likely that PBEF interacts with another potentially unique, but currently unidentified receptor.

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Cyclic AMP-Dependent Protein Kinase and Epac Mediate Cyclic AMP Responses in Human Myometrium during Pregnancy. Suren R Sooranna,[1] Peta L Grigsby,[2] Leslie Myatt,[2] Phillip R Bennett,[1] Dimitris Grammatopoulos,[3] GN Europe-Finner,[4] Mark R Johnson.[1] *[1]Imperial College, London, United Kingdom; [2]University of Cincinnati, Cincinnati, OH, USA; [3]The University of Warwick, Coventry, United Kingdom; [4]Newcastle University, Newcastle upon Tyne, United Kingdom.*

Introduction: Cyclic AMP promotes myometrial relaxation by reducing smooth muscle tone and altering pro-contractile factors. Protein kinase A (PKA) was the first effector to be characterised, but cAMP is now known to have other effectors, including cAMP receptor protein, cyclic nucleotide-gated channels and cAMP-guanine nucleotide exchange factor/exchange protein directly activated by cAMP (cAMP-GEF/Epac). Two Epac's have been identified and they consist of 4 functional domains: cAMP-binding domains, a DEP domain, a Ras exchange motif and a GEF domain. Our aim was to determine the presence of Epac's in human myometrium and to study the effect of cAMP responses on prolabour genes such as IL-8, PGHS-2, OTR and FP.

Methods: Primary human uterine myocytes were isolated from non labouring myometrium and cultured in 6 well plates until 70-80% confluent. Cells were serum starved overnight and incubated with 1μM methyl progesterone, 0.2mM sodium 8-bromo-cAMP, 0.1mM forskolin, 1μM KT5720, 75ng/mL brefeldin A and 0.1mM 8-pMeOPT-2'O-Me-cAMP either alone or in combination for 48h. RNA was extracted and converted to cDNA. Paired upper and lower segment myometrial tissue was collected at caesarean section either before or after the onset of term or pre-term labour and frozen for extraction of RNA (n=6 for PTNL, PTL, TNL and L). Copy numbers of Epac1, Epac2, Il-8, PGHS-2, OTR, FP, GAPDH and β-actin were measured by qPCR.

Results: Epac1 and Epac2 were present in the upper and lower segment of myometrium with Epac1 levels being some 10-fold higher than those of Epac2. There was no change with the onset of labour at or before term. Treatment with IL-1β for 6h significantly increased Epac2 (n=12; $p<0.012$) but had no effect on Epac1. When conditions mimicked pregnancy, (the presence of forskolin and progesterone), brefeldin A, an Epac antagonist, increased basal PGHS-2 and FP mRNA expression (n=6; $p<0.05$), but had no effect on IL-8 and tended to reduce OTR. The IL-1β-induced increase in PGHS-2 and IL-8, but not FP, was greater with the Epac antagonist.

Conclusions: These data show Epac's are present in human myometrium and that cAMP acts via Epacs to reduce PGHS-2 and FP expression during pregnancy.

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Expression of Glucocorticoid Receptor in Human Myometrium during Pregnancy and Labour. Farah Sethna, Alison J Tyson-Capper, Elizabeth A Shiells, Stephen C Robson. *Surgical and Reproductive Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom.*

Background: Inflammatory events have been implicated in the process of labour. Glucocorticoids mediate strong anti-inflammatory effects through binding to the glucocorticoid receptor (GR), which on activation translocates to the nucleus and either increases or decreases the expression of responsive genes thereby suppressing inflammation.

Objective: To characterise the expression profile for GR protein and mRNA in human myometrium during fetal maturation and parturition.

Methods: Western immunoblotting (WB) was employed to characterise GR protein expression in first (N=4) and second trimester myometrium (N=5), and in paired upper and lower segment pregnant (non-labouring, P, N=8) and labouring (L, N=8) myometrium; as compared to non-pregnant (NP, N=8) control samples. Immunofluorescence staining with confocal microscopy and RT-PCR were also undertaken.

Results: Detection of GR protein by WB revealed two bands at 90-95 KDa, representing the alternatively spliced isoforms, GR-α and GR-β. Densitometric analysis showed that GR levels decreased significantly during pregnancy and remained at very low levels at term and in labour when compared with NP samples ($P<0.001$); this decrease was seen for GR-α and GR-β. No significant temporal variations were observed in GR protein levels at term or during labour.

GR protein was localised by immunofluorescence staining to the nuclei and cytoplasm of cells. Less intense staining was apparent in P compared to NP tissues; consistent with WB data.

RT-PCR showed a consistent predominance of GR-α to GR-β mRNA in all the tissues used. The observed decrease in protein expression was also mirrored at the mRNA level: GR-α mRNA levels appeared to gradually decrease throughout gestation ($P<0.05$). Differences between the upper and lower myometrial regions were only observed in the labouring samples, where GR-α mRNA levels were significantly decreased in the lower segment ($P<0.05$). Nested PCR was additionally used to amplify GR-β, but no consistent pattern of mRNA expression was obtained.

Conclusions: These data are the first to characterise GR expression in human myometrium. Spatial and temporal variations have been found with expression evolving through the three trimesters of pregnancy and in labour. Further studies are now underway to evaluate whether GR contributes to the sequence of inflammatory events implicated in triggering term and preterm labour.

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Progestational Agents Modulation of the Immune Response: Role of the Glucocorticoid Receptor. Hua Xu, Juan M Gonzalez, Michal A Elovitz. *OBGYN; CRRWH, University of Pennsylvania, Philadelphia, PA, USA.*

Objective: Recent clinical trials suggest that progestational agents (PAs) can prevent preterm birth (PTB). A growing body of evidence suggests that cervical ripening may be the primary event in PTB. Prior work from our laboratory has demonstrated that select PAs can inhibit an inflammation induced-cytokine response in cervical cells. These studies sought to determine the mechanism by which PAs modulate the immune response.

Methods: 1) An *in vitro* co-culture model mixed with human cervical epithelial Hela cells and PMA-induced human macrophage U937 cells at epithelial/macrophage cell ratio of 5:1 was employed. 2) The co-culture was pre-treated with medroxyprogesterone acetate (MPA), progesterone (PROG) and dexamethasone (DEX) at concentrations of 1, 10, 100 and 1000 nM for 2 hrs followed by 2 day stimulation of 10μg/ml lipopolysaccharide (LPS). These experiments were repeated using Hela cells transfected with siRNA for glucocorticoid receptor (GR). The production of cytokines IL-1β, IL-6 and IL-8, IL-10 and TNF were determined using ELISAs. 3) The co-culture was pre-treated with 100nM of each PAs for 2 hours prior to LPS stimulation at 10μg/ml for 0.5, 1.5, 6, 12, 24 and 48 hours. The phosphorylation of p38 MAPK and GR and the expression of MAPK phosphatase 1 (MKP1) were determined by western-blotting.

Results: IL-1 β, IL-6 and IL-8, IL-10 and TNF were elevated by 3.8 fold, 1.7 fold, 1 fold, 4.9 fold and 4.6 fold in the co-culture model in response to LPS($P<0.001$ for all). Pretreatment of MPA and DEX, but not PROG, inhibited the LPS-induced cytokine production. The anti-inflammatory effect of MPA occurs in a dose-dependent manner. In the absence of GR, the inhibitory effect of MPA and DEX on IL-6, but not on IL-1β, was lost. MPA and DEX, but not PROG, induced the phosphorylation of GR and expression of MKP1. p38 MAPK was activated by LPS for up to 24 hrs and pretreatment of MPA and DEX attenuated this response.

Conclusions: The ability of MPA and DEX to suppress the inflammatory response in cervical tissues appears to be mediated through a GR-dependent pathway, specifically though p38 MAPK. Our studies suggest that PROG is not a significant immunomodulator in these tissues. If PROG acts similarly *in vivo* as it does *in vitro*, these studies suggest that PROG may not be preventing PTB through modulation of the immune response. It remains unclear if other PAs used clinically have biological action that is more similar to MPA or to PROG.

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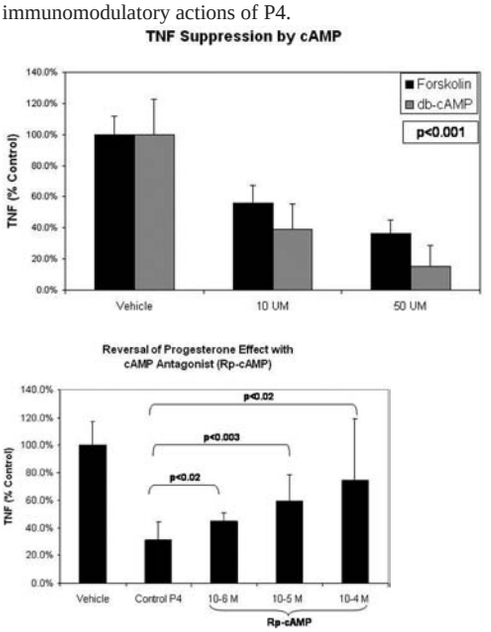
Progesterone Suppresses TNF-α Expression by Umbilical Cord Mononuclear Cells Via Cyclic AMP. Nadav Schwartz,[1] Oonagh Dowling,[2] Christine N Metz.[2] *[1]Obstetrics and Gynegology, NYU School of Medicine, New York, NY, USA; [2]Feinstein Institute for Medical Research, North Shore-LIJ Health System, Manhasset, NY, USA.*

Background: Progesterone (P4) has been shown to play a critical role in maintaining pregnancy and preventing preterm birth. These effects are likely related to its immunomodulatory properties. We investigated whether cAMP plays a role in the immunosuppressive action of progesterone on fetal mononuclear cells.

Methods: Umbilical cord blood mononuclear cells were isolated using density gradient centrifugation. To establish a P4 effect and optimize concentrations, cells were pretreated with P4 (10^{-4}M-10^{-6}M), dexamethasone (3uM) or vehicle for 1 hour prior to overnight LPS (10ng/ml) stimulation. Supernatants were assayed for TNF-α using ELISA. LDH assays confirmed the absence of a cytotoxic effect. Next, cells were pre-incubated with forskolin (adenylate cyclase activator) or db-cAMP (cAMP agonist) prior to LPS to determine the effects of cAMP on TNF production. Finally, cells were pretreated with Rp-cAMP (cAMP antagonist) prior to P4 incubation and LPS stimulation to determine whether the P4 effect was reversed by a cAMP antagonist.

Results: P4 significantly inhibited LPS-induced TNF production in a dose dependent manner, with maximum suppression observed at 10^{-4}M. Both forskolin and db-cAMP suppressed LPS-induced TNF production in a dose-related manner (Fig 1). Finally, a dose-dependent partial reversal of TNF production was observed when cells were pretreated with Rp-cAMP. (Fig 2)
Conclusions: Progesterone suppresses the inflammatory response in fetal mononuclear cells as measured by TNF expression. This effect is mimicked by adenylate cyclase activators and cAMP agonists and partially reversed by cAMP antagonists. This implicates the cAMP pathway in mediating the immunomodulatory actions of P4.



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Estrogen and Progesterone Receptor's Roles onto EPCs Proliferation. Yuko Matsubara, Keiichi Matsubara, Masaharu Ito. *Department of Obstetrics and Gynecology, Ehime University Graduate School of Medicine, Toon, Ehime, Japan.*

Objectives
Estrogen improves endothelial function after vascular injury via largely unknown mechanisms. Endothelial progenitor cells (EPCs) are known to be implicated in various vascular events requiring endothelialization. We hypothesized that estrogen and progesterone could influence the function and the change of EPCs in menstrual cycle.
Material and Methods
Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood of healthy young women in each menstrual period by density gradient centrifugation with Ficoll separating solution. PBMCs were seeded in endothelial basal medium with or without estrogen or progesterone. On the 4th day in culture, nonadherent cells were removed. On the 7th day in culture, adherent cells were incubated with Di-LDL, fixed with paraformaldehyde, and stained with fluorescein isothiocyanate-labeled lectin. The number of LDL- and lectin- positive cells was measured as EPCs using flowcytometry. The expression of estrogen and progesterone receptor mRNA in EPCs were measured by real time PCR in menstrual and luteal period.
Results
The number of EPCs was significantly increased in the menstrual and luteal period compared with the follicular phase. Estrogen and progesterone significantly increased the number of adherent EPCs dose dependently in menstrual period, but not in luteal period. The expression of estrogen-alpha receptor in menstrual period was higher than luteal period. Also, estrogen-beta receptor in luteal period was strongly expressed compared with menstrual period.
Conclusions
These results suggest the expression of estrogen receptor and progesterone receptor play important roles to regulate EPCs' proliferation during menstrual cycle.

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Aquaporin 1 (AQP1) Gene Expression Is Upregulated by Arginine Vasopressin (AVP) Acting through Cyclic AMP in Trophoblast Cells. Louiza Belkacemi, Marie H Beall, Thomas R Magee, Margaret Pourtemour, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*
Objective: Elevated levels of fetal plasma AVP are associated with the development of oligohydramnios, in part a result of AVP-mediated reduction in fetal urine production. As AVP urinary concentration effects are mediated via upregulation of renal tubular AQP1 water channels, we propose that AVP modulates placental AQP channels, influencing bidirectional maternal-fetal water flow. We sought to study the effect of AVP on trophoblast AQP1 gene expression using the first trimester-derived extravillous HTR-8/SVneo cells and the term placenta-like trophoblast carcinoma cells JEG-3.
Methods: Cultures of both cell lines were treated with a physiological concentration of AVP (0.1 nM) to determine AQP1 mRNA and protein expression. Negative controls consisted of cells incubated in medium supplemented with 1% FBS without AVP. To determine whether AVP regulation of AQP1 occurs by a cAMP signaling pathway, JEG-3 cells were preincubated with 100 µM 9-(Tetrahydro-2'-furyl) adenine (SQ22536), a cell-permeable cAMP inhibitor, before being treated with AVP (0.1 nM). Cells were incubated for 10 hrs for AQP1 mRNA expression and for 20 hrs for protein extraction at 37°C. After harvest, real time PCR and Western blotting analysis were used to detect the AQP1 mRNA and protein expression levels, respectively.
Results: AVP increased AQP1 mRNA expression in both cell lines by 1.8 and 2.8 fold after 10 hrs. AQP1 protein expression paralleled the increase seen in the mRNA ($P<0.05$). Pretreatment of JEG-3 cells with SQ22536 inhibitor completely blocked the stimulatory effect of AVP.
Conclusion: AQP1 gene expression is up-regulated by AVP in first trimester and term trophoblast cells, with a higher induction in the later. AVP activation of AQP1 gene expression occurs via a cAMP mediated pathway, as the adenyl cyclase inhibitor blocked AVP effects on AQP1 gene expression. These results suggest that increased fetal plasma AVP may contribute to oligohydramnios by an increase in AQP-mediated fetal to maternal water flow.

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Progesterone and Progesterone Receptor C in Human Myometrial and Amnion Derived WISH Cells. Laura Goodfellow, Elizabeth A Shiells, Stephen C Robson, Alison J Tyson-Capper. *Surgical & Reproductive Sciences, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom.*
Objective: Changes in expression, ratio and activity of progesterone receptors within human myometrium have been proposed as potential contributory mechanisms for the functional progesterone withdrawal effect which precedes labour. Progesterone receptor C (PR-C) is an N terminally truncated isoform of the full length progesterone receptor; PR-C has been shown to be abundant in fetal membranes, placenta and upper segment of laboring myometrium (1,2). The function and regulation of PR-C is at present unclear. In this study we aimed to investigate the regulation of PR-C in cultured human myometrial and amnion-derived WISH cells.
Methods: Myometrial primary cell cultures were prepared from non pregnant and term pregnant uteri. Both myometrial and WISH cell cultures were treated with natural progesterone (0, 100nM, 1mM, 10mM, 100mM) for 2, 6 and 24hrs. Western immunoblotting was employed using nuclear and cytoplasmic extracts prepared from treated cells to observe the effect of progesterone on a) expression and b) subcellular localisation of PR-C within both cell types. Immunofluorescent staining and confocal microscopy were also employed. Experiments were also undertaken whereby nuclear and cytoplasmic extracts were subjected to shrimp alkaline phosphatase treatment to evaluate the phosphorylation status of PR-C in response to hormone.
Results: Data indicates that a 60kDa cytoplasmic protein, representing PR-C is abundant in myometrial and amnion cell cultures. We show that expression of PR-C increases in both cytoplasmic and nuclear fractions within both cell types in response to progesterone. Evidence also suggests that PR-C is phosphorylated in a progesterone-independent manner. Concurrent treatment with progesterone and the PR-antagonists RU486 and Organon 31710 in amnion-derived cells still led to an increased abundance of PR-C but seemed to alter the phosphorylation status of PR-C.
Conclusion: PR-C expression and subcellular localisation within myometrial and WISH cells alters in response to progesterone. Speculation is generated about potential role of PR-C in reproductive tissues and its contribution to functional progesterone withdrawal.
1. Taylor, A. H., McParland, P. C., Taylor, D. J., and Bell, S. C. (2006) *Endocrinology* **147**(2), 687-693

2. Condon, J. C., Hardy, D. B., Kovaric, K., and Mendelson, C. R. (2006) *Mol Endocrinol* **20**(4), 764-775.

740

Hypogastric Neurectomy Does Not Affect Processes Associated with Remodeling of Cervix or Parturition. Jonathan W Boyd, Charlotte A Ebner, Michael A Kirby, Steven M Yellon. *Physiology, Anatomy, Pediatrics, and the Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA.*

Objective: Innervation of the uterine cervix is important for the process of parturition (*Physiol Beh 63:929,1998; J Histochem Cytochem 52:1249,2004; JSGI 12:578,2005*). The hypogastric nerve is a major pathway that innervates the uterine cervix, yet its contribution to processes associated with cervical ripening and parturition is not known. The objective of this study was to determine the effect of hypogastric nerve transection on processes associated with cervical remodeling and parturition. Methods: Time-dated pregnant rats were sham-operated or the hypogastric nerve bilaterally transected just anterior to the inferior mesenteric ganglion on day 15 post-breeding. Live pups were born spontaneously by all rats at term on days 22-23 post-breeding. On the day of birth, the cervix was excised, postfixed overnight, sectioned, and processed to evaluate collagen content and structure (NIH Image J). Sections were also processed by immunohistochemistry to assess cell nuclei density, the census of resident macrophages, and area of tissue that contained nerve fibers. Results: Hypogastric neurectomy did not affect cell nuclei density, the number of macrophages, or density of nerve fibers in the cervix. The failure of hypogastric nerve transection to affect indices of cervical remodeling is consistent with the finding that duration of pregnancy and timing of birth were not different in sham-operated and hypogastrectomized rats. Conclusions: Nerve fibers in the hypogastric nerve that innervate the lower uterus and cervix, including sympathetic and neuropeptidergic projections, are thus not essential for birth. These novel findings provide support for the contention that innervation of the uterine cervix other than through the hypogastric nerve contributes to processes associated with cervical ripening and parturition.

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The Identification and Role of the Intrauterine (pro)renin/(pro)renin Receptor System in Prostaglandin Synthesis in Pregnancy. Eugenie R Lumbers,[1,2] Della M Yates,[2] Carolyn M Mitchell,[2] Jonathan J Hirst,[1,2] Tamas Zakar.[2,3] *[1]School of Health Sciences, University of Newcastle, Newcastle, NSW, Australia; [2]Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia; [3]Obstetrics and Gynaecology, John Hunter Hospital, School of Medical Practice and Public Health, University of Newcastle, Newcastle, NSW, Australia.*

The fetal membranes and decidua contain prorenin, which requires proteolytic activation. Ngyuen (J Clin Invest. 109:1417) described a renin/prorenin receptor that conformationally activates prorenin, so that it can form Ang I from Aogen. In addition, receptor-bound prorenin can stimulate intracellular pathways directly. Prostaglandins (PGs) participate in the control of term and preterm labour, and renin can stimulate PG production by amnion and decidua when angiotensin's action is blocked (Lundin-Schiller JCEM 73:436; Mitchell Placenta 17:299). The prorenin receptor may mediate these effects.

Our aim was to find out if the prorenin receptor gene is expressed and colocalised with prorenin and prostaglandin H2 synthase-2 (PGHS-2) in human placenta, amnion, chorion and decidua. We have also determined if there is any change in the expression of the prorenin and prorenin receptor genes with labor. Placentae with attached membranes were collected after term birth either by elective caesarian section or by spontaneous labor, and prorenin, prorenin receptor and PGHS-2 mRNA levels were quantitated relative to beta-actin mRNA by real-time RT-PCR in amnion, chorion, decidua and placenta.

Before labor, mean prorenin mRNA levels were 13.8 times higher in decidua and 4.0 times higher in chorion and placenta than in amnion ($P<0.001$). There were no significant changes with labor. Proenin receptor mRNA was expressed in all tissues. Proenin receptor mRNA levels in prelabor amnion, chorion and placenta were similar, while levels in decidua were 50% of those in amnion ($P=0.02$). This low level of prorenin receptor mRNA in decidua persisted after labor ($P<0.001$). PGHS-2 mRNA expression was highest in amnion; in decidua and placenta it was only 6 and 4% of amnion. Similar differences were present after labor.

We conclude that the decidua is the principal source of prorenin within the pregnant uterus, and all gestational tissues are targets for prorenin. Decidual prorenin may affect PGHS-2 expression in amnion through the prorenin receptor forming a maternal-fetal paracrine system that stimulates PG production at labor.

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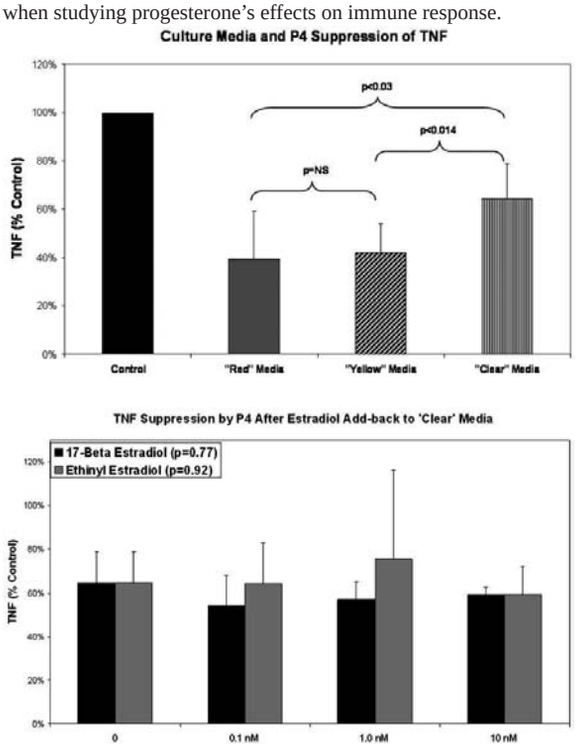
The Immuno-Suppressive Effects of Progesterone on Umbilical Cord Blood Mononuclear Cells and the Effect of Culture Media Estradiol Content. Nadav Schwartz,[1] Xiangying Xue,[2] Christine N Metz.[2] *[1]Obstetrics and Gynecology, NYU School of Medicine, New York, NY, USA; [2]Feinstein Institute for Medical Research, North Shore-LIJ Health System, Manhasset, NY, USA.*

Objective: Progesterone (P4) is known to supress the maternal immune response and similar effects have been seen with fetal cells. We sought to ascertain whether P4 action was modulated by the phenol red and/or estrogen content of the culture media.

Methods: Umbilical cord blood mononuclear cells were isolated using a density gradient centrifugation. The cells were incubated with P4 (10^{-4}M) 1 hour prior to overnight stimulation with LPS. Supernatants were assayed for TNF-α using ELISA. The following culture media were used: a)**'Red' media**: RPMI+10% FBS, b)**'Yellow' media**: phenol red-free RPMI+10% FBS, and c)**'Clear' media**: phenol-free RPMI+10% dextran/charcoal treated FBS. Finally, the 'clear' media experiments were repeated with estradiol add-back.

Results: P4 suppressed TNF production in all medias. TNF levels were suppressed to 39.4% ($p<0.06$) of controls using 'red' media and to 42.0% ($p<0.03$) in 'yellow' media. The degree of suppression was not as substantial using 'clear' media where TNF levels were 64.5% ($p<0.02$) of control. (Fig 1) Adding estradiol to the 'clear' media had little or no effect on the degree of TNF suppression. (Fig 2)

Conclusions: Progesterone exerts an immuno-suppressive effect on LPS-stimulated fetal mononuclear cells which is more pronounced when using media containing untreated fetal bovine serum. The phenol-red/estradiol content of the culture media did not modulated the P4 effect. Dextran/charcoal treatment of FBS appears to deplete the media of factors other than estradiol that are necessary for P4 to exert its full effects. Culture conditions should be optimized when studying progesterone's effects on immune response.



743

Ovine Fetal Regional Blood Flow Following Prenatal Dexamethasone (DEX) at 0.75 Gestation (G). Antonine D van Heesewijk,[1] Jan G Nijhuis,[1] Susan L Jenkins,[2] Peter W Nathanielsz,[2] Mark J Nijland.[2] *[1]Ob/Gyn, Academisch Ziekenhuis Maastricht, Maastricht, Netherlands; [2]Ob/Gyn, CPNR, UTHSCSA, San Antonio, TX, USA.*

BACKGROUND: Pregnant women at risk for premature labor receive antenatal glucocorticoids (GC) to reduce neonatal morbidity and mortality. While fetal blood pressure (BP), heart rate and vascular endothelial and

smooth muscle responses have been studied, data on regional blood flow (BF)distribution are scarce. We have studied the effect of a single DEX course on relative BF distribution within the brain, skeletal muscle, heart, omental fat, placenta and adrenal in fetal sheep at 0.75 G, the equivalent of 30 weeks.
METHODS: Fetal sheep received amniotic, jugular, carotid and femoral artery and vein catheters at 104 days G (dG). Experiments started at 110 dG in 8 DEX (2 mg) and 8 saline treated controls (CTR), each receiving 4 injections 12 h apart. Fetal blood pressure was recorded continuously (WindaQ Pro+). Microspheres ($1.86x10^6$ total; STERIspheres, BioPAL) were injected at 11:00am into fetal jugular and femoral veins before maternal i.m. injection (t0) and 2 h after the 3rd injection (t3) in both groups. Tissues were collected 12 hours after the 4th injection for assessment of BF (BioPAL).
RESULTS: Fetal BP increased above baseline after DEX (6.8+1.5 mmHg; P=0.007) with no change in CTR (-0.1+0.4 mmHg). Regional BF distribution is presented as a percent of the total counts per 100 g tissue (Table 1) and after normalization within each animal to placentomal flow, shown previously to be unchanged by DEX, in Table 2. No significant differences in flow were found.
DISCUSSION: Although DEX altered BP, regional distribution of BF among key organs was unaffected. Whether GC does not affect regional BF, or the rise in BP is the result of a non-specific, system-wide vasoconstriction, or the impact of GC on BF is similar in all organs, will be subject of further investigation that will include determination of regional vascular resistance and absolute flow.

Table 1	CTR		DEX	
	t0 (n=4)	t3 (n=3)	t0 (n=7)	t3 (n=6)
Frontal cortex	5±1	9±2	6±1	6±1
Femoral muscle	4±0	5±1	6±1	6±1
Omental Fat	3±1	3±1	6±1	7±1
Heart	18±3	22±5	19±3	20±3
Placentome	12±2	18±1	17±2	19±2
Adrenal	58±6	43±7	46±4	41±3

Table 2	CTR		DEX	
	t0 (n=4)	t3 (n=3)	t0 (n=7)	t3 (n=6)
Frontal cortex	0.4±0.1	0.5±0.1	0.4±0.1	0.4±0.1
Femoral muscle	0.3±0.0	0.3±0.0	0.3±0.1	0.3±0.1
Omental Fat	0.2±0.0	0.2±0.1	0.4±0.1	0.4±0.2
Heart	1.5±0.2	1.2±0.2	1.1±0.2	1.1±0.3
Placentome	1	1	1	1
Adrenal	5.3±1.3	2.5±0.6	2.9±0.5	2.3±0.3

Table 1: Relative distribution of BF between organs studied. **Table 2:** Blood flow normalized within animals to plancentomal flow.

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Effects of Infusion of ACTH on Expression of Adrenal ACTH-R and StAR mRNA with or without ACTH-R Knockdown. Yixin Su, Luke C Carey, James C Rose. *Department of Obstetrics and Gynecology, Wake Forest University Health Science, Winston Salem, NC, USA.*
Background: Previously we showed that Adrenocorticotropic (ACTH) can upregulate mRNA expression of its receptor and StAR in the fetal sheep adrenal in vivo and in vitro. Our recent studies suggest that partial suppression of ACTH receptor expression with siRNA markedly attenuates functional responses to stimulation in fetal and adult adrenocortical cells. Here we aimed to determined effects of infusion ACTH on expression of adrenal ACTH-R and StAR mRNA with or without ACTH-R knockdown.
Materials and Methods: Fetuese of 131 days of gestational age (dGA) were infused with ACTH-(1-24) for 24h. Controls received saline infusion. Adrenals cells were obtained dispersed, transfected with receptor targeted siRNA or Scrambled siRNA and subsequenently stimulated with ACTH. Cells and media were harvested for measurements of gene expression and contisol levels. Concentrations of cortisol were determined by RIA. RNase Protection Assay (RPA) was used to measure the ACTH-R or StAR mRNA levels. Data are compared using two-way ANOVA.
Results:1. ACTH-R and StAR mRNA levels were significantly higher in ACTH infused fetuses than those from saline infused fetuses. 2. ACTH-mediated increased expression of ACTH-R and StAR mRNA was accompanied by increase in cortisol output in both groups. 3. SiRNA knockdown ACTH-R mRNA expression in both groups, this was accompanied by marked reductions in cortisol response in both ($p<0.05$). 4. Fetal plasma cortisol concentrations increased over the infusion period in ACTH infusion group.
Conclusion: This observation supports the idea that developmental increases in ACTH receptor expression are essential for maturation of cortisol secretory capacity by the adrenal. These results indicated that ACTH is the major regulator of cortisol production as well as the expression of key genes essential for cortiosl biosynthesis in the fetal adrenal.
Supported by NIH grant HD-11210.

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Mouse Fetal Gastro-Intestinal Tract Is a Stress Hormone Target. Jayaraman Lakshmanan, John D Richard, Marie H Beall, Sharon K Sugano, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*
Objective: Evidence in adult rats indicates that CRF and its receptor subtypes, CRF-R1 and CRF-2, mediate stress responses in the GI tract. CRF-R1 is a potent stimulator while CRF-R2 is an inhibitor of GI motility at times of stress. We recently hypothesized that stress-induced in utero meconium passage in fetuses is analogous to stress-induced defecation in adult rats and likely mediated by CRF pathway. In support, we demonstrated hypoxia-induced meconium passage in conjunction with marked increases in fetal plasma CRF levels. As the mouse is a common experimental model, with known genome, we sought to determine the presence of CRF-R1 and CRF-R2 receptors in mouse fetuses.
Methods: Time-dated CD-1 pregnant mouse were anaesthetized and fetuses (n=10) were collected at e17 (term = e19). Whole GI tracts were dissected, fixed in Bouin's solution, paraffin embedded and sectioned at five micron thickness. Sections were immunostained with rabbit polyclonal antibodies to CRF-R1 and CRF-R2 by ABC technique with Vector ABC reagents. Immunoreactivity was identified as brown staining using 3',3 diaminobenzamidine as chromagen. Slides were counter stained with Mayer's hematoxylin and examined under the microscope. Immunoreactive signals in the smooth muscle layers of GI tracts were quantified by image analysis using Image Pro 4.01 plus software.
Results: Immunostaining for both CRF-R1 and CRF-R2 was seen in the smooth muscle layers of all lumens examined (7 lumens per section). The immunostaining intensity expressed as intensity over density (IOD) in arbitrary units for CRF-R1 (Maximal IOD: 0.369±0.024 AU and minimal IOD: 0.177±0.13AU) and for CRF-R2 (Maximal IOD: 0.318±0.015 AU and minimal IOD: 0.240±0.020 AU) greatly varied between lumens.
Conclusion: The mouse fetal GI tract expressed both CRF-R1 and CRF-R2 receptors, suggesting that the mouse is an appropriate model for fetal-stress induced neurovisceral motor responses. The non-uniform distribution of CRF receptors in the fetal GI tract suggests the existence of regional differences in the expression patterns of CRF-receptors of both types. We speculate that mouse devoid of CRF and CRF-R1 or R2 receptors will be useful to confirm the participation of CRF pathway in stress-induced in utero meconium passage.

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Nephron Number and Hypertension Following Antenatal Glucocorticoids: Is There a Causal Relationship? Jorge P Figueroa, Angela G Massmann, Jie Zhang, James C Rose. *Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*
Antenatal exposure to glucocorticoids (GC) is associated with a reduction in nephron number and hypertension in adult life. One of the mechanisms for the development of hypertension is thought to be the long term effect of single nephron hyperfiltration. However, in most studies there is only a 20–30% reduction in nephron number with no change in GFR. Thus, although a reduction in nephron number may be a contributing factor it is unlikely the only variable. The aim of the present study was to evaluate nephron mass, renal function and blood pressure in a cohort of adult sheep exposed antenatally to betamethasone.
METHODS: Pregnant sheep were treated with two IM doses of betamethasone (BM, 0.17 mg/kg) or vehicle (CTR) 24-hs apart at 80 days gestational age and allowed to deliver at term. At 1.5 yr of age in female (F) and male (M) offspring, glomerular filtration rate (GFR) was measured as inulin clearance and effective renal plasma flow (ERPF) as PAH clearance. Blood pressure was measured though an indwelling catheter in the femoral artery over a 48-hour period. Nephron number was measured by the acid maceration technique. Data Mean±SEM were analyzed by ANOVA and/or two sample t test.
RESULTS: Antenatal BM was associated with an elevation in arterial blood pressure and a reduction in nephron number in both F and M offspring. In contrast, a reduction in GFR and ERPF was present only in males. Plasma and urinary electrolytes as well as urinary protein excretion were not different from CTR.
CONCLUSION: Our data show that prenatal exposure to a single course of GC at 0.55 gestation has long-term effects on blood pressure regulation. Interestingly, while the decrease in nephron number was of similar magnitude in M and F, evidence of alterations in renal function was present only in males. These suggest that the decrease in renal function observed in males is not solely a consequence of the decrease in nephron number. Furthermore, it is possible that different mechanisms are responsible for the elevation in arterial blood pressure observed in M and F. HL 68728; HD P01 HD04784.

	Nephron number x 10^5		Mean AP mm Hg		GFR ml/min/m^2		ERPF ml/min/m^2	
	CTR	BM	CTR	BM	CTR	BM	CTR	BM
Males	9.4±0.4	7.1±0.1*	98±2.7	105±2.4*	1.1±0.08	0.9±0.05*	7.6±0.69	7.1±1.16*
Females	9.4±0.5	6.5±0.4*	96±1.9	105±2.4*	1.0±0.10	1.1±0.04	7.0±0.79	7.8±0.97

*$p<0.05$ from CTR

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Effect of Intrarenal Infusion of Angiotensin-(1-7) (Ang 1-7) in Male Sheep with or without Prenatal Exposure to Betamethasone (B). Jianli Bi,[1] Stephen Contag,[1] Jorge P Figueroa,[1] Lijun Tang,[1] Mark C Chappell,[2] James C Rose.[1] *[1]Center of Research for Obstetrics and Gynecology; [2]Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Winston Salem, NC, USA.*

Objective

To evaluate the effects on blood pressure, urine output, sodium excretion and GFR of the intrarenal infusion of angiotensin 1-7 in the male sheep with or without prenatal exposure to B.

Methods

We studied male sheep which had received either B (n=4) or vehicle (n=3) at 80-81 days gestation and were born at term. Catheters were placed in the femoral artery, femoral vein, left renal artery and the bladder. The right kidney was removed. After 5 days recovery the sheep received an infusion of Ang 1-7(1ng/kg/min) with or without its antagonist, [D-Ala^7]-ANG-(1–7)(D-ALA, 10ng/kg/min), into the renal artery for 24 hours. Blood pressure, GFR, urine output and sodium excretion were measured before and after the infusion. Two-Way Analysis of variance (ANOVA) was used to test mean values between the groups.

Results

With or without D-ALA, MAP and urine output didn't change significantly during Ang 1-7 infusion. However, the infusion of Ang 1-7 resulted in a decrease (change from baseline) ($p=0.029$) in Na^+ excretion of 0.41±0.15 meq/kg/24h in the vehicle animals and 0.12±0.05 meq/kg/24h in the B animals. There was no significant change in GFR during Ang 1-7 infusion. GFR was lower in the B group during the combined Ang 1-7 and D-ALA infusion (91.5±3.5ml/min vs 117.0±15 in vehicle group, $p=0.03$).

Conclusion

Intrarenal infusion of Ang 1-7 at 1ng/kg/min is followed by a significant decrease in sodium excretion but no significant change in urine output and GFR. The decrease tended to be greater in the vehicle animals and was not markedly attenuated by the antagonist D-ALA. This suggests the effect of Ang 1-7 may be mediated by a receptor other than the MAS receptor for Ang 1-7 and may be of lesser magnitude in animals exposed to B before birth. The reduced GFR in the B suggests prenatal steroid exposure can affect renal function.

Supported by NIH grant HD 04784.

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Widespread Distribution of Corticotrophin Releasing Factor-Binding Protein in Fetal Rat Gastrointestinal Tract: A Local Inhibitor for CRF-Mediated Stress Responses. Jayaraman Lakshmanan, John D Richard, Sharon K Sugano, Avish Arora, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: We recently demonstrated that fetal in utero meconium passage is a neuroviseral motor response to maternal hypoxic stress. Based on the marked increases in plasma CRF levels in hypoxic fetuses we hypothesized that in utero meconium passage is mediated by CRF pathways. In CRF-target tissues such as brain and placenta, a specific CRF-binding protein (CRF-BP) of 37kDa co-exists with CRF. Several in vitro studies suggest that CRF-BP can compete with CRF-R1 and CRF-R2 receptor subtypes for CRF and Urocortin I (a CRF analog) and prevent stress responses elicited by both stress hormones. In the present study we examined fetal rat gastrointestinal tract for CRF-BP in an attempt to define its role in prevention of stress hormones-mediated in utero meconium passage.

Methods: Paraformaldehyde fixed paraffin embedded whole GI tracts of fetal rats at day 21 of gestation (n=10) were subjected to immunohistochemistry with polyclonal antibody to human CRF-BP precursor (sc-1824, Santa Cruz Biotechnology, CA) using ABC technique with 3, 3'-diaminobenzidine as a chromagen. Immunoreactive intensities were quantified by Image Pro 4.01 software and expressed in arbitrary units (AU). For western blot analyses, whole fetal GI tracts (n=6, four GI tracts per pool) were homogenized in detergent buffer containing protease inhibitors and the homogenate supernatants were subjected to electrophoresis and immunoblotting with polyclonal antibodies using chemiluminescent reagent.

Results: CRF-BP antibody elicited strong positive staining at multiple sites throughout rat fetal GI tract. The most notable sites are: crypt cells (0.282±0.031 AU), enterocytes: (0.314±0.007 AU), myenteric ganglia: (0.325±0.324 AU) and submucosal ganglia: (0.343±0.035AU), serosal surface: (0.177±0.005 AU) and circular and longitudinal smooth muscle: (0.154±0.25 AU). Western blot analysis identified two major bands of 150 and 58 kDa in size and minor bands with molecular masses of 41, 37, 27 and 25 kDa.

Conclusion: We conclude CRF-BP, the key regulator of CRF and Urocortin-dependent stress responses is expressed in rat fetal gut at 21 days of gestation. The high molecular CRF-BP species is analogous to the molecular mass of recombinant CRF-BP secreted by cell lines transfected with CRF cDNA. CRF-BP may play an important role in preventing CRF-mediated in utero meconium passage.

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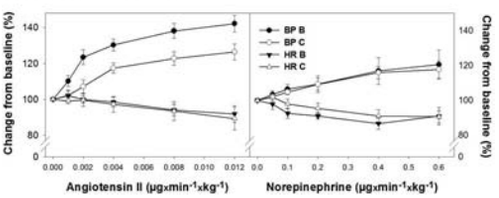
Antenatal Exposure to Betamethasone Enhances the Vascular Response to Angiotensin II in Adult Sheep Offspring. Victor M Pulgar, Angela G Massmann, Jorge P Figueroa. *Department of Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

Fetal exposure to high levels of glucocorticoids (GC) is considered to be one of the mechanisms underlying Fetal Programming. We have shown that antenatal exposure to GC induces hypertension and alterations in *in vitro* vascular reactivity in the adult offspring. The aim of this study was to investigate the *in vivo* responses to vasomotor agonists in the hypertensive offspring at 1.5 years of age.

METHODS: Pregnant sheep were treated with two IM doses of betamethasone (BM, 0.17 mg/kg) or vehicle 24 h apart at 80 days of gestational age and allowed to deliver at term. Adult sheep were studied at 1.5 yr of age. Under halothane general anesthesia, intravascular catheters were inserted in femoral artery and vein. Blood pressure and heart rate (HR) responses to increasing concentrations of intravenously administered angiotensin II (0.002-0.012 µg/Kg/min) and norepinephrine (0.1-0.6 µg/Kg/min) and to an L-NAME (2 mg/Kg) bolus were studied. Vascular reactivity was analyzed by curve fitting of either the blood pressure or HR responses to obtain the maximal response. Data are expressed as mean±SEM of change from baseline. Statistical significance was established using t test.

RESULTS: BM-exposed animals displayed a higher blood pressure response to At-II (AtII MAX 142±4 vs 101±5 % of baseline, $p<0.05$, Figure left panel). No differences in blood pressure or heart rate responses to norepinephrine infusion were detected in BM when compared to control animals (Figure 1 right panel). Maximal response to L-NAME was also elevated in BM-exposed animals but did not reach statistical significance with the current sample size.

CONCLUSION: We have shown that antenatal GC treatment significantly increases blood pressure in sheep. Here we show an enhanced *in vivo* response to At-II. This response may contribute to the increased blood pressure observed. The greater response to L-NAME most likely represent an increased NO production as a compensatory mechanism to the higher blood pressure observed. HL 68728.



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Effect of Prenatal Betamethasone Exposure on Responsiveness of Pituitary Cells Isolated from Adult Sheep. Luke C Carey,[1,2] Jorge P Figueroa,[1,2] James C Rose.[1,2] *[1]Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA; [2]The Center of Research for Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

Women at risk of delivering preterm are frequently treated with glucocorticoids to facilitate fetal lung maturation. Animal studies have revealed that prenatal exposure to glucocorticoids may alter aspects of hypothalamic-pituitary adrenal functioning in later life. In this study we examined the effects of clinically relevant prenatal betamethasone treatment on the responsiveness of pituitary cells (in terms of adrenocorticotropin, ACTH, secretion) isolated from adult sheep. Time-dated pregnant sheep were injected with betamethasone (0.17 mg/kg) or vehicle on days 80 and 81 of gestation. Thereafter pregnancy was allowed to continue unimpeded and offspring were born. Pituitaries were isolated from adult sheep aged between 6 and 9 months and cells were dispersed and plated at a density 2×10^5 cells per well in 48 well plates. After 48h, cells were stimulated with normal medium, 10nM arginine vasopressin (AVP), 100nM AVP, 1nM corticotropin releasing factor (CRF) or 10nM CRF for 2h. Medium was collected and analyzed for ACTH using a commercially available kit. ACTH secretion (given as % increase from untreated cells) was similar between the control and betamethasone groups following 10nM

AVP (21.9 ± 4.3 vs. 20.4 ± 6.6) and 1nM CRF (10.0 ± 2.8 vs. 10.7 ± 5.0). At higher stimulatory concentrations of both AVP and CRF, ACTH secretion remained similar in control cells (24.1 ± 4.9 and 11.8 ± 5.9 respectively), but was significantly decreased in betamethasone cells (9.9 ± 7.5 and 2.4 ± 4.3 respectively). These findings suggest that prenatal exposure to clinically relevant doses of betamethasone can alter pituitary responsiveness to both AVP and CRF in adulthood.

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Acute Hypoxic Stress-Induced In Utero Meconium Passage Accompanies a Total Depletion of Corticotrophin Releasing Factor Content from Placental Cells: Evidence That Stress-Induced In Utero Meconium Passage Is Mediated by Peripheral (Placenta) CRF Pathway. Jayaraman Lakshmanan, Sureshbabu N Ahanya, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: Newborn meconium (MEC) passage normally occurs within the first 24-48 h after birth. In contrast, more than half a million infants born annually in the US pass MEC in utero. Neither the physiological mechanism(s) nor causes for in utero MEC passage are well understood, though both fetal maturation and stress are associated. We recently reported in utero MEC passage and marked increases in plasma CRF levels in term fetal rats exposed to maternal hypoxic stress. We hypothesize that stress-induced in utero MEC passage is mediated by the CRF pathway in a manner analogous to stress-induced defecation in adult rats. In the present investigation we examined placental CRF content prior to and following maternal hypoxia, to determine the source of increased plasma CRF.

Methods: Time-dated pregnant rats (n=6) were exposed to a paradigm of graded, stepwise hypoxia on day 22 (term=22) (Pediatr. Res. 61: 176-179, 2007). Control pregnant rats were exposed to 21% oxygen for a similar duration as experimental animals. At the end of the study, placentas were harvested, fixed in 4% paraformaldehyde and paraffin embedded. Sections (n=6 per placenta) were subjected to immunohistochemical analyses with rat/human CRF antibody (Peninsula Laboratory) by ABC technique. Immunoreactivities on placental sections were quantified using the Image Pro 4.01 software and intensity expressed as arbitrary unit (AU). All values are mean± SEM.

Results: In control maternal rats exposed to normoxia, positive staining for CRF was seen in decidua (0.206±0.015 AU) and in all three major placental cells types (Giant trophoblast cells: 0.143±0.019 AU, Spongiotrophoblast cells: 0.133±0.003 AU and Labyrinth cells: 0.083±0.008 AU.) In contrast, in animals exposed to hypoxia, there was a total absence of CRF staining in the placental cells, with only positive CRF staining seen only in the decidua (0.197±0.006 AU).

Conclusion: The total absence of CRF staining in both the basal and labyrinth zones in placentas of pregnant rats subjected to hypoxic stress suggests that placental cells rapidly release CRF into the maternal and fetal circulation in response to hypoxic-stress. Our findings support our hypothesis that the peripheral CRF pathway mediates in utero MEC passage.

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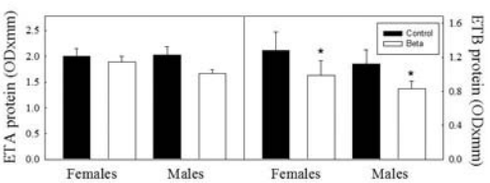
Antenatal Betamethasone Exposure at 80 Days Gestation Alters Kidney Medulla Endothelin B Receptor (ETB) Expression in Adult Sheep Offspring. Angela G Massmann, Jie Zhang, Jorge P Figueroa. *Department of Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

OBJECTIVE: In rats and sheep, exposure to glucocorticoids (GC) in the perinatal period induces hypertension in adult life. Recent evidence suggests that endothelin B (ETB) receptor plays an important role in the regulation of sodium balance and blood pressure. Renal medulla is an important site of expression and action of ETB receptor. Furthermore, in kidney it is the medulla (KM) where the highest concentrations of immunoreactive ET-1 and ETB receptor are found. The aim of this study was to measure ETA and ETB expression in adult sheep kidney exposed antenatally to GC.

METHODS: Pregnant sheep were treated with two IM doses of betamethasone (BM, 0.17 mg/kg) or vehicle (V) 24 hours apart at 80 days of gestational age and allowed to deliver at term. At 1.5 yr of age, male and female offspring exposed to either vehicle or BM were euthanized and the kidneys harvested. Kidney medulla (KM) was obtained by sharp dissection. ETA and ETB protein and mRNA expression were evaluated by western blot and RNAase protection assay. Data are expressed as Mean±SEM and were analyzed by two way ANOVA.

RESULTS: A significant decrease in ETB (F=5.166, P=0.030) but not ETA protein was observed in KM of BM exposed male and female adult sheep. At the mRNA level, BM exposure also affected ETB, but not, ETA expression. However, BM treated animals had higher mRNA levels than control sheep (F=7.05; P=0.01).

CONCLUSION: Our data show that prenatal exposure to a single course of GC at 0.55 gestation has long-term effects. The activation of ETB receptor by ET-1 inhibits sodium transport function in the collecting duct and the selective gene deletion of the ETB in medulla results in hypertension.Therefore, our finding of a significant reduction in ETB protein suggests that there may be an impairment in the kidney's ability to regulate sodium reabsorption. The functional relevance of the alterations in ETB protein expression as well as the discrepancies in mRNA regulation need to be established. HL 68728; HD P01 HD04784.



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Maternal Glucocorticoid Administration in Early Pregnancy and the Relationship between 11βHSD2 mRNA in Ovine Placenta and Fetal Weight across Gestation. MC Audette,[1] KL Connor,[1] T Braun,[1] S Li,[2] TM Moss,[2,3] DM Sloboda,[2,3,4] JP Newnham,[2,3] JRG Challis.[1,5] *[1]Physiology, University of Toronto; [2]School of Women's and Infants' Health, University of Western Australia; [3]Women and Infants Research Foundation, University of Western Australia; [4]The Liggins Institute, University of Auckland; [5]Obstetrics, Gynecology and Medicine, The University of Toronto.*

In the placenta, 11β-Hydroxysteroid Dehydrogenase Type 2 (11βHSD2) regulates the transfer of cortisol. Maternal glucocorticoid (GC) administration has been shown to affect the ovine fetal growth trajectory and regulate 11βHSD2 expression in mid and late gestation. However, little is know about the effects of GC administration in early gestation. We hypothesized that maternally administered low-dose dexamethasone (DEX) in early gestation would affect 11βHSD2 expression, which will subsequently alter fetal birth weight. Pregnant ewes were randomized and received 4 intramuscular injections consisting of either saline (2ml saline/ewe) or DEX (0.14 mg/kg) given 12 hours apart starting on 40 days of gestation (dG). The animals were sacrificed at 50, 100, 125, and 140dG and fetal weights were recorded and placental tissue was collected. qRT-PCR was used to measure 11βHSD2 placental mRNA expression. Statistical analysis was done by ANOVA with Student's t-test post-hoc. Overall, there was no significant effect of DEX treatment on placental 11βHSD2 mRNA expression across gestation. However at 50dG, there was a significant increase in 11βHSD2 expression after DEX (p=0.026). There was an increase in placental 11βHSD2 mRNA progressively from 50dG (mean = 0.45) to 140dG (mean =1.48), independent of treatment. Overall, a positive correlation between 11βHSD2 and fetal weight (R^2=0.43) was apparent at 50dG and at 140dG (p=0.065) in both control (p=0.01) and DEX (p=0.13). Significant positive correlations between placental 11βHSD2 and fetal weight (R^2=0.45) were found in females (p=0.01) and a similar trend was found in male fetuses (p=0.07). We conclude that in the sheep placenta, 11βHSD2 expression increases throughout gestation and may respond, at least transiently in early gestation, to elevations in maternal GC. The positive correlation between 11βHSD2 and fetal weight is consistent with the thesis that the fetal growth trajectory may be influenced by maternal cortisol levels and that placental 11βHSD2 is an important mediator of these effects.

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Antenatal Glucocorticoids (GC) Profoundly Change Renal and Cerebral Vascular Tone in the Aged Rat. Carolin Schinzel, Daniel Reymann, Otto W Witte, Matthias Schwab. *Department of Neurology, Friedrich Schiller University Jena, Germany.*

Antenatal GC exposure induces hypertension in the young adult rat (Neuroendocrinol;1996;64:412) and increases femoral vascular resistance in the lamb (JPhysiol;2003;547:61). Unpublished own examinations in preparation of this study have shown age dependency of the vascular reactivity. Vascular contractility of the middle cerebral artery (MCA) decreased in response to

K^+ and noradrenaline (NA) between 3mo and 2,5y of age ($p<0.01$). Vascular relaxation to acetylcholine (Ach) but not to PGE2 decreased during the same time ($p<0.01$). Vascular contractility of the renal artery (RA) increased in response to K^+ ($p<0.05$).

Aims: To examine if antenatal GC alter vascular reactivity in the aged individual when cardiovascular diseases are predominant. We studied the RA because its vascular tone is involved in modulation of arterial pressure control (AmJPhysiol;2002;283:R441) and the MCA because depressive disorders that are associated with dysregulation of the HPA axis (JCEM;1997;82:234) increase stroke mortality for unknown reasons (AmJPsychiatry;2003;160:1823). To be clinically relevant, we administered dexamethasone at the dose used to enhance lung maturation in babies threaten premature labor.

Methods: Pregnant dams received saline (n=6) or 170µg/kg dexamethasone (n=6) i.p. at day E19/20 equivalent to 2x12mg dexamethasone administered to a 70kg pregnant woman. At 2.5 years of age, vascular response of the RA and MCA to endothelium-dependent and independent mediators was measured using wire myography. Vessels were inspected histologically for intact endothelium.

Results: Basal vasoconstrictory and dilatory responses to all mediators were less pronounced in the MCA than in the RA probably reflecting autoregulatory properties of cerebral vessels ($p<0.05$). After prenatal dexamethasone exposure, contractility but not sensitivity to K^+ and NA was enhanced in the MCA and even more pronounced in the RA ($p<0.05$). Relaxation to Ach and PGE2 was similarly diminished in both vessels after precontraction with NA ($p<0.05$).

Conclusions: Prenatal dexamethasone exposure at the dose used clinically increases renal and cerebral vascular tone in the aged rat by endothelium-dependent and independent mechanisms. This effect may be a potential mechanism of fetal programming of cardiovascular diseases in later life.

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Prenatal Betamethasone Exposure Alters Renin Angiotensin System Enzyme Expression in Adult Male Sheep. Jennifer G Smith,[1] Shashima Nakahara,[1] Brian Westwood,[2] Jorge Figueora,[1] Mark C Chappell,[2] James C Rose.[1] *[1]Obstetrics and Gynecology, Wake Forest University, Winston Salem, NC, USA; [2]Hypertension and Vascular Disease Center, Wake Forest University, Winston Salem, NC, USA.*

Objective:

Betamethasone (BMZ) therapy during pregnancy to improve fetal lung maturity may result in long term side effects, including hypertension, during adulthood. The kidney is an important organ which regulates systemic blood pressure via the local renin angiotensin system (RAS). The major enzymes of the intrarenal RAS include angiotensin converting enzyme (ACE), angiotensin converting enzyme 2 (ACE2), and neprilysin. The hypothesis of this study is that prenatal BMZ exposure will result in alterations of the enzymes regulating the intrarenal RAS. Specifically, sheep that are treated with BMZ in utero will have higher amounts of ACE activity in kidney cortex compared to control animals.

Materials and Methods

Pregnant sheep of known mating date were either treated with vehicle (n=7) or with two doses of BMZ (n=6), 0.17mg/kg, 24 hours apart at 80 days of gestation. Renal cortex from the male offspring was harvested at 19-24 months of age. Cortical membranes were then solubilized and incubated at 37°C with either ^{125}I-Ang I or ^{125}I-Ang II in the presence or absence of lisinopril to inhibit ACE activity, MLN4760 to inhibit ACE2 activity, or SCH39370 to inhibit neprilysin activity. The metabolic products were separated by reverse-phase high performance liquid chromatography (RP-HPLC). The rate of enzyme activity was quantified by calculating the area under the curve for each product and converted to fmol of product per mg protein per minute of incubation. Statistical analysis was performed using Student's t-test. $p<0.05$ was considered significant.

Results:

BMZ treatment resulted in a significant increase in ACE activity compared to control animals ($p=0.0073$). ACE2 and neprilysin levels were not significantly different between treatment groups. When expressed as a ratio of ACE/ACE2 activity, BMZ treated animals exhibited a ~2.5 fold greater proportion of ACE activity versus control animals ($P=0.04$).

Conclusions:

This study demonstrates that BMZ exposure during fetal life results in a significant increase in renal ACE activity during adult life. This increase in enzyme activity would be expected to be associated with increased levels of Ang II in the kidney and Na^+ retention, possibly underlying the development of hypertension.

HD47584, HD17644.

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Term Fetal Distal Colon Is Totally Wired by Intrinsic- and Extrinsic-Corticotrophin Releasing Factor (CRF) Nerve Fibers: Mechanism of *In Utero* Meconium Passage. Jayaraman Lakshmanan, Noboru Oyachi, Sharon K Sugano, Guo L Liu, Michael G Ross. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: We recently hypothesized that stress-induced in utero meconium passage in term fetuses utilizes peripheral and/or central CRF pathways in a manner analogous to stress-induced defecation in adult rats. As participation of central CRF pathway requires the CRF circuitry system in brain-gut axis, we exploited immunohistochemical techniques to address whether term fetal colon is wired by CRF-nerve fibers.

Methods: Fetal distal colon segments (n=6) were dissected from term ovine fetuses (146-147 d gestation). In addition, lumbosacral spinal cord with spinal roots and dorsal root ganglia (DRG) (n=3) were dissected from ovine fetuses, and vagal nerve trunk with nodose ganglia (n=3) were dissected from mouse fetuses. Paraffin sections fixed either in Bouin's solution or Zamboni's were subjected to immunohistochemistry with rabbit polyclonal antibodies specific to ovine-CRF (oCRF). Immunoreactivity on the sections was identified by standard ABC technique, immunostaining quantified by Image-Pro Plus software and expressed (intensity over area) as arbitrary units (AU).

Results: oCRF antibody elicited strong positive staining on the serosal surface (port of entry for extrinsic nerve fibers) in distal colonic segments (0.112±0.057AU). A sub-population of enteric neurons elicited strong positive staining (0.165±0.001 AU). Intense oCRF staining also evident in nerve fibers spanning the smooth muscle layer and lamina propria. The oCRF antibody also elicited strong positive immunostaining in spinal cord motor neurons (0.129±0.004 AU) and DRG sensory neurons of large size (0.141±0.006 AU) in ovine fetal spinal cord-DRG preparations. Spinal roots also elicited positive oCRF immmunoreactivity of similar intensity (0.133±0.016 AU) as those motor and sensory neurons

Conclusion: CRF immunostaining in serosal layers indicates that the distal colon receives CRF nerve fibers from projection neurons, while staining in spinal motor neurons, DRG sensory neurons and spinal roots suggests that the CRF neural network is a component of spinal visceral pathway.

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Finally, at Term Precipitous Drops in Urocortin-I (UCN-1) Innervation Occurs in Ovine Fetal Distal Colon: Dynamics of Colonic Stress Circuitry System Prior to Birth. Jayaraman Lakshmanan,[1] Guong L Liu,[1] Sharon K Sugano,[1] Monica Ferrini,[2] Reuben Lakshmanan,[1] Michael G Ross.[1] *[1]Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA; [2]Dept. of Urology, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: We hypothesize that stress-induced in utero meconium passage is mediated by the CRF pathway. UCN-I, similar to CRF, is a potent contractility inhibitor of preterm ovine fetal distal colon, which has abundant CRF-R2 receptors. Both UCN-1 and CRF thus may inhibit colonic motility and prevent preterm in utero meconium passage via CRF-R2 receptors. However, UCN-I is reported to stimulate contractility of adult rat colonic smooth muscle strips more strongly than CRF. We sought to study the pattern of UCN-I innervation in distal colon of ovine fetuses to delineate whether UCN-1 functions as a facilitator or inhibitor of colonic propulsive motility.

Methods: Bouin's solution fixed, paraffin sections of very preterm (VPT: 118-120 days gestation), preterm (PT: 130-132 days), near term (NT: 140-142) and term (T: 146-147days) ovine fetal distal colon rings were subjected to immunohistochemistry with polyclonal antibodies to human UCN-1 (1:500 Sigma) by ABC system. Intensity of UCN-1 immunostaining in smooth muscle layers and myenteric neurons were quantified by Image-Pro Plus software and expressed (intensity/area) in arbitrary units (AU). Differences over time were assessed with ANOVA.

Results: UCN-1 antibody elicited positive staining in smooth muscle layers and enteric neurons in distal colon at all four gestational ages investigated. The UCN-1 immunoreactivity as quantified by image analysis is as follows: Muscularis muscosa (AU): VPT 0.313±0.004, PT 0.193±0.009, NT 0.176±0.007, T 0.180±0.014; Longitudinal muscle layer (AU): VPT 0.340±0.013, PT 0.232±0.024, NT 0.234±0.014, T 0.117±0.009; Circular muscle layer (AU): VPT 0.284±0.009, PT 0.224±0.009, NT 0.162±0.008, T 0.106±0.010; Myenteric neurons (AU): 0.326±0.017, PT 0.221±0.008, NT 0.180±0.021, T 0.192±0.017; Submucosal Neurons (AU): VPT 0.310±0.020, PT 0.197±0.014, NT 0.232±0.014, T 0.120±0.004. In all groups very preterm was significantly greater than term ($p<0.05$).

Conclusion: UCN-1 immunoreactivity in all three muscle layers, as well as

myenteric and submucosal neurons, is highest at very preterm as compared to more mature gestations. These results suggest that the reduction of UCN-1 inhibitory function may facilitate stress-induced meconium passage at term.

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Effect of Intrarenal Infusion of Angiotensin II (Ang II) in Male Sheep with or without Prenatal Exposure to Antenatal Betamethasone (B). Stephen A Contag,[1] Jianli Bi,[1] Jorge P Figueroa,[1] Lijun Tang,[1] Mark Chappell,[2] Jim C Rose.[1] *[1]Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA; [2]Hypertension, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

Objective:
To evaluate the effect on blood pressure (BP), urinary output (UOP), sodium excretion (NaE) and GFR of the intrarenal infusion of Ang II in the male sheep after prenatal exposure to B.
Methods:
We studied male sheep which had received either B (n=3) or vehicle (n=3) at 80-81 days gestation. Catheters were placed in the femoral artery and vein, left renal artery and in the bladder. A right side nephrectomy was performed. After 5 days of recovery, the sheep were infused with Ang II (1 ng/kg/min) with or without candesartan (10 ng/kg/day) or PD123319 (PD 500µg loading dose and 10 ng/kg/min infusion) into the renal artery over 24 hours. BP, GFR, UOP and NaE were measured before and after the infusion. Two-way analysis of variance was used to test mean values between the groups.
Results:
BP and UOP did not change with Ang II infusion with or without candesartan. The infusion of PD did not affect UOP but did increase BP in the B sheep (p=0.03). Ang II decreased the NaE in both groups(p=0.0007). Candesartan increased NaE among controls(controls 0.16 and B 0.56 mEq/kg/h, p=0.048). PD infusion decreased NaE among controls but this was not significant. Baseline GFR was higher among vehicle compared to B animals (p=0.0172). During Ang II infusion there was a decrease in GFR among control compared to B sheep (-71.17 vs. -18.46 ml/min, p=0.0081). This difference was not seen during candesartan or PD infusion.
Conclusion:
Ang II infusion led to a decrease in NaE in both groups and GFR among controls but not B animals. Infusion of Ang II with candesartan increased NaE while PD decreased NaE among controls but not B animals. This suggests that prenatal steroid exposure can alter AT2 receptor mediated response in renal function by decreasing the effect of Ang II on renal sodium excretion. The differences in GFR suggest that this may be due to an effect on tubular sodium reabsorption rather than glomerular perfusion.

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Antalarmin Antagonism of ACTH-Induced Cortisol Secretion by Ovine Adrenal Cells Probably Not at ACTH Receptor. Nancy K Valego, James C Rose. *Center of Research for OB/GYN, Wake Forest U. School of Medicine, Winston-Salem, NC, USA.*

In addition to regulating the HPA axis, CRH and its type 1 receptor (CRHR-1) occur peripherally and in the female reproductive system. Late in human pregnancy, a surge in placental CRH probably stimulates adrenal secretion of cortisol and DHEAS requisite to parturition. We previously reported that, in dispersed fetal or adult ovine adrenal cortical cells, 24 hour incubation with the specific CRH-R1 antagonist, antalarmin (ANT), significantly reduced both cyclicAMP and cortisol responses to ACTH, suggesting an interaction with ACTH-R (like CRH-R1, a G-protein-coupled membrane receptor). However, forskolin (FSK; direct stimulant of adenylyl cyclase)-stimulated cortisol secretion was also attenuated by 24 hour co-incubation with ANT. Our objective was to clarify the effect of ANT on binding of ACTH to its receptor after a short (2.5 hours) treatment.
METHOD: ACTH binding: The adrenals from adult sheep were obtained at necropsy and the cortex cells dispersed and plated @ 200000 cells/well. After 48 hours in culture, wells were rinsed 2x and refilled with serum-free DMEM/F12 containing vehicle (DMSO) with or without ANT. After 2.5 hours, wells were washed very gently and the binding assay (adapted from Rainey et al; J Biol Chem,264:21474,1989) completed. Triplicate vehicle or ANT wells were treated with I^{125}-tyrosine^{23}human ACTH (1-39) with or without 10^{-6} M ACTH (for non-specific binding). After 1 hour, wells were chilled, washed, and the cells lysed and counted on a gamma counter.
FSK treatment: Cells were treated as above except that FSK (10^{-6} M) was added to the wells. After 2.5 hours, medium was removed and stored @ -80ºC for cAMP EIA and cortisol RIA.

Results (mean±SE) of 2.5 hour incubation on ACTH binding and FSK-induced secretion.

	VEHICLE	ANTALARMIN
ACTH binding (net CPM) n=4	3024±415	2966±463
Cortisol (ng/ml/2.5hr) n=7	144±20	126±19* (p=.012)
cAMP (pMol/ml/2.5hr) n=12	9.1±2.0	4.8±1.1* (p=.003)

* indicates significant difference from vehicle alone.

CONCLUSION: The CRH-R1 antagonist, antalarmin, attenuates ACTH-stimulated cortisol secretion but not by preventing ACTH receptor binding. However, its effect is early in the secretory process and is associated with a reduced cAMP response.

Supported by NIH grant HD11210.

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Plasma Renin Concentration in Female Sheep Following Antenatal Betamethasone Exposure. L Kantorowicz,[1] NK Valego,[1] JP Figueroa,[1] MC Chappell,[2] L Tang,[1] JC Rose.[1] *[1]Ob/Gyn, Wake Forest University, Medical School, Winston-Salem, NC, USA; [2]Hypertension and Vascular Disease Center, Wake Forest University.*

OBJECTIVE
Glucocorticoids are often administered to pregnant women to prevent neonatal respiratory distress syndrome. Prenatal steroid treatment increases blood pressure in adult sheep. Exposure to excess corticosteroids before birth is hypothesized to be a key mechanism underlying the fetal origins of adult disease hypothesis and effects on the renin-angiotension system (RAS) may modulate the steroid-induced increases in blood pressure.
We therefore sought to determine if renin processing and secretion were altered in adult female sheep exposed antenatally to betamethasone (β) and to compare them with data from males studied previously.
METHODS
Pregnant sheep were randomized to receive 2 doses of 0.17 mg/kg of β or vehicle, at 80 and 81 days of gestation; the offspring were studied at 6 and 18 months of age.
In 17 female offspring, active renin concentration (ARC) and total renin concentration in plasma were measured by RIA of angiotensin I generated by incubation with excess substrate. Prorenin concentration (PRC) is the difference between total and active renin. Nine β or control exposed female animals born at term (142-148 days of gestation) were brought from the farm at 12- 32 months of age, and had vascular and bladder catheters placed. Five days after surgery, a sodium load of hypertonic NaCl (0.0275 meq/kg/min at 0.55 ml/min) was given for 60 min. Blood samples were obtained.
Data are expressed as mean SEM and were analyzed by *t* test.
RESULTS
The PRC was significantly higher in the females than in the males (17.74± 1.15 vs 8.90± 1.46 p<0.0001) but there was no effect of prenatal steroid treatment. ARC was similar in both genders. However, ARC was a significantly greater percent of the total plasma renin concentration in the males (33.9± 12.7 vs 13.88± 3.06 p<0.0001)
During the Na infusion experiment, the β exposed females had lower ARC than did the control females (0.40± 0.08 vs 1.28± 0.20 p<0.05).
CONCLUSION
The data suggest that prenatal exposure to β didn't alter the processing and secretion of renin in adult female sheep. Prenatal steroid treatment does not appear to alter the effect of gender on plasma renin levels in adult sheep.It seems unlikely that the elevated blood pressure seen in adult ewes after prenatal β exposure is the result of increased secretion or processing of renin.
Supported by HD 47584 and HD17644.

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Ontogenic Patterns and Effect of Long Term Hypoxia (LTH) on Melanocortin 2 Receptor Accessory Protein (MRAP) Expression in the Ovine Fetal Adrenal Cortex. Dean A Myers,[1] Kimberly Hyatt,[1] Malgorzata Mlynarczyk,[2] Kanchan M Kaushal,[2] Charles A Ducsay.[2] *[1]Ob/Gyn, Univ. Oklahoma HSC, Oklahoma City, OK; [2]Ctr. for Perinatal Biol., Loma Linda Univ., Loma Linda, CA.*

Background: MRAP is a recently discovered protein with alpha and beta isoforms, a common amino terminal region and divergent C-terminal sequences generated by alternative splicing. In human and mouse MRAP plays an essential role in the generation of a functional, G-protein coupled ACTH receptor (Melanocortin-2 receptor; MC2R) but has not been described for ruminants.

We previously reported that LTH fetal sheep exhibited elevated basal $ACTH_{1-39}$ yet decreased expression of key steroidogenic enzymes and the MC2R in the adrenal cortex while basal cortisol levels were not different from control. We hypothesized that MRAP could play a key role in mediating the effect of LTH as well as developmental regulation of fetal adrenal cortisol synthesis in fetal sheep. The goals of the present study were to determine the ontogenic pattern of MRAP expression in the sheep fetus and to determine if LTH alters MRAP expression.

Methods: We searched the bovine genomic sequence database (www.ncbi.nlm.nih.gov/genome/guide/cow/) and found a sequence with >90% homology to the human MRAP N-terminal 68 residues, with partial homology to the beta isoform in the carboxyl region (~65%). Using primers based on the bovine sequence, we confirmed the presence of MRAP in the ovine fetal adrenal cortex. Adrenal cortical tissue was collected from sheep fetuses from 105-120 (n=5) and near term (142-145; n=5) days of gestation (dG) as well as from LTH (n=6) fetuses (exposed to high altitude hypoxia from ~40 to 139-141 dG) and age-matched normoxic controls (n=6). Cyclophilin was used as a housekeeping mRNA. Data are expressed in fg mRNA/50 ng total RNA.

Results: The expression of MRAP, based on quantitative RT-PCR, was low between 105-120 dG (11.72 ± 4.2) and increased ($p<0.05$) approx. 5-fold near term (140-145 dG; 50.6 ± 7.3). Levels of mRNA for MRAP were highly correlated to CYP17 mRNA in individual samples. MRAP expression in control (25.1 ± 2.5) and LTH (26.5 ± 4.2) did not differ between groups.

Conclusions: MRAP may represent a major gene governing adrenocortical function and development during late gestation. Unlike expression of the MC2R, MRAP does not appear to be regulated by LTH and would thus support greater coupling/functionality of this receptor in the LTH fetal adrenal. (Supported my NIH grants HD33147 and HD31226).

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Gender Differences in Key Enzymes of the Renin Angiotensin System in Adult Sheep. Jennifer G Smith,[1] Brian Westwood,[2] Shashima Nakara, Jorge Figueroa,[1] Mark C Chappell,[2] James C Rose.[1] *[1]Obstetrics and Gynecology; [2]Hypertension and Vascular Disease Center, Wake Forest University, Winston Salem, NC, USA.*

Objective

Gender differences in hypertension are well described and there is growing evidence that the regulation of the renin angiotensin system (RAS) is influenced by sex hormones. The major enzymes of the RAS include angiotensin converting enzyme (ACE), angiotensin converting enzyme 2 (ACE2), and neprilysin. The peptide products of these enzymes have opposing actions. Angiotensin II (Ang II), the product of ACE, is a potent vasoconstrictor. On the other hand, the peptide products of ACE2 and neprilysin, Ang (1-7) and Ang (1-4), exhibit vasodilatory properties. Thus, modifications in the relative proportions of these enzymes and their peptide products can result in alterations of systemic blood pressure. The purpose of this study was to describe the gender differences in angiotensin converting enzyme (ACE), angiotensin converting enzyme 2 (ACE2), and neprilysin (NEP) enzyme activities in adult sheep kidney cortex.

Materials and Methods

Renal cortex from male (n=5) and female (n=8) sheep were harvested at 15-31 months of age. The tissue membranes were solubilized and incubated at 37°C with either ^{125}I-Ang I or ^{125}I-Ang II in the presence or absence of lisinopril to inhibit ACE activity, MLN4760 to inhibit ACE2 activity, or SCH39370 to inhibit neprilysin activity. The metabolic products were then separated by reverse-phase high performance liquid chromatography (RP-HPLC). The rate of enzyme activity was then quantified by calculating the area under the curve for each product and converted to fmol of product per mg protein per minute of incubation. Statistical analysis was performed using Student's t-test. $p<0.05$ was considered significant.

Results

ACE activity was over 4 times greater (1282 ± 103.5 vs. 303.3 ± 118.4 fmol/mg/min, $p<0.0001$), ACE2 activity was 2.5 times greater (1826 ± 312.5 vs. 721.2 ± 86.4 fmol/mg/min, $p=0.02$) and NEP activity was nearly 2 times greater (761.3 ± 73.09 vs. 401.5 ± 39.81 fmol/mg/min, $p=0.0081$) in female kidney cortex.

Conclusions

In adult sheep, key enzymes of the intrarenal RAS have signficantly greater activity in female kidney cortex. These findings suggest that there are fundamental, gender specific, differences in the regulation of the enzymes of the intrarenal RAS. The physiologic significance of these findings remain to be elucidated.

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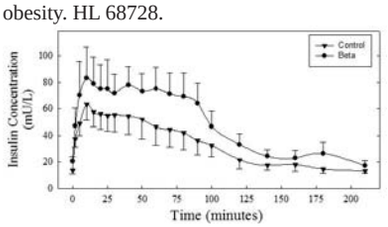
Effects of Diet-Induced Obesity on Insulin Sensitivity in Adult Sheep Exposed Antenataly to Glucocorticoids. Jie Zhang, Angela G Massmann, Victor M Pulgar, Jorge P Figueroa. *Department of Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.*

In rats and sheep exposure to glucocorticoids (GC) in the perinatal period is associated with a reduction in nephron number and hypertension in adult life. Furthermore, antenatal exposure to GC alters glucose tolerance in animals and in people. The aim of the present study was to determine 1) if insulin resistance is a contributing factor for the development of hypertension in adult sheep exposed antenatally to GC and 2) if diet-induced obesity has a more pronounced effect in sheep exposed antenatally to GC.

METHODS: Pregnant sheep were treated with two IM doses of betamethasone (BM, 0.17 mg/kg) or vehicle (CTR) 24-hours apart at 80 days gestational age and allowed to deliver at term. At 9 mo of age, female sheep were randomly allocated to be fed at either 100% of recommended nutritional allowance or ad libitum for three months. Sheep were chronically instrumented under general anesthesia to place intravascular catheters. Insulin sensitivity was evaluated both by iv glucose tolerance test (IVGTT) and euglycemic clamp (HEC)techniques. For the IVGTT a 0.25 g/Kg glucose bolus was used and for the HEC 4mU/Kg human insulin was used. Data Mean±SEM were analyzed by ANOVA and/or two sample t test.

RESULTS: Ad lib fed sheep gain > 50% of the original weight. Antenatal BM was associated with an elevation in basal and IVGTT plasma insulin values. As shown on the figure, diet-induced obesity significantly increased insulin plasma levels during IVGTT in BM-exposed adult female sheep ($F=18.982;p<0.001$). Insulin sensitivity derived from HEC was significantly decreased by obesity in BM-exposed adult female sheep.

CONCLUSION: Our data show that prenatal exposure to a single course of GC at 0.55 gestation has long-term effects on glucose metabolism regulation. BM-exposed sheep exhibit alterations in glucose tolerance, hyperinsulinemia and insulin resistance. These abnormalities are exagertated by diet-induced obesity. HL 68728.



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Syngergistic Induction of 11β-Hydroxysteroid Deyhdrogenase Type 1 Expression by Cortisol and Interleukin-1β in Human Fetal Lung Fibroblasts. Z Yang,[1] P Zhu,[1] CM Guo,[1] L Myatt,[2] K Sun.[2] *[1]School of Life Sciences, Fudan University, Shanghai, China; [2]Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA.*

Objectives: Glucocorticoids acting via glucocorticoid receptor (GR), serve as crucial hormones in fetal lung maturation. Glucocorticoids and pro-inflammatory cytokines to induce 11b-hydroxysteroid dehydrogenase type 1 (11b- HSD1) which converts inactive cortisone to active cortisol, but their effect on 11b-HSD1 expression has not been addressed in human fetal lung. We examined the interactions and mechanism of cortisol and interleukin-1b (IL-1b) effect on 11b-HSD1 in human fetal lung fibroblasts (HFL-1 cells).

Methods: The expression of 11b-HSD1 in HFL-1 was examined with immunocytochemistry and PCR. 11b-HSD1, prostaglandin H synthase-2 (PGHS-2) and cytosolic phospholipase A2a (cPLA2) mRNA levels in cultured human fetal lung fibroblasts treated with cortisol and IL-1b were measured with real time PCR. The roles of GR and C/EBPs in the effect of cortisol and IL-1b were studied using a GR antagonist (RU486) and transfection of plasmid carrying C/EBP-specific dominant-negative gene (CMV500-A/CEBP).

Results: HFL-1 cells expressed a high level of 11b-HSD1. Both cortisol (10^{-8}-10^{-6}M) and IL-1b (0.1-10 ng/ml) induced 11b-HSD1 mRNA expression in a concentration-dependent manner, an effect blocked by the mRNA transcription inhibitor 5,6-dichlorobenzimidazole riboside (75 μM). RU486 (10^{-6} M) blocked the induction of 11b-HSD1 by cortisol. Induction of 11b-HSD1 mRNA expression by cortisol (10^{-6} M) was synergistically increased by co-treatment with IL-1b (0.1- 10 ng/ml) in a concentration-dependent manner. In contrast, the induction of cPLA2 and PGHS-2 expression by IL-1b was inhibited by cortisol,

suggesting a different mechanism of interaction. Transfection of the cells with C/EBP-specific dominant-negative plasmid attenuated induction of 11b-HSD1 mRNA expression by either cortisol or IL-1b. These data suggest induction of 11b-HSD1 expression by cortisol is a GR dependent process involving C/EBPs, which also mediate induction of 11b-HSD1 expression by IL-1b.

Conclusions: Cortisol and IL-1b synergistically induce 11b-HSD1 expression in human fetal lung fibroblasts. This would lead to greater local cortisol production, perhaps providing either a self-attenuating mechanism for control of inflammation or a mechanism for enhancing fetal lung maturation when the fetus is exposed to cytokines e.g. with infection-induced preterm labor.

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Do alterations in Placental 11β-Hydroxysteroid Dehydrogenase (11βHSD) Activities Explain Differences in Fetal Hypothalamic Pituitary Adrenal Function Following Periconceptional Undernutrition or Twinning in Sheep? KL Connor,[1] PL van Zijl,[2] CW Rumball,[2,3] AL Jaquiery,[2,3] JE Harding,[2,3] MH Oliver,[2,3] FH Bloomfield,[2,3] JRG Challis.[1,4] *[1]Physiology, University of Toronto; [2]Liggins Institute, University of Auckland; [3]National Research Centre for Growth & Development, New Zealand; [4]Obstetrics, Gynaecology & Medicine, University of Toronto.*

Periconceptional undernutrition (PCUN) leads to activation of fetal hypothalamic pituitary adrenal (HPA) function, whereas twinning results in delayed fetal HPA activation. We hypothesized that these differences in fetal HPA activity were the result of altered patterns of expression of placental of 11βHSD isozymes and hence of the maternal glucocorticoid (GC) effect on the fetus. We developed a mass spectrometric assay for the measurement of 11βHSD-1 and -2 activities and validated this method against a widely used thin layer chromatography method. Sheep were randomly assigned to ad libitum (N control) concentrates throughout gestation or were undernourished (UN) from 60 days before until 30 days after mating to reduce maternal body weight by 15%, with ad libitum feeding thereafter. Placentomes were collected on days 50, 85, 120, and 131 of gestation (term, 147d) and 11βHSD-1 and -2 activities were determined by measuring the rate of interconversion between cortisone to cortisol. With UN, there was a trend towards lower 11βHSD-2 activity at d50 ($p=0.1$), a significant reduction at d85 ($p<0.05$), but no difference at d120 or d131. 11βHSD-1 activity was not different between N and UN animals at any time. There was no effect of twinning on 11βHSD-1 or -2 at d50. However, with twins both 11βHSD-1 ($p=0.01$) and -2 ($p<0.05$) activities increased at d85. 11βHSD-1 activity was reduced in twins ($p=0.06$) at d120 but was higher than any singleton pregnancies at d131 ($p=0.06$). 11βHSD-2 was not different between singletons and twins at either d120 or d131. Overall, 11βHSD-2 was lower in placentae of male compared to female fetuses in late gestation; 11βHSD-1 was higher in male than female fetuses. There was no interaction with UN in either sex. We conclude that PCUN and twinning result in alterations of placental 11βHSD activities in sheep. Modifications in these enzymes during critical periods of fetal development may affect transplacental transfer or placental generation of GCs reaching the fetus potentially influencing the timing of activation of the fetal HPA axis, fetal maturation and later life development.

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Maternal Undernutrition Influences Offspring Adrenal Expression of Steroidogenic Enzymes in a Gender Specific Manner. Naseem Khorram, Thomas Magee, Mina Desai, Michael G Ross, Omid Khorram. *Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA, USA.*

Objective: To determine the adrenal mRNA expression of enzymes involved in the glucocorticoid (GC)/mineralocorticoid (MC) synthesis pathway in neonatal and adult offspring of maternal food restricted (MFR) rats.

Methods: Pregnant Sprague-Dawley rats had 50% MFR from day 10 of gestation until delivery. Control animals had ad libitum food. Offspring were sacrificed on day 1 of life (p1) and at 6 months (n= 6-8 per group). Adrenals were dissected and snap frozen in liquid nitrogen for later extraction of RNA. Real time RT-PCR using specific rat primers was used to quantify mRNA levels (18S as control). We evaluated the expression of 11-beta hydroxylase (CYP11B1), aldosterone synthase (CYP11B2), ACTH receptor (MCR2), P450 side chain cleavage enzyme (CYP11A1), STAR protein, 11 β-hydroxysteroid dehydrogenase type 1 (HSD1) and type 2 (HSD2), glucocorticoid receptor (GR), and mineralocorticoid receptor (NR3C2). Fold changes in mRNA expression in controls and MFR offspring was compared by Student's t-test.

Results: There was a marked downregulation in expression of CYP11B1 (P=.01), CYP11B2 (P=.04), HSD2 (P=.03), P450 (P=.05), ACTH receptor (P=.05), STAR (P=.01) and NR3C2 (P=.02) mRNA in p1 MFR offspring, with no changes in HSD1 and GR. Gender specific differences were found in the adult MFR offspring. In the male MFR offspring the expression of HSD1 and GR were significantly upregulated with a trend towards an increase in ACTH receptor (P=.07) whereas in the female MFR the expression of ACTH receptor (P=.02) was increased and NR3C2 (P=.03) and CYP11B2 were decreased (P=.04). In combined data for adult male and female offspring the expression of ACTH receptor was significantly (P=.02) increased.

Conclusion: These results indicate that MFR has a suppressive effect on steroidogenic enzymes of the newborn offspring regardless of gender. This may be an adaptive mechanism in the fetus/newborn to offset the high circulating maternal glucocorticoids in response to undernutrition. In adult male MFR offspring, increased HSD1 indicates an increase in GC synthesis whereas in the females there were no changes in GC synthesizing enzymes with a suppression of MC synthesizing pathway. The common finding of an increased ACTH receptor expression in both genders would suggest an increased sensitivity of adult MFR offspring adrenals to the effects of stress.

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Effect of Gender on Adrenal Growth and IGF Expression in the Fetal Sheep during Late Gestation. Christelle Ucinek,[1,2] Sheridan Gentili,[1] Severence M MacLaughlin,[1] I Caroline McMillen.[1] *[1]Sansom Institute, UniSA, Adelaide, South Australia, Australia; [2]Discipline of Physiology, Adelaide University, Adelaide, South Australia, Australia.*

Objective: During gestation the fetal adrenal undergoes phases of active growth (40-90d), quiescence (100-120d) followed by reactivation (>135d) before birth. Insulin like growth factors play an important role in stimulating adrenal growth throughout late gestation. Interestingly the prepartum activation of the adrenal is delayed in the female compared with the male fetus, but the mechanisms underlying this delay are unknown.

Hypothesis: We hypothesize that there are gender specific differences in the gestational profile of adrenal IGF2 mRNA expression between male and female fetuses.

Methods: A total of 37 twin fetuses were used in this study. Post mortem was performed at either 110-112d, 125-134d or 137-145d gestation. Adrenal mRNA expression of IGF1, IGF2, IGF1R, IGF2R and CYP17 was determined by qRT-PCR.

Results: Fetal weight was not different between males and females, and increased ($P<0.0001$) with increasing gestational age. The relative adrenal weight was lower however after 125d when compared to the earlier gestation age group ($P<0.0001$). Adrenal IGF1 mRNA expression was lower ($P<0.0001$) at 137-145d when compared to the earlier gestation age groups. Interestingly, IGF1R expression was highest at 125-134d ($P<0.0001$). There was an interaction between the effects of age and gender on adrenal IGF2 and IGF2R mRNA expression such that the expression was higher in males compared to females at 125-134d ($P<0.01$), but was not different to either the earlier or later gestational ages. There was no effect of either gender or gestational age on adrenal CYP17 mRNA expression.

Conclusions: It has been speculated that a delay in prepartum activation of the adrenal in female fetuses may be due to gender specific differences in the intra-adrenal bioavailability of IGF2. In this study we have demonstrated there is an increase in both IGF2 and IGF2R mRNA expression in males compared to female fetuses. This may be evidence that adrenal IGF2 expression plays a role in the earlier activation of the adrenal gland in male fetuses.

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Long Term Hypoxia Enhances ACTH-Induced Cortisol Secretion in the near Term Ovine Fetal Adrenal In Vitro. Vladimir E Vargas,[1] Tshepo M Monau,[1] Kanchan M Kaushal,[1] Dean A Myers,[2] Charles A Ducsay.[1] *[1]Center for Perinatal Biology, Loma Linda University, Loma Linda, CA; [2]Ob/Gyn, University of Oklahoma Health Sci. Ctr., Oklahoma City, OK.*

Background: We have previously shown that in response to a secondary stressor, *in vivo* cortisol secretion is elevated in long term hypoxic (LTH) ovine fetus despite lower ACTH receptor mRNA expression, and no differences in plasma adrenocorticotropic hormone (ACTH) levels when compared to normoxic controls. The present study was designed to determine the potential mechanism(s) of this enhanced cortisol secretion. Specifically we tested the hypothesis that post receptor signaling events including cAMP production and expression of steroidogenic acute regulatory protein (StAR) are enhanced following LTH.

Methods: For the LTH group, pregnant sheep were maintained at high altitude (3,820 m) from day 30 to near term. On days 138-141 (term = 146 days), fetal adrenal glands were collected from LTH (n=6) and age-matched, normoxic

control fetuses (n=6). Dispersed adrenal cortical cells (2.5 x10^5 cells/tube; in duplicate) were challenged with 10^{-8}M ACTH. Samples were collected at time 0 (baseline), 5, 15, 30, and 60 min after ACTH treatment, and tissue and media samples were frozen for determination of cortisol, cAMP, and StAR.
Results: Cortisol output (ng/2.5x10^5 cells) was higher in the LTH group compared to the control, ($p< 0.01$) at 15min (3.3 ± 0.5 vs. 1.0 ± 0.1), 30min (7.7 ± 0.6 vs. 1.9 ± 0.1), and 60min (10.9 ± 0.7 vs. 2.5 ± 0.1). Peak cAMP levels (fmol/2.5x10^5cells) were observed at 30 min but did not differ between control (81.8 ± 10.4) and LTH (77.9 ± 17.8) adrenal cells. Western analysis demonstrated that StAR protein expression was higher in the LTH adrenal cortex compared to control ($p<0.05$) at 0 min (1.02 ± 0.07 vs. 0.59 ± 0.05), and at 60 min (1.26 ± 0.04 vs. 0.65 ± 0.05), relative optical density units.
Conclusions: Results from the present study taken together with those of previous *in vivo* studies suggest that the enhanced cortisol output in LTH fetuses is the result of increased adrenal ACTH sensitivity. This enhanced sensitivity is not due to differences in cAMP generation. StAR, which is a key element involved in cholesterol transfer at the level of the mitochondrial membrane appears to play a key role in the enhanced cortisol response to ACTH in the LTH group. (NIH grants HD31226, HD33147 and LLU-NIH IMSD 2R 25GM060501-05).

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Maternal High Protein Died during Pregnancy Affects the Blood Pressure of Adult Offspring Via the Up-Regulation of Placental 11beta-HSD2 Gene Expression. Hiroaki Itoh,[1] Makoto Kawamura,[2] Shigeo Yura,[2] Haruta Mogami,[2] Tsuyoshhi Fujii,[2] Norimasa Sagawa.[3] *[1]Obstetrics and Gynecology, National Hospital Organization Osaka National Hospital, Osaka, Japan; [2]Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Kyoto, Japan; [3]Obstetrics and Gynecology, Mie University School of Medicine, Tsu, Japan.*
Objective: Epidemiological evidences suggested that undernutrition *in utero* develops risk factors for adult cardiovascular disorders, such as blood pressure increase. The present study was designed to prove the hypothesis that maternal iso-caloric high protein diet alleviates blood pressure increase in the adult offspring by affecting placental 11beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2) expression. **Methods:** The 30 % calorie restriction was applied to pregnant mice by using either standard protein diet (SPD; 20% casein protein; SPD-UN) or high protein diet (HPD;40% casein protein; SPD-UN). In some groups, these pregnant mice were sacrificed at 18.5 d.p.c.; then maternal and fetal plasma as well as placental tissues were corrected. While in other groups, systolic blood pressure was measured in the offspring at 16 wks. Fetal and maternal corticosterone levels were measured by ELISA. The placental 11beta-HSD2 gene expression was measured by quantitative TaqMan PCR.
Results: Systolic blood pressure in the SPD-UN offspring at 16 weeks (106 mmHg) was higher than that in SPD-NN offspring (99.3 mmHg). By contrast, systolic blood pressure in the HPD-UN offspring (97.5 mmHg) was similar to that in SPD-NN offspring. Maternal calorie restriction with SPD and HPD caused similar elevation of maternal plasma corticosterone concentrations. By contrast, fetal plasma corticosterone levels in HPD-UN offspring (151 ng/ml) was lower than those in SPD-UN offspring (208 ng/ml), indicating the amelioration of fetal exposure to high corticosterone by maternal HPD. The placental 11beta-HSD2 gene expression in the HPD-UN was 72% higher than that in SPD-UN, suggesting that maternal HPD augmented placental 11beta-HSD2 gene expression in the placenta and probably facilitated the inactivation of maternal cortocsterone in the process of placental transfer to fetal circulation.
Conclusion: The preset study suggests that maternal high protein ingestion may elevate placental 11beta-HSD2 gene expression and ameliorate blood pressure increase in the adult offspring via modification of fetal exposure to maternal corticosterone.

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Role of Mineralocorticoid Receptors in Control of ACTH and Lung Liquid Composition in the Preterm Fetal Sheep. Maureen Keller-Wood,[1] Jarret McCartney,[1] Nathan Jesse,[2] Charles Wood.[3] *[1]Pharmacodynamics, University of Florida, Gainesville, FL, USA; [2]Pediatrics, University of Florida, Gainesville, FL, USA; [3]Physiology and Functional Genomics, University of Florida, Gainesville, FL, USA.*
Elevated cortisol levels at birth exert critical maturational effects through action at glucocorticoid receptors, GR. In contrast, the low cortisol concentrations in the preterm fetus, before the time of increased fetal cortisol secretion, are near to the Kd for cortisol at the higher affinity mineralocorticoid receptor, MR. We hypothesized that endogenous cortisol in the fetus exerts physiologic actions at MR in tissues without appreciable cortisol-inactivating enzyme, 11βHSD2, including the fetal lung and brain. We therefore tested the effect of infusion of a MR-antagonist, RU26752 (MRa, 1.68 mg/h over 12h) into 120-130 day fetal sheep. We tested for effects on lung liquid production rate, lung liquid and plasma electrolytes, plasma ACTH and cortisol, and hematocrit at 10-12h after start of the infusion. Although there was no significant difference in the liquid production rate in fetuses infused with RU26752, the ratio of Na^+ to K^+ in lung liquid was significantly greater in MRa-treated fetuses than in the control fetuses (34.7±1.6 vs 30.2±1.7). Plasma ACTH was significantly greater in the MRa-treated fetuses than in control fetuses at 12h (657±262 vs 171±20 pg/ml); in the MRa-treated fetuses, ACTH concentrations at 12h were greater than in the same fetuses at 0h (124±16 pg/ml), wheras there was no increase in plasma ACTH in the control fetuses (0h: 100±19 pg/ml). Similarly, plasma cortisol concentrations at 11 and 12h were greater in the MRa infused fetuses than in control fetuses (12h: 18.2±5.5 vs 7.0±1.4 ng/ml), and cortisol increased significantly over time in the fetuses infused with MRa, but not in control fetuses (0h, MRa: 4.7±1.5, control: 2.9±0.5 ng/ml). Infusion of MRa did not alter fetal plasma electrolyte, fetal blood pressure or fetal heart rate. However, fetal packed cell volume was significantly increased at 9-12h of infusion of MRa (12h: 37±1 vs 32±1 %) and fetal PCO2 was significantly greater at 12h of infusion of MRa as compared to control fetuses (61.0±2.0 vs 53.5±0.8). These results suggest that endogenous cortisol concentrations in the preterm fetus exert negative feedback control of ACTH via action at MR in the fetal brain, and play a role in regulation of fetal lung liquid composition and in fetal fluid balance.

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Preparturient Increases in Fetal ACTH Secretion Are Cyclooxygenase-2 (COX-2) Dependent. Charles E Wood. *Dept. Physiology and Functional Genomics, University of Florida College of Medicine, Gainesville, FL, USA.*
Maturation of the fetal hypothalamus-pituitary-adrenal axis is critical for the timely somatic development of the fetus and readiness for birth. In sheep, increased preparturient activity of the fetal HPA axis appears to be responsible for triggering parturition itself. This study was designed to test the hypothesis that prostaglandin generation, mediated by COX-2 in the fetal brain, is critically important for stimulation of the preparturient increase in fetal ACTH secretion. Singleton fetal sheep were chronically catheterized with vascular, amniotic, and lateral cerebral ventricular catheters. Nimesulide, a selective COX-2 inhibitor, was infused (1 mg/day, n=9), and vehicle (50% dimethylsulfoxide, n=6) was infused. Arterial blood samples were drawn from fetuses at 48 hour intervals for measurement of plasma hormone concentrations and arterial blood gases. In the vehicle-treated fetuses, fetal plasma ACTH, POMC, and cortisol concentrations increased exponentially ($p<0.001$ by ANOVA) before spontaneous parturition at 146±0.3 days. In the nimesulide-treated fetuses, fetal plasma ACTH and POMC were constant and did not increase prior to parturition (p=NS by ANOVA). In contrast, fetal plasma cortisol increased independent of ACTH ($p<0.001$ by ANOVA) and the fetuses were born spontaneously on 148±0.3 days gestation. The slight delay in spontaneous parturition in the nimesulide-treated fetuses was statistically significant ($p<0.01$ by Mantel-Cox test). Intracerebroventricular nimesulide infusion did not decrease fetal plasma concentrations of Prostaglandin E2, demonstrating that the action of the nimesulide was restricted to the fetal brain. Fetal blood gases were normal in all of the fetuses, and there were no differences in blood gases between groups. We conclude that the spontaneous increase in fetal ACTH and POMC prior to parturition is COX-2 dependent. However, we also conclude that the increase in fetal plasma cortisol concentration can occur independent of increases in fetal ACTH, suggesting that the increase in cortisol can result from increases in adrenal sensitivity to ACTH. The results of this study demonstrate that the timing of parturition in the sheep is not dependent upon increased ACTH secretion, and the results suggest that parturition is regulated primarily by changes in adrenal sensitivity.

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Expression of Extracellular Signal-Regulated Kinase1/2 and p38 Mitogen-Activated Protein Kinase in the Invasive Trophoblasts at the Human Placental Bed. Kyung Chul Moon,[1] Joong Shin Park,[2] Errol R Norwitz,[3] Dong-Il Kim,[1] Kyung Jun Oh,[2] Chan Wook Park,[2] Jong Kwan Jun,[2] Hee Chul Syn.[2] *[1]Pathology, Seoul National University College of Medicine, Seoul, Korea; [2]Obstetrics & Gynecology, Seoul National University College of Medicine, Seoul, Korea; [3]Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Background: Mitogen-activated protein kinases (MAP kinases) participate in

signal transduction pathways that control embryogenesis, cell differentiation, cell proliferation and cell death. The roles of extracellular signal-regulated kinase1/2 (ERK1/2) and p38 MAP kinase in the differentiation and invasion of human trophoblasts have been studied. However, the in vivo expression and activation of ERK1/2 and p38 at the placental bed has not been elucidated. ***Methods:*** The study group consisted of placental bed biopsy tissues obtained from the pregnancies without preeclampsia (n=24) and with preeclampsia (n=8) between 31 and 40 weeks of gestation. We evaluated the expressions and phosphorylations of ERK1/2 and p38 MAP kinase in the invasive trophoblasts in the placental bed tissues using immunohistochemistry. ***Results:*** p38 and phospho-p38 MAP kinase were not detected in invasive trophoblasts in cases or controls. ERK1/2 and phospho-ERK1/2 were positive in invasive trophoblasts albeit with variable staining. Phosphorylation of ERK1/2 was significantly less frequent in invasive trophoblasts in placental bed biopsies from women with preeclampsia compared with normotensive controls. ***Conclusion:*** These findings suggest that preeclampsia is associated with decreased activation of ERK1/2 in invasive trophoblasts in vivo.

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G1 Phase Cell Cycle Regulators Are Altered in Placentae from Preeclamptic Pregnancy. Jocelyn Ray,[1,2] Livia Deda,[1,2] Andrea Jurisicova,[1,2] Isabella Caniggia.[1,2] *[1]Ob/Gyn, Mount Sinai Hospital, Toronto, ON, Canada; [2]Physiology, University of Toronto, Toronto, ON, Canada.*

Objective: Placentae from preeclamptic pregnancies are characterized by an excess of immature hyperproliferative trophoblast cells, however the molecular mechanisms regulating cell cycle progression in this pathology are unclear. Our aim was to examine the expression of G1 phase cell cycle regulators in normal and preeclamptic placentae and to establish whether the hyperproliferative state of trophoblast cells in preeclampsia may result from a developmental delay.

Methods: Human placental samples were collected from normal pregnancies throughout gestation (n=60) and from severe early onset preeclamptic (n=20), and age-matched control placentae (n=15). Protein expression of cyclin E1, cyclin D1, cyclin D3 and cell cycle inhibitors, p15, p16, p21, and p27 was assessed by Western Blot analysis. Spatial and temporal localization of cyclins E1, D1, D3, p21 and p27 was determined by fluorescence immunohistochemistry. Expression of cyclin E1 and cyclin D1 mRNA was evaluated by qPCR analysis.

Results: Immunohistochemical analysis showed cyclin E1 and cyclin D3 to be localized to cytotrophoblast (CT) cells of the chorionic villi; additionally cyclin E1 was also expressed in the extravillous trophoblast cells of the anchoring columns. In contrast, cyclin D1 expression was predominantly restricted to the villous stroma. Cell cycle inhibitor p27 was expressed in both CT and syncytiotrophoblast (ST) cells whereas p21 was restricted to the ST. During normal placentation, levels of both cyclin E1 and cyclin D3 were high in the first trimester and decreased with advancing gestation. The expression of cyclin D1, p27 and p16 showed an inverse correlation to cyclins E1 and D3 whereby their expression increased towards term. Levels of p21 and p15 remained constant throughout pregnancy. Preeclamptic placentae showed a significant increase in both cyclin E1 and p27 and a decrease cyclin D1 and p15 expression, as compared to age-matched control tissues. Interestingly, preeclampsia was associated with an increased number of cyclin E1 positive progenitor CT cells in the floating villi and an increased expression of p27 in the endothelial cells lining the villous vessels.

Conclusion: Preeclampsia displays an altered expression of G1 phase cell cycle regulatory molecules portraying an expression profile that closely resembles that of first trimester placentae. (Supported by CIHR, OWH/IGH).

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Angiogenic Growth Factors and Pregnancy Outcome: A Cautionary Tale from High Altitude. Olga Kovalenko,[1] Nicholas P Illsley,[1] Tatiana Torricos,[2] Shauna Williams,[1] Marcus Borges,[1] Stacy Zamudio.[1] *[1]Obstetrics, Gynecology & Women's Health, UMD-New Jersey Medical School, Newark, NJ, USA; [2]Instituto Boliviano de Biología de Altura, Universidad de San Andreas Mayor, La Paz, Bolivia.*

Introduction: High altitude residence is associated with an increased incidence of preeclampsia. We have shown a number of physiological and biochemical parameters are intermediate between high-altitude and preeclamptic pregnancies. We have also demonstrated elevated, HIF-1-mediated placental and circulating sFlt-1 at high altitude. We sought to correlate circulating levels of PlGF, free VEGF and sFlt-1 with maternal and fetal oxygen tensions. We hypothesized that circulating sFlt-1 and free VEGF would be increased at 3600 m, and that PlGF, known to be up-regulated by higher oxygen tension, would be decreased.

Methods: We collected both serum and plasma samples, the latter treated with inhibitors of platelet activation. Maternal and umbilical samples were obtained from 77 and 65 healthy mother-infant pairs living at 400 m and 3600 m respectively. PlGF, free VEGF and sFlt-1 were measured in both serum and plasma using commercially available ELISA kits (R&D). Data were log-transformed and analyzed by unpaired student's t test or ANOVA as appropriate.

Results: PlGF did not differ between altitudes. Free VEGF (7±2 pg/ml vs. 12±2 pg/ml, $p<.05$) and sFlt-1 (25.7±2.4 vs. 31.4 ±2.2 ng/ml) were increased at 3600 m. However concentrations of all the angiogenic growth factors were reduced when platelet activation was prevented (-35±3% PlGF; -85±5% free VEGF; -24±2% sFLT-1, $p<.001$). Cord blood free VEGF was 100-fold greater than in the mothers, but inhibition of peripheral cell activation abolished detectable levels in 95% of the babies. Similar results were obtained for sFlt-1. Thus, while free and total maternal circulating VEGF and sFlt-1 are elevated at high altitude, these increases are due to activation of peripheral blood cells. Moreover, free VEGF does not exist in detectable amounts in human pregnancy. There was no relationship between variation in the angiogenic growth factors and maternal or fetal oxygen tensions.

Conclusion: A significant proportion of circulating angiogenic growth factors measured in the maternal and fetal circulations are artifacts of the techniques used for blood collection.

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Differential Gene Expression in Severe Preeclamptic Versus HELLP Syndrome Placentas. Maureen Lee,[1] Paul Norton,[2] Curtis Cook,[4] Richard Leach,[3] James Resau.[2] *[1]Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL, USA; [2]Van Andel Institute, Grand Rapids, MI, USA; [3]Department of Obstetrics, Gynecology and Reproductive Biology, Michigan State University, Lansing, MI, USA; [4]Maternal-Fetal Medicine, Spectrum Health Hospital, Grand Rapids, MI, USA.*

Objective:

The objective of this study is to identify candidate genes responsible for the variance between severe preeclampsia (SPE) and HELLP syndrome (HS).

Study Design:

Placental biopsies from SPE (n=6) and HS (n=2) were collected. Diagnosis of SPE was confirmed by blood pressure and protein criteria. HS was diagnosed in preeclamptic patients who developed characteristic laboratory abnormalities. Placental tissues were embedded in OCT for sectioning, H&E staining and RNA isolation (Invitrogen, Carlsbad). Gene expression data was obtained by hybridizing fluorescently labeled reverse transcription products to spotted cDNA microarrays. The microarrays were produced by the Laboratory of Microarray Technology at the Van Andel Institute (Grand Rapids, MI) using a custom microarrayer.

Microarrays were scanned using an Agilent G2505B scanner. Images were analyzed using GenePix 5.0 (Axon). LimmaGUI was used to generate lists of discriminating genes for these data, while DAVID provided functional annotations.

Results:

There were 310 differentially expressed genes between SPE and HS ($p<.05$). Among these candidate genes, 233 were up-regulated and 77 were down-regulated. The most up-regulated genes are 5(3)-deoxyribonucleotidase (dNT-2), superoxide dismutase 3, hydroxy-delta-5-steroid dehydrogenase, caspase 9, and general transcription factor IIH. Further analysis of functional groups revealed the most enriched gene categories are related to cellular energy (mitochondria), cell cycle regulation, and protein metabolism.

Conclusion:

The underlying role of the placenta in the development of HS is currently unknown. This study shows that there is a relatively mdest number of genes that are differentially expressed between HS versus SPE placentals. Thes data provide the molecular context for placental changes seen in this variant of preeclampsia and provides an opportunity to study the role of the effected genes in disease variance.

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Pre Exposure to Mitogenic Doses of Growth Factors Associated with IUGR and Preeclampsia Impair Subsequent Uterine Artery Endothelial Cell eNOS Activation Responses to ATP. Shannon M Gifford, FuXian Yi, Mary A Grummer, Ian M Bird. *ObGyn, University Wisconsin, Madison, Wi, USA.*

Growth factors play an important role in normal placentation and promote uterine angiogenesis during pregnancy. Nonetheless, IUGR and preeclampsia are associated with an elevation of placental bFGF, EGF or VEGF. Increased uterine blood flow not only relies on increased angiogenesis but also enhanced vasodilation. In the wound healing literature it is clear that at the same time growth factors increase angiogenesis at the wound site, they also impair cell-cell communication by closure of CX43 gap junctions via ERK-1/2, and thereby reduce vasodilator production. In this study we considered if high levels of growth factors, each of which can activate ERK-1/2 in uterine artery endothelial cells (UAEC), are capable of blocking UAEC eNOS activation. We have found ATP (100uM) acts via P2Y2 heptahelical receptor coupled to PLC-Beta3 to activate eNOS in UAEC. We have also shown that UAEC from pregnant ewes communicate via CX43 gap junctions and that increasing cell density from 20% to 100% confluence is associated with an increasing number of cells showing detectable Ca2+ responses in response to Ca2+ mobilizing agonists such as ATP. Uncoupling of these gap junctions by Gap27 results in a reduction of % responding cells and a corresponding 50% loss in overall ATP-stimulated eNOS activation. We herein report for the first time that prolonged recording (30 min) of the effects of EGF, bFGF and VEGF in Fura-2 loaded cells reveals a smaller and delayed (5-10 min) Ca2+ elevation in NP-UAEC and P-UAEC. Of note, the maximum % cells responding to ATP is as much as 91% in P-UAEC, yet the maximum observed for these other agonists is between 4.4 and 19.1% at confluence. Nonetheless, if P-UAEC are subsequently challenged with ATP, the number of cells responding to ATP is halved at high cell density. Thus prior exposure of P-UAEC to mitogenic doses of growth factors strongly impairs subsequent responses of all UAEC to agonists such as ATP. As such it would appear that maximally mitogenic doses of growth factors are indeed capable of negatively affecting uterine artery endothelial NO production by altering the vasodilatory response to those agonists working though heptahelical receptors coupled to PLC-B3 in UAEC. Supported by NIH Grants HL079020 HD 38843.

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Localization and Expression of Human Telomerase Reverse Transcriptase (hTERT) in Placentas from Patients with Progressive Manifestations of Preeclampsia. Yali Xiong,[1] Dan Lieberman,[2] Eliezer J Holtzman,[3] Barbara Hoffman,[2] Enrique Hernandez,[1] Ossie Geifman-Holtzman.[1] *[1]Obstetrics & Gynecology, Temple University School of Medicine, Philadelphia, PA, USA; [2]Fels Institute, Temple University School of Medicine, Philadelphia, PA, USA; [3]Nephrology and Hypertension Institute, Tel-Aviv University, Sheba Medical Center, Ramat-Gan, Israel.*

Objective: To correlate the localization and expression of placental Human Telomerase Reverse Transcriptase (hTERT) with progressive clinical manifestations of preeclampsia.

Study Design: 61 placental biopsies were collected freshly. The study group (32 patients): preeclampsia (14), severe preeclampsia (10), HELLP syndrome (4) and eclampsia (4) and control group (29 patients) uncomplicated pregnancies. Total RNA was isolated and reversely transcribed to c-DNA. The mRNA level of TERT was detected with a probe-specific real-time quantitative PCR assay using ß-actin as the reference gene. Crossing point (Cp) values were obtained during the PCR amplification and the relative expression level of hTERT equals to $2^{(Cp\ hTERT\ -Cp\ ß\text{-}actin)}$. Statistical analysis was performed using the student's t test. Immunohistochemistry (IHC) staining was employed to localize hTERT protein on placenta tissue sections using ABC method, incubation with rabbit anti-hTERT antibody followed by application of a goat anti-rabbit antibody with results evaluation using microscope.

Results: The ratio of average hTERT mRNA levels were higher in the study group versus control both in preterm (1.71 versus 0.88, $p<0.05$) and term deliveries (1.66 versus 1.15, $p<0.05$). The hTERT mRNA ratio in sub-groups of preeclampsia were 1.81(HELLP Syndrome), 1.80(severe preeclampsia), 1.76(eclampsia), and 1.54(preeclampsia). In both the preeclamptic (ratio: 1.78 versus 1.54, $p<0.05$) and control groups (ratio: 1.13 versus 0.93, $p<0.05$). The IHC staining showed that the hTERT protein was expressed in trophoblast cells and endothelial cells in placentas. The overall expression of hTERT in preeclamptic group was elevated compared to control. Infarction necrosis was present in some of the preeclamptic sections noted to be with lower hTERT expression.

Conclusions: This work demonstrated increased hTERT expression and localization in placentas of patients with hypertensive disease. This provides novel data on the possible role of telomerase in placentas from pregnancies associated with progressive clinical manifestations of preeclampsia.

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Direct Imaging of Intact Vessels Shows that Reduced Ca2+ Responses in Human Umbilical Vein Endothelium of Preeclamptic Pregnancy Are Temporally Associated with a Blunted Nitric Oxide (NO) Production. FuXian Yi, Jennifer Krupp, Dinesh Shah, Ian M Bird. *OB/Gyn, University Wisconsin, Madison, WI, USA.*

The umbilical vein is a critical conduit connecting the fetus to the placenta. Clearly both appropriate vessel formation and sustained vasodilatory function is necessary for normal development. Normal Human Umbilical Vein Endothelial (HUVE) vasodilation in response to a number of agonists is associated with prolonged agonists stimulated changes in Ca2+ signaling and indirect evidence suggests an associated nitric oxide production. Previous studies have shown the Ca2+ response of HUVE is in fact impaired in preeclamptic pregnancy but no direct measure of NO was made nor the temporal relationship between Ca2+ and NO formation clearly established. More direct and simultaneous imaging of real time changes in the levels of Ca2+ and NO in individual umbilical vein endothelial cells on the surface endothelium of intact vessels is now possible. The purpose of this study is thus to directly demonstrate in preeclampsia, the previously reported decreased ability of the endothelial cell to signal Ca2+ responses is indeed temporally associated with a corresponding loss of associated nitric oxide production, thereby diminishing normal vasodilation. STUDY DESIGN: Umbilical veins from the cords of normal vs preeclamptic subjects (obtained with authorized consent) were imaged using Fura2 and Daf-2 dyes to detect Ca2+ and NO respectively in real time in response to stimulation with ATP (100uM). RESULTS: These representative graphs depict the changes in Ca2+ and NO in the endothelium of the human cord umbilical vein. Recordings show that while normal and preeclamptic (PE) subjects both respond to ATP with an acute Ca2+ response, the normal cord includes a sustained secondary phase which is indeed associated with sustained NO production. In contrast, the preeclamptic cord shows a poor Ca2+ secondary phase and this is associated with a blunted NO response. CONCLUSION: This preliminary data supports the proposal that normal umbilical vein endothelial cell function allows sustained Ca2+ response and associated NO production, while in preeclampsia, endothelial dysfunction leads to shorter duration Ca2+ responses and associated NO production. Supported by NIH Grants HL079020 HD 38843 and R&D funding by Dept OBGyn.

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Differential Expression of TGF-β Receptors ALK1 and ALK5 in Placentae from Preeclamptic and IUGR Pregnancies. Yoav Yinon,[1] Jing Xu,[1] Tullia Todros,[2] John Kingdom,[1] Martin Post,[3] Isabella Caniggia.[1] *[1]Depart. of Ob/Gyn, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; [2]Depart. of Ob/Gyn, University of Turin; [3]Depart. of Pediatrics, Hospital for Sick Children, Toronto.*

Objective

TGFβs are involved in the regulation of trophoblast differentiation and invasion, and we have previously reported that TGF-β3 is over expressed in pre-eclamptic placentae. TGFβs signal via a receptor complex composed of type II (TβR-II) and type I (TβR-I) receptor. To date, seven type I receptors, designated as activin receptor-like kinase (ALK1-7) have been identified. Our aim was to investigate the expression pattern of ALK1 and ALK5 receptors, known to exert TGFβ signaling, in preeclamptic and IUGR placentae.

Methods

Human placental tissue throughout gestation was used in order to determine the development profile of the receptors. In addition, placental tissue from preeclamptic and IUGR pregnancies and from age matched controls was collected. All IUGR pregnancies were characterized by absence of end diastolic velocity in the umbilical artery and had no evidence of preeclampsia.

Expression of ALK1 and ALK5 mRNA was measured by real-time PCR analysis, and protein by Western Blot analysis using ALK1 and ALK5 antibodies.

Results

Immunoblot analysis demonstrated a unique developmental profile whereby ALK1 expression increased with advancing gestation. In preeclamptic placentae ALK5 expression was significantly increased compared to preterm and term controls, whereas ALK1 expression was significantly decreased. Preeclamptic placentae also exhibited decreased phosphorylation of Smad1, a TGFβ signaling molecule, which is activated by ALK1 and increased phosphorylation of

Smad2, which is triggered by ALK5. The expression of ALK1 and ALK5 in IUGR placentae differed from that of preeclampsia as both ALK1 and ALK5 mRNA levels were significantly increased in IUGR compared to preterm and term controls. However, only ALK5 expression was significantly increased in IUGR placentae at the protein level, while no differences in ALK1 protein levels were noted between IUGR and controls.

Conclusions

Imbalance between ALK1 and ALK5 signaling pathways might play a role in the pathogenesis of preeclampsia and IUGR. As TGFβ signaling via either ALK1 or ALK5 has been found to differentially regulate vasculogenesis, changes in these signaling pathways may contribute to the altered vasculogenesis found in these pregnancy-related disorders. (Supported by CIHR and OWH/IGH).

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Stress Inducible Gene Gadd45a Regulates sFlt-1 Secretion Via p38 Pathway Activation in Preeclampsia. Yali Xiong,[1] Jennifer S Tront,[2] Dan Lieberman,[2] Barabra Hoffman,[2] Eliezer J Holtzman,[3] Yajue Huang,[4] Enrique Hernandez,[1] Ossie Geifman-Holtzman.[1] *[1]Obstetrics & Gynecology, Temple University School of Medicine, Philadelphia, PA, USA; [2]Fels Institute, Temple University School of Medicine, Philadelphia, PA, USA; [3]Nephrology and Hypertension Institute, Tel-Aviv University, Sheba Medical Center, Ramat-Gan, Israel; [4]Pathology, Temple University School of Medicine, Philadelphia, PA, USA.*

Objective: To reveal the stress inducible protein Gadd45a and its downstream signal pathway activation in placentas with preeclampsia and their regulation in the secretion sFlt-1 in HUVEC cells.

Study Design: 61 placental biopsies were collected freshly from 32 patients, the study group: preeclampsia (14), severe preeclampsia (10), HELLP syndrome (4) and eclampsia (4) and 29 patients, the control group who had uncomplicated pregnancies. Total protein was isolated from placental tissue. Gadd45a and its downstream signal proteins--phospo-p38, phospho-Mkk3/Mkk6 were assessed by western blot. Immunohistochemistry (IHC) staining was employed to localize the expression of Gadd45a and sFlt-1 proteins in placenta tissue sections using ABC method. HUVEC cells were cultured to 80-90% confluence and were divided into 3 groups: control, stress induction (sorbitol, 0.3M, 4h), p38 inhibition (SB-203580, 1ug/ml, 5ug/ml and 10ug/ml, 1 hour) + sorbitol (0.3M, 4h). Total protein was isolated from cells and the supernatant of HUVEC was collected. Western blot was processed to detect the induction of Gadd45a and phospho-p38. Supernatant sFlt-1 was measured with an EIA kit and the results were read at 450nm wavelength.

Results: Gadd45a protein was elevated in the preeclamptic placentas with its downstream proteins (MKK3 and p38) activation compared with control. Over-expression of Gadd45a and sFlt-1 in preeclamptic placentas was observed with IHC staining. In HUVEC cells, Gadd45a was induced by sorbitol, triggering the activation of the downstream p-38 pathway and the accumulation of sFlt-1 in the supernatant. The up-regulation of sFlt-1 secretion by inducing Gadd45a was depleted when treated with p-38 inhibitor.

Conclusions: Our study reveals that Gadd45a and its down stream p-38 pathway were activated in preeclamptic placentas and this stress inducible signal pathway regulates the secretion of sFlt-1, which is a key player in preeclampsia. It provides novel evidence that links placental stress to sFLT-1 secretion via the Gadd4.

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Trophoblast Adipose Triglyceride Lipase (ATGL) Expression Is Upregulated in Preeclampsia. Beth A Plunkett,[1] Jennifer A Doll,[2] Emily J Su,[1] Serdar E Bulun,[1] Mona Cornwell,[2] Susan E Crawford.[2] *[1]Obstetrics and Gynecology, Northwestern University, Chicago, IL, USA; [2]Pathology, Northwestern University, Chicago, IL, USA.*

Objective: Preeclampsia is characterized by placental endothelial cell dysfunction and elevated maternal triglyceridemia (TG). TG traverse the placenta in a process of uptake followed by lipolysis with subsequent release of fatty acids to the fetus. Although three placental lipases (hormone sensitive lipase, endothelial lipase and lipoprotein lipase) have been identified, they do not account for all lipolytic activity. Here, we introduce a new lipase, adipose triglyceride lipase (ATGL), which is responsible for the hydrolysis of triglycerides to diglycerides in adipocytes and was recently identified as a receptor for endothelial cell modulator pigment epithelium-derived factor. The purpose of this study is to determine if expression of ATGL, a potential modulator of both lipid metabolism and vasculature, is upregulated in preeclampsia.

Methods: Immunohistochemical studies were performed on placental tissues from normal pregnancies (n=5) and those complicated by severe preeclampsia (n=5) with anti-ATGL antibodies. The degree of positivity in the trophoblasts and endothelial cell was scored (1=none, 2=spotty, light, 3=consistent, dark). To determine if ATGL is a product of placental endothelial cells (PLEC), microvascular cells were isolated from normal placental tissue. Purity of the sample was confirmed using flowcytometry (>2/3 positivity for Factor 8 antigen). ATGL was detected immunohistochemically and via Western blot using anti-ATGL antibodies.

Results: Mean ATGL expression in preeclamptic trophoblast was significantly higher than normal placentas (3.0 ± 0 standard deviation versus $2.3 \pm .39$, $P = 0.018$). Endothelial expression was not significantly different in preeclamptic ($2.6 \pm .41$) versus normal placentas ($2.3 \pm .39$, $P>0.05$). ATGL stained intensely and demonstrated a beaded pattern in the endothelial cells, suggestive of a lipid droplet pattern. Immunohistochemistry of PLEC and Western blot analysis of cell lysates revealed strong immunopositivity for ATGL, although at a smaller size than anticipated.

Conclusion: These findings demonstrate that a novel lipase, ATGL, is produced by trophoblasts and is upregulated in severe preeclampsia. PLEC express high levels of ATGL, suggesting that ATGL could prove to be important in the vascular dysfunction and lipid abnormalities characteristic of preeclampsia.

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Increased Endothelial Chymotrypsin-Like Protease (Chymase) Expression Is Responsible for Endothelial Activation in Preeclampsia. Yuping Wang, Yang Gu, Yanping Zhang, David F Lewis. *Obstetrics and Gynecology, LSUHSC-Shreveport, Shreveport, LA, USA.*

Objective: Endothelial (EC) activation is an important component of inflammatory phenotypic changes in preeclampsia (PE). Our previous study showed enhanced chymotrypsin-like protease (CLP)/chymase expression in the maternal vessel endothelium in women with PE. In this study, specific effect of placental-derived CLP on EC activation was examined.

Methods: Human uterine microvascular endothelial cells (UtMVECs) were used. Placental conditioned medium (CM) was prepared by culturing villous explants from normal and PE placentas. Confluent UtMVECs were treated with placental CM with or without depletion of chymotrypsin. EC adhesion molecule expressions for ICAM, VCAM, P-selectin and E-selectin were determined by a colorimetric assay at OD 450nm. Depletion of chymotrypsin from CM was performed by immunoprecipitation. To further determine if activation of endogenous CLP/chymase in ECs is responsible for up-regulation of P-selectin and E-selectin expression, chymase siRNA was applied to EC culture before the cells were treated with normal or PE CM and then EC adhesion molecule expressions were examined. Data was expressed as mean ± SE and analyzed by ANOVA. A p level < 0.05 was set for statistically different.

Results: 1) Expressions of VCAM, P-selectin and E-selectin, but not ICAM, were significantly increased in PE-CM treated UtMVECs compared to those of normal-CM treated cells and untreated controls, $p<0.01$; 2) There was no difference for adhesion molecule expression in UtMVECs between normal-CM treated with untreated controls; 3) UtMVECs transfected with chymase siRNA significantly reduced P-selectin and E-selectin expressions when exposed to PE-CM, $p<0.05$.

Conclusion: Placental-derived CLP/chymase is responsible for activating ECs and inducing EC adhesion molecule expression. Activation of EC chymase may be directly related to the inflammatory phenotypic changes that occur in ECs in PE. (Supported NIH grants HD36822 and HL65997).

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Corin Expression and Regulation in the Myometrium of Women with Preeclampsia. C Mason,[1] IA Buhimschi,[2] C Buhimschi,[2] P Swaan,[1] CP Weiner.[3] *[1]Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD, USA; [2]Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT, USA; [3]Obstetrics and Gynecology, University of Kansas School of Medicine, Kansas City, KS, USA.*

OBJECTIVE: Corin is a transmembrane serine protease that is important in processing natriuretic peptides (NPs) and maintaining normal blood pressure. Genetically modified mice without corin function develop a syndrome during pregnancy similar to preeclampsia. Corin is present in the pregnant uterus and a deficiency in the enzyme may lead to hypertension and preeclampsia. We tested the hypothesis that corin expression was increased in human myometrium from women with preeclampsia.

METHODS: Myometrium was obtained from 4 groups of women at the time of primary cesarean section: (i) preterm no labor with preeclampsia (n=3, PTsPE, 28.9 weeks); (ii) preterm labor (n=3, PTL, 30.9 weeks); (iv) term no labor (n=3, TNL, 39.6 weeks); (v) term labor (n=3, TL, 39.7 weeks). Microarray

gene profiling was performed using Affymetrix Human Genome U133 Plus 2.0 Arrays. Women who were not in active labor at the time of delivery (TNL) served as the control group. Conventional and real time PCR was performed to verify corin expression in the arrays was directionally accurate. Corin protein expression was examined by western blot.

RESULTS: Compared to TNL control, corin levels decreased nearly 2-fold in myometrium from women in term labor (TL). There was a small increase in corin gene expression of preeclamptic women (PTsPE) and little-to-no change in myometrium from women in preterm labor (PTL). These results were confirmed by PCR analysis.

CONCLUSION: Blood volume surges during pregnancy, increasing the potential for hypertension and preeclampsia in the mother. The corin-NP control system could contribute to this condition. However, there are no reports on corin message or protein levels in women with preeclampsia. Our preliminary results suggest that preeclampsia is not a consequence of a corin deficiency (decreased corin ≠ preeclampsia).

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The Expression of gp130 at Implantation Site: Implication for the Pathogenesis of Preeclampsia. Chih-Feng Yen,[1,2,3] Murat Basar,[1] Lynn Buchwalder,[1] William Murk,[1] Umit A Kayisli,[1] Joseph Huang,[1] Frederick Schatz,[1] Charles J Lockwood.[1] *[1]Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA; [2]Obstetrics and Gynecology, Chang Gung Memorial Hospital and University, Tao-Yuan, Taiwan; [3]Graduate Institute of Clinical Medical Sciences, Chang Gung University, Tao-Yuan, Taiwan.*

Objective: gp130 is a common shard signal transducing subunit of the IL-6 cytokine family which is critical for implantation, and viewed as marker of endometrial blastocyst receptivity. Preeclampsia (PE) is highly related to the restricted trophoblast invasion, which leads to impaired spiral artery remodeling. Our hypothesis was that the poor placentation of PE is associated with the altered expression of gp130 at the implantation site.

Methods: Human decidua from patients with PE and uncomplicated term deliveries (n = 9, respectively) were immunostained for gp130. The intensity and distribution of immunostaining on decidual cells and extravillous traphoblasts were evaluated with HSCORE. Statistical analysis of the data was performed using Student's t-test and Kruskal-Wallis one way ANOVA on ranks followed by post hoc test.

Results: Immunostaining of gp130was significantly higher in decidual cells of patients with PE compared with normal specimens, with HSCORE (Median, [interquartile range]) value 180 [142.5-185.0] and 120 [20-135], respectively ($P<0.05$). In PE specimen, the HSCORE of decidual cells was also significantly higher than that of trophoblast (40 [30-50]; $P<0.05$). There were no difference in the comparison of HSCORE of trophoblasts between the PE and normal specimens, and in normal specimens between decidal cells and trophoblasts.

Conclusions: The increase of gp130 expression in the decidual cells of preeclamptic placenta may be implied to the pathogenesis of poor placentation in preeclampsia.

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Effect of Hypoxia on Renin Secretion by Human Decidual Cells *In Vitro*. Xiu-Yu Yi, Ziming Yu, Dinesh M Shah. *Department of Obstetrics and Gynecology, University of Wisconsin, Madison, WI, USA.*

Background and Objective: The uteroplacental renin-angiotensin system (RAS) plays an important role in regulating the uteroplacental blood flow and pressure during human gestation. And aberrant regulation of this local RAS has been suggested to be associated with the pathogenesis of preeclampsia where placental hypoxia may have a causative role in the clinical syndrome. We have previously demonstrated that decidual cells are the major source of renin at the human uteroplacental interface, but little is known regarding the human decidual renin expression in hypoxic conditions. Therefore, the present study was undertaken to determine the effect of hypoxia on renin secretion by human decidual cells *in vitro*.

Methods: Full-term normal human placentas were obtained within one hour of vaginal deliveries or Cesarean sections. Decidual cells were isolated from the decidua parietalis. After an initial culture for 2~3 days in a serum-containing medium, the decidual cells were exposed to normoxia or hypoxia (10% or 3% oxygen) in a serum-free medium for 24 hours. The culture supernatants were then harvested and subject to Western blot analyses of renin protein contents.

Results: A dominant band of renin at approximately 47 kD was detected in all samples. When compared with the cells cultured in the normoxic condition, the cells cultured in both hypoxic conditions (i.e. 10% and 3% oxygen) had significantly lower renin protein contents in their culture supernatants.

Conclusion: Our data for the first time show that hypoxia down-regulates renin secretion in human decidua, suggesting a link between the uteroplacental RAS and oxygen tension during human gestation.

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The Effect of Nucleated Fetal Red Blood Cells Derived from Preeclamptic Patients on Endothelial Progenitor Cell Proliferation. Keiichi Matsubara, Emiko Abe, Yuko Matsubara, Shinji Hyodo, Masaharu Ito. *Obstetrics and Gynecology, Ehime University School of Medicine, Toon, Ehime, Japan.*

Objectives

Inadequate uteroplacental circulation results in placental ischemia and the development of preeclampsia (PE). Endothelial progenitor cells (EPCs) are thought to be a key player in the fetal angiogenesis. Vascular endothelial growth factor (VEGF), which is up regulated in PE, is involved in EPCs proliferation. Recently, it was reported that fetal nucleated red blood cells (NRBCs) have the capability to generate VEGF. We hypothesized that NRBCs could influence EPCs proliferation in the placenta and may be involved in the pathogenesis of PE.

Material and Methods

Mononuclear cells (MNCs) were isolated from the umbilical venous blood of normal pregnant women and preeclamptic patients by density gradient centrifugation. MNCs were incubated with anti-CD71 antibody conjugated with microbeads. NRBCs were collected using a Mini MACS separator. NRBCs were incubated for 24 hours with or without angiotensin II (Ang II) and erythropoietin (EPO). VEGF and placental growth factor (PlGF) concentrations in the supernatant were measured using ELISA. Also, PBMCs without NRBCs were seeded in endothelial basal medium with or without NRBCs using a Boyden chamber. These samples were incubated for 7 days with or without Ang II and EPO. The adherent cells were incubated with Di-LDL, fixed with paraformaldehyde, and stained with fluorescein isothiocyanate- labeled lectin. Di-LDL and lectin positive cells was considered to represent EPCs and the number was measured using flowcytometry.

Results

The number of NRBCs derived from umbilical venous blood was significantly increased in PE. Both Ang II and EPO significantly increased VEGF concentration in the supernatant of NRBCs derived from normal pregnant women. However, Ang II and EPO did not influence the NRBCs' VEGF production in PE patients. PlGF was not detectable in the supernatant. The number of EPCs in the umbilical venous blood was significantly decreased in PE and the number was not changed by NRBCs. On the other hand, the number of EPCs was significantly decreased in the culture with NRBCs. EPO significantly increased the number of EPCs in PE at the lower concentration of EPO compared with normal pregnant women.

Conclusions

It appears that fetal NRBCs may inhibit fetal EPCs proliferation in PE. Since EPO reduced the inhibitory reaction of NRBCs without VEGF production EPO may affect EPCs proliferation independently of NRBCs.

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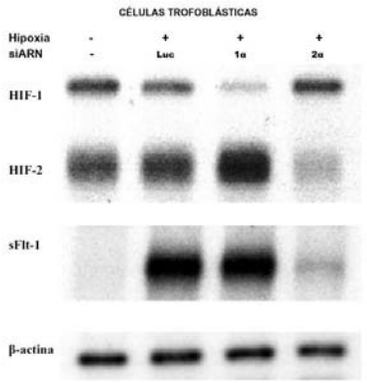
Relationship between the Hypoxia-Inducible Factor-2α (HIF-2α) and the Receptor sVEGF-R1/sFlt-1: Implication for Phatophysiology of Preeclampsia. Julio E Valdivia-Silva,[1,3] Juan C Gonzalez-Altamirano,[2] Keisy Lopez-Molina.[3] *[1]Immunology, Biomedical Research Institute - UNAM, Mexico, Federal Distrite, Mexico; [2]Cardiology and Torax Surgery, CASE EsSalud Hospital from Arequipa, Arequipa, Peru; [3]Oncoimmunology and Vascular Biology, Immunology Research Group- School of Medicine- UNSA, Arequipa, Peru.*

The trophoblast invasion is critical for the establishment of the uteroplacental circulation. At early phases of this process local oxygen pressure in the placenta is lower, that pathologically in preeclampsia remain constant. Because of this, is important to understand the response of placental cells against these stimuli. In the present work, we use primary cultures of trophoblast cells, fibroblasts of the villous, and human umbilical endothelial cells, isolated of preterm and term placentas (with and without preeclampsia), to explore the effect of the oxygen pressure in the expression and synthesis of VEGF, sVEGFR-1/sFlt-1, HIF-1α and-2α. Our results show that the low pressure of oxygen resulted in a significant increase of the mRNA and the protein of the receptor sVEGF-R1 selectively in the CTs. The VEGF's expression and synthesis was raised in three

cellular types, but the free protein (not bounded to sVEGF-R1) of the CTs was diminished. On the other hand, the expression of the ARNm of HIF-1α or -2α in cells was comparable in all the types of placentas, nevertheless, the protein HIF-2α was more increased in the CTs of preeclamptic placentas. To evaluate the relation of HIF-2α and the increase of the receptor sVEGFR-1, we used siRNA-HIF2α. In response to the inhibition, the expression of the receptor sVEGF-R1 diminished dramatically. The blockade of HIF-2α did not alter VEGF's expression. Our data are the first that propose that the protein of the factor of transcription HIF-2α is one of the molecules involved in the selective expression of the receptor sVEGF-R1 in trophoblast cells during hypoxia. **Figure:** Reduction of the expression to soluble receptor Flt-1/sVEGFR-1 after transfection with siRNA-HIF2α in trophoblast cells. siRNA= interference RNA, Lu=luciferase. Luc-siRNA was used as control.



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Effects of Estradiol on Synthesis, Secretion, and Activation of von Willebrand Factor in Endometrial Endothelial Cell. Shumei Zhao, Chainarong Choksuchat, Michael S Scholfield, Todd D Deutch, Thomas D Kimble, David F Archer. *Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA, USA.*

Objectives: Heavy menstrual bleeding (HMB) is a serious clinical condition affecting 30% of women. Due to inefficient and ineffective medical interventions, women with HMB often elect endometrial ablation or hysterectomy to eliminate HMB symptoms. Morbidity and loss of fertility linked to these surgical treatments support the search for more effective medical remedies. von Willebrand Factor (vWF), a principle initiator of blood clotting produced by endothelial cells. Women with von Willebrand Disease (vWD), have a high incidence of HMB indicating poor clotting. These findings suggest vWF heavily impacts the amount of blood loss during menstruation. Estrogen stops/reduces HMB and has been used to treat HMB in women with vWD, although the mechanism(s) is unknown. This proposal addresses the hypothesis that estradiol (E_2) increases the synthesis and activation of vWF, promoting clotting. **Materials and Methods:** Immortalized human endometrial endothelia cells (HEECs) were used for the studies. To determine if E_2 increases synthesis of vWF, we treated HEECs with E_2 at 0.001μM, 0.01μM and 0.1μM for 24 hours. vWF protein and mRNA levels were determined by Western blotting and real time PCR, respectively. To establish if E_2 can convert vWF from an inactive to an active conformation, the release of activated vWF by cells into culture medium will be assessed by ELISA. Decreased ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif) activity results in increased amounts of active vWF. Rrelease of activated ADAMTS13 will be determined by FRET. To ascertain if E2 regulated vWF secretion is by genomic pathway, HEECs will be exposed to the estrogen receptor antagonist ICI 182,780. ELISA and FRET will assess the release of active vWF and ADAMTS13, respectively. **Results:** Western blotting demonstrated E_2 increases vWF mRNA and protein levels in a dose-dependent manner in HEECs.**Conclusion:** The project will determine if E_2 acts at the HEECs to increase synthesis, secretion and activation of vWF. Preliminary results show E_2 increases intracellular vWF protein and mRNA levels in HEECs, supporting our hypothesis that E_2 increases synthesis of vWF in HEECs in vitro. If E_2 increases activated vWF, it could reduce/stop HMB by increasing activated vWF, providing justification for a clinical trial of E_2 to treat HMB.

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Over-Expression of VEGF-D Does Not Induce Lymphangiogenesis in the Mouse Endometrium. Peter A Rogers,[1] Jacqui F Donoghue,[1] Marc G Achen,[2] Steven A Stacker,[2] Jane E Girling.[1] *[1]Obstetrics & Gynaecology, Monash University, Melbourne, Victoria, Australia; [2]Ludwig Institute for Cancer Research, Melbourne, Victoria, Australia.*

Background: The human endometrial functionalis has reduced lymphatics compared to the basalis and myometrium[1]. This study examines the distribution of lymphatics in mouse uterus and investigates if over-expression of the lymphangiogenic growth factor vascular endothelial growth factor-D (VEGF-D) stimulates growth of new endometrial lymphatic vessels.

Methods: The distribution of uterine lymphatics was examined in C57BL/6JxCBA mice collected during the oestrus cycle, early pregnancy and following oestrogen and progesterone treatment. Human 293EBNA cells with/without stable transfection of VEGF-D were injected into the uterine horn of NOD/SCID mice. Uteri were collected after 4 weeks. Serial sections were immunostained with Lyve-1 and/or VEGFR3 (lymphatic endothelial cell markers), CD31 (blood endothelial cell marker), MAB286 (human VEGF-D), MAB1278 (human mitochondria) and PCNA (proliferative cell nuclear antigen).

Results: Lymphatic vessel profiles were mostly found in the connective tissue between the longitudinal and circular muscle layers of the myometrium. They were rare in the endometrium and only observed in 24% of the sections. When present in endometrium, lymphatic vessel profiles were usually situated adjacent to the endometrial/myometrial border. 293EBNA tumours formed inside and outside the uterine horn of both the control (n=4 of 6) and VEGF-D group (n=3 of 6). Localization of 293EBNA cells within the mouse uterus was confirmed by anti-human mitochondrial expression. VEGF-D immunostaining confirmed that transfected 293EBNA cells expressed VEGF-D in vivo. Over-expression of VEGF-D did not stimulate endometrial lymphangiogenesis, although there was an increase in vessel diameter of lymphatics in the myometrium adjacent to tumours. Initial analysis shows no significant effect of VEGF-D 293EBNA cells on endometrial blood vascular density or endothelial cell proliferation.

Conclusions: Minimal lymphatics are present in the mouse endometrium, as is the case for lymphatic vessels in the human endometrial functionalis. The lack of endometrial lymphangiogenesis in response to VEGF-D suggests the presence of an inhibitory factor limiting lymphatic growth in this tissue.

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Thrombin Enhances Vascular Endothelial Growth Factor (VEGF) Expression in Term Decidua: Implications for Abruption-Related Preterm Birth. Victoria V Snegovskikh, Lynn F Buchwalder, Rebecca Caze, Mizanur Rahman, Graciela Krikun, Frederick Schatz, Charles J Lockwood, Errol R Norwitz. *Ob/Gyn, Yale, New Haven, CT, USA.*

OBJECTIVE: In early pregnancy, decidual-derived VEGF mediates angiogenesis and is required for implantation and placentation. The role of decidual VEGF in later pregnancy is poorly understood. Decidual hemorrhage (placental abruption) generates excess thrombin and is a major risk factor for pPROM and preterm birth. This study compares immunohistochemical (IHC) localization of VEGF in decidual tissue sections from term pregnancies complicated by abruption and gestational age-matched controls, and investigates the effect of thrombin on VEGF expression by cultured human term decidual stromal cells (DSCs).

STUDY DESIGN: IHC was performed on serial sections of term placental tissues (no labor) with (n=5) and without (n=5) abruption. Purified term DSCs were passaged until >99% free of CD45+ cells by FACS. Confluent DSCs were primed with 10^{-8} M estradiol (E2), 10^{-7} M medroxyprogesterone acetate (MPA), both, or vehicle for 7 days. After 24h incubation in defined medium with corresponding steroids ± thrombin (0.5-2.5 IU/mL), conditioned supernatants were analyzed for VEGF by ELISA. Extracted total RNA was used to assess VEGF mRNA levels by quantitative RT-PCR using established primers.

RESULTS: VEGF expression was localized by IHC primarily to DSCs in placental tissue sections, and was increased in tissues from placental abruption vs controls. In term DSCs, thrombin increased VEGF secretion in a dose-dependent fashion irrespective of the hormonal milieu (eg, 2.17-fold stimulation by 2.5 IU/mL thrombin from 5.05±1.23 to 12.62±3.01 pg/mL per mcg protein for E2+MPA; p=0.034). This effect was abrogated by the thrombin inactivator, hirudin. VEGF mRNA were similarly increased by thrombin with or without steroid hormones (eg, 2.9-fold for E2+MPA; p<0.05).

CONCLUSIONS: Placental abruption is associated with increased VEGF expression in term decidual tissues in vivo with thrombin enhancing VEGF

mRNA and protein expression in term DSCs in vitro. Excess thrombin-mediated VEGF expression in term decidua aberrantly increases endothelial cell permeability to further generate thrombin by continuous exposure of tissue factor-expressing decidual cells to circulating factor VII. Thrombin-enhanced matrix metalloproteinase expression in term DSCs would degrade decidual and fetal membrane extracellular matrix to induce pPROM and preterm birth.

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Basal Directional Release of Angiotensin II by Endothelial Cells Stimulated by Chymotrypsin-Like Protease (CLP)/Chymase. Yuping Wang, David F Lewis, Yang Gu. *Obstetrics and Gynecology, LSUHSC-Shreveport, Shreveport, LA, USA.*

Objective: Chymotrypsin-like protease (CLP)/chymase is a serine protease which plays a major role in angiotensin II (Ang II) generation in the human heart. Our previous study showed a higher CLP activity in the maternal plasma in women with PE than in normal pregnancies. We also found enhanced chymase expression in the maternal vessel endothelium in women with PE. In this study, we determined if CLP could promote endothelial cell (EC) generation of Ang II.

Methods: We specifically examined basal directional release of Ang II by cultured ECs. ECs were grown on cell culture insert (6 well/plate, 8 micron pore size). When ECs reached confluence, chymotrypsin (chy) at concentrations of 0.25, 0.5, 1.0, 2.5, and 5.0 μg/ml were added to the upper chamber of the cell insert. After 24 hours of culture, medium in the lower chamber was collected. Medium concentrations of Ang II were measured by enzyme-linked immunoassay (EIA). All samples were measured in duplicate. Data are expressed as mean ± SE and analyzed by ANOVA. A p level < 0.05 was considered statistically different.

Results: Chymotrypsin produced a concentration-dependent increase in basal directional release of Ang II by cultured ECs, control: 1.016 ± 0.065 μg/ml; chy 0.25: 1.284 ± 0.281 μg/ml; chy 0.5: 1.204 ± 2.06 μg/ml; chy 1.0: 2.726 ± 0.829 μg/ml; chy 2.5: 4.499 ± 1.467μg/ml ($p<0.01$); chy 5.0: 5.159 ± 1.087 μg/ml ($p<0.01$), respectively. Data are means from 6 independent experiments.

Conclusion: Apical exposure of ECs with chymotrypsin-like protease could promote basal directional release of Ang II. Our result implicates that in PE, elevated CLP levels in the maternal circulation are very likely to affect EC generation of Ang II. Basal directional released Ang II may bind to its receptor on underlying vascular smooth muscle cells and contribute to the increased vasoconstriction in PE. (Supported NIH grants HD36822 and HL65997).

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VEGF-Activated ERK2/1 Pathway Is Regulated by Caveolin-1 in Placental Artery Endothelial Cell Caveolae. Wu Xiang Liao,[1] HongHai Zhang,[1] Jing Zheng,[2] Dong-bao Chen.[1] *[1]Reproductive Medicine, University of California San Diego, La Jolla, CA, USA; [2]Ob/Gyn, University of Wisconsin-Medison, Madison, WI, USA.*

VEGF stimulates angiogenesis and vasodilation critical for dramatic rises in materno-feto interface blood flows directly linked to fetal growth/survival. Extracellular signal-regulated kinase (ERK2/1) pathway mediates partially VEGF-induced angiogenic and vasodilatory responses in placental endothelial cells (EC). It is, however, unknown how this VEGF-induced signaling is organized in placental EC. **Objectives**: Ovine fetoplacental artery EC (oFPAEC) and its transformed counterpart, SV40-OF to test whether: 1) VEGF-activated ERK2/1 signaling is compartmentalized in the caveolae and disruption of caveolae interferes VEGF-induced ERK2/1 activation and; 2) caveolin-1, the structure protein of caveolae, regulates VEGF-stimulated ERK2/1 phosphorylation. **Methods**: oFPAEC or SV40-OF cells were cultured in MCDB-131/10% FBS/antibiotics. Serum-starved subconfluent (~80%) cells were treated with rhVEGF (0 to 100 ng/ml) for various times. Caveolae were disrupted by β-cyclodextrin (β-CD, 10 mM, 60 min) or caveolin-1 scaffolding domain (Cav-SD, 5 μM, 2 hr). SV40-OF cells were used for fractionation of caveolae membranes by discontinuous sucrose gradient (45%/35%/5%) ultracentrifugation. Activation of ERK2/1 signaling pathway were analyzed by Western-blotting with specific antibodies. **Results**: In total cell extracts, VEGF stimulates ERK2/1 phosphorylation in a time- and dose-dependent manner. ERK2/1 phosphorylation maximized by VEGF (10 ng/ml) at 5-10 min, which was abrogated by β-CD or Cav-SD. All the molecules for compromising the ERK2/1 signaling module, PLCγ1, PKCα, Src, Ras, Raf-1, MEK2/1 and ERK2/1, were detectable in purified caveolae membranes positive for various markers including caveolin-1, eNOS, flotillin-1, and β-adaptin. In caveolae, VEGF dramatically increased phosphorylated ERK2/1 without altering total ERK2/1 in a time-dependent manner similar to that in total cell extracts, which also maximized at 5-10 min. Pretreatment with β-CD or Cav-SD blocked VEGF stimulation of ERK2/1 phosphorylation in caveolae. **Conclusion**: VEGF activates the ERK2/1 signaling pathway in caveolae and caveolae integrity is essential for VEGF-activated ERK2/1 signaling pathway. We conclude that caveolae/caveolin-1 serves as a platform for compartmentalizing the VEGF-induced ERK2/1 signaling pathway in placental EC (HL74947 and HL70562).

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Hypoxia Upregulates GCM1 in Human Placenta. David McCaig, Fiona Lyall. *Institute of Medical Genetics, University of Glasgow, Glasgow, United Kingdom.*

INTRODUCTION: Studies in transgenic mice have shown that a variety of genes regulate the differentiation of trophoblast cells. These genes include GCM1. GCM1 is also expressed in the human placenta. Placental GCM-1 protein has been reported to be reduced in pre-eclampsia, In view of the close link between hypoxia, hypoxia-reoxygneation, pre-eclampsia, placental development and the reported reduction in GCM1 we hypothesised that GCM1 expression would be affected by hypoxia.

AIM: The aim of this study was to determine the effects of hypoxia on GCM1 expression in the human placenta. Two model systems were used; (1) free floating villous explants and (2) cultured primary cytotrophoblast and syncytiotrophoblast cells as described previously*.

METHODS: Explants or cell cultures were exposed to either hypoxia or hypoxia followed by re-oxygenation. Western blot analysis was used to assess GCM1 protein levels. Bands on the gels were quantified using scanning densitometry. Statistical differences (n=6 experiments for both models used) were calulated by ANOVA and Turkey's post-hoc test.

RESULTS: GCM1 protein was detectable at a low level in villous explants maintained for 7h in 20% O_2. A striking increase in GCM1 protein was observed when villous explants were incubated for 1h in 0% O_2 ($p<0.002$). Incubation of villous explants for 1h in 0% O_2 followed by re-oxygenation for 6h in 20% O_2 resulted in a marked decline in GCM1 protein ($p<0.002$). Expression of GCM1 was also analysed in primary cytotrophoblast and syncytiotrophoblast cultured in 18% O_2 or reduced oxygen (2% O_2) conditions. GCM1 protein was not detected in any of the experimental conditions used.

DISCUSSION: The present study has shown that acute hypoxia increases GCM-1 protein in villous explants. The experiments with purified trophoblast do not support a role for hypoxia increasing GCM-1 in these cells under the experimental conditions used. The present findings are in keeping with the complex effects of oxygen depending on the conditions used. The observed hypoxic effects on GCM1 warrant further investigation.

***REFERENCE:**Newby D, Cousins F, Myatt L, Lyall F. Heme oxygenase expression in cultured human trophoblast cells during in vitro differentiation: Effecs of hypoxia. Placenta 2005;26:201-9.

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The Effect of Acute Alcohol Exposure on Histone3-lys9 Modification in the Mid-Gestation Embryonic Lung. Xiangyuan Wang,[1] Debra Wolgemuth,[1,2,3] Laxmi Baxi.[1] *[1]OB/GYN; [2]Genetics & Development; [3]Human Nutrition, Columbia University Medical Center, New York, NY, USA.*

Objective

Maternal alcohol abuse during pregnancy produces an array of birth defects comprising fetal alcohol syndrome. Lung development depends on a balance between cell proliferation and apoptosis. We have previously shown that the acute alcohol exposure in the mid-gestation embryo can delay lung development and induce apoptosis. Acute exposure to ethanol of selected tissues in mouse embryos has been reported by others to initiate apoptosis within 12 hours after exposure and result in histone modifications. Specifically, histone acetylation and deacetylation are involved in transcriptional activation and repression, respectively, but can also involve apoptosis. In the present study, we have investigated the effect of alcohol on acetylation of histone3 at lysine9 (AcH3Lys9) in the mid-gestation embryonic lung.

Study Design

Pregnant C57BL/6J mice at day 13.5 of gestation (E13.5) were injected intra-peritoneally with 2 doses of 25% ethanol (3.75g/kg), 4 hr apart (Alcohol-exposed: AE) or with Ringers solution (Controls: C). AE and five C fetuses were retrieved 12 and 24 hr later and the lungs were fixed and processed for morphological evaluation and staining with rabbit polyclonal anti-AcH3Ly9 antibody. The entire lung tissue field was evaluated for the levels of AcH3Lys9

staining and scored as (-) to (++++). Three areas were selected randomly from each sample and the total number of cells and staining positive cells were counted in the bronchial epithelium and in the mesenchyme.

Results

Twelve hr after alcohol exposure at E13.5, the morphology of AE embryonic lungs was normal. However, high levels of AcH3Lys9 were detected in 50% of the bronchial epithelial cells and 50% of the mesenchymal cells. The expression level in both lineages decreased 24 hr after alcohol exposure. In the controls, the expression of AcH3Lys9 was virtually undetectable in both the bronchial epithelium and the mesenchymal cells.

Conclusions

Our previous study showed that AE E13.5 lungs significantly increased apoptotic cell in both bronchial epithelium and mesenchyme 16 hours after alcohol treatment. We now observe that elevated expression of AcH3Lys9 in the embryonic lung preceded the observation of apoptosis, suggesting that alteration in the acetylation of H3 could be one of the molecular mechanisms involved in the induction of apoptosis following acute alcohol exposure.

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Effects of Maternal Vitamin C and E Supplementation on Lipid Peroxide Levels, Total Antioxidant Ability, and Antioxidant Levels in the Umbilical Venous Plasma. Yoon Ha Kim,[1] Tae-Bok Song,[1] Cheol Hong Kim,[1] Jong Woon Kim,[1] Moon Kyoung Cho,[1] Sung Yeul Yang,[2] Bong Whan Ahn.[2] *[1]Obstetrics & Gynecology, Chonnam National University Medical School, Gwangju, Korea; [2]Biochemistry, Chonnam National University Medical School, Gwangju, Korea.*

Objective: Our purpose was to evaluate the effect of vitamin C and E supplementation on lipid peroxide levels, total antioxidant ability, and antioxidant levels in the umbilical venous plasma.

Materials and methods: Women at risk for preeclampsia (nullipara, previous preeclampsia, chronic hypertension) were recruited at 15 to 20 weeksgestation and randomly assigned to receive either 1000 mg of vitamin C and 400 IU of vitamin E (study group, n=20) or placebo (control group, n=20) daily until delivery. Umbilical venous blood were collected after full term delivery. Lipid peroxide levels, oxygen-radical absorbance capacity (ORAC) values, antioxidant levels were measured by each method (thiobarbituric acid reaction, Cao's method, and high performance liquid chromatography).

Results: 1. The lipid peroxide levels in the umbilical venous plasma of study group were significantly lower than that of control group (2.22±0.06 vs. 2.56±0.12 nmol/mg protein, $p<0.05$). 2. The ORAC values in the umbilical venous plasma of study group were significantly higher than that of control group (15010.1±649.4 vs. 11804.6±463.7 U/mL, $p<0.05$). 3. The α-tocopherol levels in the umbilical venous plasma of study group were significantly higher than that of control group (130.2±10.5 vs. 78.7±4.4 nmol/mL, $p<0.01$). 4. There were no significant differences in the ascorbic acid, uric acid, β-carotene, retinol, and γ- tocopherol levels in the umbilical venous plasma between control and study group.

Conclusion: This suggested that maternal vitamin C and E supplementation may affect the oxidant-antioxidants balance of the utero-placenta unit and fetus.

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Placental TNF-Related Apoptosis-Inducing Ligand (TRAIL) in Normal Pregnancy and Pre-Eclampsia. Xilian Bai, Jenny E Myers, Philip N Baker, John D Aplin, Ian P Crocker. *Maternal and Fetal Health Research Group, The University of Manchester.*

Introduction: Enhanced placental trophoblast apoptosis is well known to occur in pre-eclampsia. However, the potential role of membrane associated or soluble TRAIL, an apoptosis-inducing ligand, and its death receptor, DR5, is not known. We tested the hypotheses that the TRAIL/TRAIL-Receptor system is compromised in pre-eclampsia and that soluble TRAIL is a circulating factor which triggers the vascular complications of pre-eclampsia.

Method: This study was conducted on placental samples and plasma (EDTA) from women with uncomplicated pregnancies (n=16) and with pre-eclampsia (n=16) at 35-40 weeks gestation. Protein expression levels and tissue localisation of TRAIL and DR5 were defined in villous tissue by Western blotting and immunohistochemistry. Soluble TRAIL (sTRAIL) was measured in maternal plasma from both groups using a commercial ELISA (Diaclone).

Results: Placental villous TRAIL and DR5 protein was unaltered in pre-eclampsia compared to normal pregnancy. Whilst there was differential distribution of TRAIL and DR5 within the component cells, this also was unaffected in pre-eclampsia. Within the villi, TRAIL was mainly confined to the cytoplasm and perinuclear regions of cytotrophoblast, syncytiotrophoblast, stromal cells and the fetal capillary endothelium. Conversely, DR5 was restricted to trophoblast only, distributed evenly between cytoplasm, plasma membranes and nucleus. sTRAIL was present in plasma from non-pregnant women of childbearing age [391.6 (312.4-646.8), median (interquartile range), pg/ml, n=6]. However, there was no significant increase either in pregnancy [372.3 (319.6-442.3) pg/ml, n=12] or pre-eclampsia [301.8 (209.0-435.5) pg/ml, n=13] (Kruskal-Wallis test, $p>0.05$).

Conclusions: These results suggest that placental villous TRAIL is not adversely regulated in pre-eclampsia. The absence of DR5 in stromal and endothelial cells may increase resistance to apoptotic stimuli in cells of the villous core. The co-localisation of TRAIL and DR5 in trophoblast suggests a role in autocrine regulation of cell turnover in this cell type.

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Use of Laser Capture Microdissection (LCMD) To Measure Levels of Syncytial Fas Ligand Expression in Human Placenta. Seth Guller, Yula Y Ma, Vikki M Abrahams, Gil Mor. *OB/GYN & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Introduction: Preeclampsia is associated with apoptosis of the syncytial layer of placenta and the release of particulate and soluble factors which are deleterious to maternal endothelial function. The goal of the current study was to determine whether laser capture microdissection (LCMD) and Western blotting could be used to assess levels of syncytial Fas ligand (FasL), a key protein in the apoptotic cascade.

Methods: Frozen sections of term placenta delivered from uncomplicated pregnancies at term were used for study (n=3). Following staining with Mayer's hematoxylin (n=3), LCMD (Leica Instruments) of an intact terminal villus was carried out using a focused laser pulse directed to the area of interest using a microscope. In the first round of microdissection the placental villus core consisting of fibroblast, macrophages, fetal vessels, and connective tissue, were removed. In the second round of LCMD, the syncytial layer of that same villus was removed and collected in lysis buffer containing detergent and protease inhibitors, and the number of nuclei per sample was recorded. This procedure was repeated until syncytial tissue from 10-15 villi were collected. Electrophoretic separation of lysate proteins was then carried out, and Western blotting and immunodetection using an anti-FasL antibody was performed.

Results: We observed that FasL was detected in microdissected syncytial specimens at a molecular weight of approximately 37 kDa (Figure), consistent with our previous reports. It is of note, that Western blotting of samples containing approximately 5000 (Lane 1), 2000 (Lane 2), 1000 (Lane 3), and 500 (Lane 4) nuclei revealed that FasL could be reliably detected in specimens containing as few as 1000 nuclei.

Conclusions: Since FasL is a cytokine of low to moderate abundance in placenta, this suggests that LCMD coupled with Western blotting will be a valuable methodology to elucidate pathways of syncytial apoptosis and pathophysiology in pregnancies complicated by preeclampsia.

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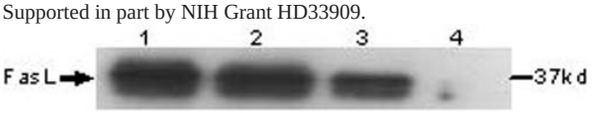

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Reduced Tyrosine Phosphorylation of Inducible Nitric Oxide Synthase in Placentae from Severe Preeclamptic Pregnancy. Carlos Escudero,[1] Rose Webster,[2] Leslie Myatt,[2] Luis Sobrevia.[1] *[1]Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics & Gynaecology, Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Department of Ob/Gyn, University of Cincinnati, Cincinnati, OH, USA.*

Background: The placenta of preeclamptic pregnancies shows oxidative and nitrative stress. We have shown low protein abundance but paradoxically high activity of inducible nitric oxide synthase (iNOS) in the placenta with severe preeclampsia compared with normal pregnancies. Protein nitration and phosphorylation are post-translational modifications possibly involved in NOS activity.

Objectives: Examine iNOS localization and tyrosine phosphorylation in the preeclamptic placenta and the effect of a peroxynitrite generator (SIN-1) on iNOS expression in primary human placental microvascular endothelial cell (hPMEC) cultures.

Methods: Placental lysates from normal (n=3), mild (n=2) and severe (n=3) preeclamptic pregnancies were western blotted for iNOS and

antiphosphotyrosine. Immunolocalization of iNOS was performed in the same tissues. hPMEC were used to determine effect of SIN-1 (1mM) on iNOS protein expression (30 min, 4 and 8 h).

Results: iNOS protein was detected in microvascular endothelium, smooth muscle and syncytiotrophoblast of the placenta. Inducible NOS levels in placentas from severe preeclampsia were significantly decreased compared to normal ($p<0.05$) but were not different from mild preeclampsia. Aligning western blots of anti-phosphotyrosine and iNOS revealed a phosphorylated band corresponding to the molecular weight of iNOS. The proportion of tyrosine phosphorylated iNOS was reduced by ~35% in severe preeclampsia compared with normotensive. Preliminary data with IP for phosphotyrosine and cross-blotting with iNOS confirmed this finding. SIN-1 treatment decreased iNOS protein abundance at 4 and 8 h in hPMEC.

Conclusions: Our results demonstrate decreased tyrosine phosphorylation of iNOS in preeclamptic placenta. Phosphorylation of tyrosine in iNOS has been reported to negatively regulate its activity. We therefore postulate that decreased phosphorylation of iNOS may be responsible for the increased catalytic activity of the enzyme that we have previously observed. The decreased levels of iNOS observed on SIN-1 treatment may be due to nitration, an effect analogous to preeclampsia, where the presence of peroxynitrite has been well established. It remains to be seen if protein nitration has an effect on enzyme stability.

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Maternal Periodontal Disease Is Associated with Oxidative Stress in Early Pregnancy. Ashley Hickman,[1] Kevin Moss,[2] James Beck,[2] Steven Offenbacher,[3] Michael Ruma,[1] Kim Boggess.[1] *[1]Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, University of North Carolina, Chapel Hill, NC, USA; [2]Department of Dental Ecology, School of Dentistry, University of North Carolina, Chapel Hill, NC, USA; [3]Department of Periodontology, School of Dentistry, University of North Carolina, Chapel Hill, NC, USA.*

OBJECTIVE:

8-isoprostane (8-iso), a prostaglandin-like compound formed *in vivo*, is a reliable measure of oxidative stress which occurs in response to many different stimuli, including infection. Periodontal disease is a chronic oral infection associated with fetal growth restriction and preeclampsia. Our objective was to determine if maternal periodontal disease is associated with oxidative stress as measured by 8-iso.

METHODS:

A secondary analysis was conducted using prospective data from the Oral Conditions and Pregnancy Study. A cohort of healthy women enrolled <26 weeks underwent oral health examination and serum sampling. Maternal periodontal disease was categorized as healthy, mild, or moderate-severe by clinical criteria. Maternal serum was analyzed for 8-iso by ultra-sensitive ELISA. Elevated 8-iso was defined by a value ≥75th %tile. Maternal factors associated with elevated 8-iso were determined using chi-square or t-tests as appropriate. A logistic regression model was created to determine adjusted odds ratios (95th %CI) for elevated 8-iso.

RESULTS:

791 women had complete data for analysis. The median 8-iso level (IQR) was 1,806 (16 - 81,870 pg/dL). Using bivariate analysis, moderate-severe periodontal disease, non-white race, use of WIC/food stamps, unmarried status, obesity, and lack of insurance were associated with elevated 8-iso. The logistic regression model for elevated 8-iso is shown below.

Maternal Factors

	Adjusted OR (95thCI)*
Mild periodontal disease	1.2 (0.8 - 1.9)
Moderate-severe periodontal disease	2.5 (1.4 - 4.4)
White race	0.7 (0.5 - 1.1)
WIC/food stamps	1.8 (1.2 - 2.6)
Maternal age	1.0 (1.0 - 1.0)
Smoking	1.1 (0.7 - 1.7)

*Adjusted for variables above

CONCLUSION:

Maternal periodontal disease and utilization of public assistance are associated with oxidative stress in pregnancy. The relationship between periodontal disease, social hardship, oxidative stress, and adverse pregnancy outcome remains to be determined. Antioxidant therapy could represent novel therapy for the prevention of adverse pregnancy outcome.

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Severe Transient Immunological Thrombocytopenia in a Preeclampsia Patient Whose Bone Marrow Production of Platelets Had Been Restricted. Toshihiro Yoshimura. *Obstetrics and Gynecology, NTT-West Kyushu General Hospital, Kumamoto-city, Kumamoto, Japan.*

Introduction: Idiopathic myelofibrosis is a chronic myeloproliferative disorder in which clonal haemopoetic stem cell proliferation is accompanied by reactive fibrosis.

Case report: A 23-year-old primiparous woman was referred to our Hospital for prenatal care after 12 weeks of gestation. Her medical history was significant for idiopathic myelofibrosis, and congenital agenesis of the spleen. The laboratory workup showed the patient's white blood cell count to be 5,500/μL; hemoglobin, 8.0 gm/dl; and platelet count, 38,000/μL. Her bleeding time was normal (3 min). Until the 34th week, the patient's pregnancy was uneventful. During the 35th week, however, the patient's platelet count declined to 16,000/μL, when proteinuria became evident. Her bleeding time was prolonged to 13 min. The coagulation system was normal. By the 37th week, the patient's blood pressure was elevated to 150/100 mmHg, and her urinary protein excretion exceeded 2 g/day. Our initial diagnosis was thrombocytopenia due to bone marrow suppression. The patient had no history of platelet transfusion, but we expected it would increase the platelet count, particularly since in the absence of destruction by the spleen, the lifespan of the platelets would be prolonged. This was not the case, however. Elective induction of labor was carried out after 38 weeks. At the same time, the patient was transfused with 10 units of platelets, but her platelet count remained unchanged (20,000/μL). It was then that we realized that immunological thrombocytopenia may be involved, and massive doses (400 mg/kg) of gamma globulin were given intravenously for one day. Thereafter, platelets (15 units) were again transfused, this time raising the platelet count to 110,000/μL. Cesarean section was promptly carried out under general anesthesia. We later found that the patient's serum platelet associated immunoglobulin (PAIgG) level was positive, though antiplatelet and antinuclear antibodies were negative. The postoperative course was uneventful. The maternal platelet count declined to the pretransfusion level within 2 days after delivery, but gradually increased to the prepregnancy level by 2 weeks.

Comment: This idiopathic myelofibrosis patient in whom bone marrow production of platelets had been severely restricted demonstrates that immunological destruction may play a major role in the development of thrombocytopenia in preeclampsia.

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Early L-Arginine Therapy Improves Notably the Fetal Growth in Preeclamptic Women. A Randomized Controlled Trial. Keisy Lopez-Molina,[1] Juan C Gonzalez-Altamirano,[2] Julio E Valdivia-Silva.[1,3] *[1]Oncoinmunología y Biología Vascular, Facultad de Medicina, Universidad Nacional San Agustín, Arequipa, Peru; [2]Cardiología y Cirugía de Tórax, Hospital Nacional CASE EsSalud, Arequipa, Peru; [3]Inmunología, Instituto de Investigaciones Biomédicas, México, Distrito Federal, Mexico.*

OBJECTIVE: To assess the benefit of early administration to L-arginine, the precursor to nitric oxide, on fetal growth.

METHODS: One hundred women with preeclampsia were randomized to receive either l-arginine or placebo until day 1 postpartum. 96 live singleton infants were born after preeclamptic pregnancies and compared those with other 50 control infants. Birth size was expressed as the ratio between observed and expected birth weights, and infants smaller than two standard deviations from expected birth weights were classified as small for gestational age (SGA). All the women had neither previous precedents of the preeclampsia nor other factors that they cause SGA.

RESULTS: No significant differences existed between the groups with preeclampsia before randomization. Preeclampsia was associated with a 8% (95% confidence interval [CI] 6%, 9%) reduction in birth weight. 4 women with HELLP syndrome had to leave the study. The risk of SGA was three times higher (relative risk [RR] = 3.4; 95% CI 2.8, 7.0) in infants born after preeclampsia without L-Arginine therapy than in control pregnancies, and two times higher (relative risk [RR] = 1.8; 95% CI 0.7, 2.5). in infants born after preeclampsia with L-Arginine therapy.

CONCLUSION: Fetal growth improve markedly with L-arginine therapy in women with preeclampsia.

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What Are the Roles Played by Peroxynitrite in Preeclamptic Women? Yoshikatsu Suzuki,[1] Tamao Yamamoto,[1] Yoshimasa Watanabe,[2] Takeo Itoh,[2] Hidetaka Izumi.[3] *[1]Obstetrics and Gynecology, Nagoya City University, Nagoya, Japan; [2]Pharmacology, Nagoya City University, Nagoya, Japan; [3]Obstetrics and Gynecology, Izumi Women's Hospital, Fukuoka, Japan.*

Aim

Preeclampsia is characterized by hypertension plus proteinuria. It is hypothesized that the endothelial cell function might be activated by placental faculty in early pregnancy and the activation might cause the vascular disease in preeclampsia. Peroxynitrite, which is produced by combination with superoxide (O_2^-) and nitric oxide (NO), is strong oxidant as well as O_2^-.We investigated whether or not the localization of peroxynitrite in both placenta and resistance artery might play important roles of developing preeclampsia.

Method

Omental arteries or placentas were obtained from severe preeclamptic and term-normotensive pregnant women at cesarean section. They were stained by ant-nitrotyrosine (NT, a marker of peroxynitrite) antibody. Furthermore, the concentration of NT was measured in omental arteries. In formed consent was obtained from all patients in written. Preeclampsia was diagnosed according to the criteria of Japan Society of Obstetrics and Gynecology.

Results

The localization of NT was seen in placentas obtained from sever preeclamptic women (7/10), although it was not seen from normotensive pregnant women (0/8). The localization was also seen in placentas from 5 of 7 severe preeclamptic women with intrauterine fetal restriction. In omental arteries from both groups, the localization of NT was seen to same degree, and the concentration of NT was similar (0.788±0.161 for sever preeclampsia and 0.984±0.209 for normotensive pregnant women).

Conclusions

From these results, it was suggested that an increase in O_2^- production, a decrease in NO as well as peroxynitrite production in the placentas might cause the vascular dysfunction and subsequently damage uteroplacental blood circulation in preeclampsia. However, the role played by peroxynitrite in resistance artery might be different and more complicated.

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Regulation of Matrix Metalloproteinase-1 and -3 in First Trimester Decidual Cells: Implications in the Pathogenesis of Preeclampsia. William Murk, Ceyda Oner, Lynn Buchwalder, Umit A Kayisli, Aydin Arici, Frederick Schatz, Charles J Lockwood. *Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Background: Preeclampsia (PE) is associated with shallow cytotrophoblast (CT) invasion of the decidua, leading to impaired vascular transformation and poor uteroplacental perfusion. CT invasion requires the selective proteolysis of the peri-decidual cell (DC) extracellular matrix. We hypothesized that IL1β and TNFα, cytokines that have been linked to PE, may induce aberrant expression of the matrix metalloproteinases (MMP) 1 and 3 in DCs, thereby preferentially degrading the decidual ECM and interfering with sequential CT invasion.

Methods: Immunostaining for MMP-1, MMP-3, and vimentin (a DC marker) was performed on decidua from normal (n=5) and preeclamptic (n=4) women, and staining intensities were evaluated by HSCORE. Confluent, leukocyte-free first trimester DCs were primed with 10-8 M estradiol (E2) or E2 + 10-7 M medroxyprogesterone acetate (MPA), and then switched to a defined medium with E2 +/- MPA with or without 1 ng/mL of IL1β or TNFα. Secreted MMP-1 and MMP-3 levels were measured by ELISA (n=8) and confirmed by Western blotting. Quantitative RT-PCR assessed MMP-1 and MMP-3 mRNA levels (n=3).

Results: Tissue staining revealed that MMP-1 and MMP-3 levels in preeclamptic decidua (HSCORE mean±SEM: 217±41 and 205±11, respectively) were significantly higher than in normal decidua (140±22 and 148±25, respectively; $p<0.05$). In cultured first trimester DCs incubated with E2, TNFα increased secreted MMP-1 and MMP-3 levels by 13±2 and 8±2-fold, respectively, while IL1β increased them by 17±3 and 28±13 fold, respectively ($p<0.05$). In parallel incubations with E2+MPA, basal MMP-1 and MMP-3 output were lowered by approximately 70% and 60%, respectively, while TNFα- and IL1β-elicited MMP-1 and MMP-3 levels were blunted by 40-60%. Western blotting confirmed the ELISA results, and mRNA levels corresponded to changes in MMP-1 and MMP-3 secreted protein levels.

Conclusions: Over-expression of MMP-1 and MMP-3 in decidual cells may promote PE by disrupting decidual ECM and impairing normal CT invasion. The high levels of IL1β and TNFα associated with PE may be contributing to this over-expression. Our in vitro observations that MPA blunts TNFα- and IL1β-elicited MMP expression suggests that exogenous progestin may offer a novel therapeutic approach in preventing PE.

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A Polymorphism in the Fetal COMT Gene Is Associated with Preeclampsia. Lori H Walsh,[1] Lu Minyan,[1] Mary D Sammel,[2] Roberto Romero,[3] Jerome F Strauss III.[1] *[1]Obstetrics and Gynecology, Virginia Commonwealth University, Richmond, VA, USA; [2]Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA, USA; [3]Perinatology Research Branch, NICHD, Hutzel Hospital, Detroit, MI, USA.*

2-Hydroxyestrogens are metabolized by catechol-O-methyl transferase (COMT) to produce 2-methoxyestradiol (2-ME), a compound with diverse biological activities including inhibition of HIF-1α, a transcription factor that mediates cellular response to hypoxia. Circulating levels of 2-ME and placental COMT activity are significantly reduced in preeclampsia, raising the possibility that reduced production of 2-ME contributes to the pathophysiology of preeclampsia by altering placental response to hypoxia. Genetic variation in the COMT gene is linked to COMT activity and has been associated with intrauterine fetal growth restriction. We determined if a SNP in Exon 4 of the COMT gene (rs4818), which does not change amino acid sequence (136Leu136), but reduces COMT mRNA translation, was associated with preeclampsia. We analyzed COMT genotypes in paired DNA samples extracted from maternal and cord blood from normal pregnancies and pregnancies complicated by preeclampsia by allele discrimination. The study population was predominantly (>98%) African-American. The frequency of the minor rs4818 "G" allele, which is associated with low COMT activity, was similar in maternal cases and controls (Cases: 19%; Controls: 23%, P=0.5), but was significantly greater in fetal DNA from 35 pregnancies complicated by preeclampsia compared to 55 control pregnancies (G allele frequency Cases: 34%; Control: 16%; $P<0.001$). Likewise, fetal carriage of the rs4818 "G" allele conferred a significantly greater risk of preeclampsia (Odds Ratio: 1.74; 95% C.I.: 1.22, 2.48, $p<0.0045$). There was also a significant discordance between paired maternal and fetal rs4818 genotypes with significantly greater discordance for the "G" allele in fetuses hosted in preeclamptic pregnancies (Odds Ratio: 3.17; 95% C.I.: 1.19, 8.49). We conclude that genetic variation in the COMT gene is associated with risk of preeclampsia, possibly through a mechanism involving reduced production of 2-ME. Supported by NIH P60MD002256.

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Smoking Is Associated with Elevated ADMA in Preeclampsia. Michael P Frank,[1] Robert W Powers.[1,2] *[1]Magee-Womens Research Institute, Pittsburgh, PA, USA; [2]Obstetrics & Gynecology, University of Pittsburgh, Pittsburgh, PA, USA.*

Objective: Cigarette smoke exposure paradoxically reduces the risk of preeclampsia. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide synthase (NOS), an independent risk factor for cardiovascular mortality, ADMA is elevated in women who develop preeclampsia, and ADMA has been reported to be both higher and lower in smokers. The objective of this study was to investigate the concentration of ADMA in pregnant smokers and nonsmokers with and without preeclampsia.

Study Design: Case-control study of 120 women with uncomplicated pregnancy (controls), and 57 women with preeclampsia matched for gestational age at sample collection. ADMA was measured by HPLC. Cigarette smoke exposure was determined by questionnaire and confirmed by plasma cotinine. Data are mean±SD. Analysis was by two factor ANOVA with Fishers post-hoc testing, significance accepted at $p<0.05$

Results: As previously reported, maternal plasma ADMA concentrations were higher in women with preeclampsia compared to controls ($p<0.001$). In addition, the concentration of ADMA was significantly higher in preeclampsia smokers compared to controls and preeclampsia nonsmokers ($p<0.05$). In contrast, there was no difference in ADMA concentration between control smokers and nonsmokers.

Conclusion: These data may suggest a differential effect of cigarette smoke exposure on circulating ADMA concentrations between women who do and do not develop preeclampsia. Previous data has suggested that cigarette smoking is associated with lower ADMA in low risk elderly patients, and higher ADMA in high-risk subjects with diabetes. Therefore, the data in preeclamptic and non-preeclamptic subjects may be consistent with these studies, however, the underlying biological explanation for this differential effect has yet to be determined.

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	Control-nonsmokers (n=61)	Control-smokers (n=59)	Preeclampsia-nonsmokers (n=29)	Preeclampsia-smokers (n=28)
Gestational age at sample collection (weeks)	34.3±0.48	34.1±0.57	34.3±0.69	34.2±0.78
Plasma cotinine (ng/ml)	1.6±3.7	61.3±56.9*	0.8±1.5	30.1±43.5*
ADMA (µmol/L)	0.43±0.06	0.46±0.07	0.54±0.09*	0.62±0.28*‡

Data: mean±SD, *: p<0.05 vs. control-nonsmokers; ‡: p<0.05 vs. preeclampsia-nonsmokers.

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Pregnancy Decreases Cerebrovascular Resistance and Increases Blood-Brain Barrier Permeability during Acute Hypertension: A Role in Eclampsia? Marilyn J Cipolla, Lisa V Bullinger. *Neurology, Ob/Gyn and Pharmacology, University of Vermont, Burlington, VT, USA.*

Objective: Eclampsia is similar to hypertensive encephalopathy in which an acute elevation in blood pressure causes autoregulatory breakthrough, hyperperfusion and edema formation. We previously reported that the pressure of breakthrough was similar between nonpregnant (NP) and late-pregnant (LP) rats, but only LP animals developed edema. This study tested the hypothesis that LP animals have decreased in cerebrovascular resistance (CVR) and hyperperfusion in response to breakthrough vs. NP. We further hypothesized that acute hypertension would cause greater blood-brain barrier (BBB) permeability in LP rats due to elevated hydrostatic pressure.

Methods: *In vivo* models of BBB permeability and cerebral blood flow (CBF) were used in NP (n=16) and LP (d19-20; n=16) rats that were either normotensive or hypertensive (NP-HTN, LP-HTN) by infusion of phenylephrine to raise mean arterial pressure. Permeability was determined in anterior and posterior brain regions by calculating the flux of 70kD dextran into the brain tissue, measured by a fluorescent spectrophotometer after flushing the vasculature with saline. CBF and CVR were measured by infusion of 15µm fluorescent microspheres and determined based on the flow rate and fluorescence intensity of a reference sample for each animal. Animals were ventilated to maintain blood gases within normal ranges (PO_2>100mmHg, PCO_2 =35-45mmHg).

Results: Although the pressure change was similar between NP and LP (↑83 and 85 mm Hg), LP animals responded to acute hypertension with hyperperfusion. CBF increased from 86 ± 10 to 242 ± 22 ml/100g/min in NP (181%) and 94±9 to 318±31ml/100g/min in LP (238%; p<0.05 vs. NP). Hyperperfusion in LP animals was associate with decreased CVR vs. NP (0.7±0.07 vs. 0.5±0.05 mm Hg/(ml/100g/min); p<0.05). BBB permeability was significantly increased in LP animals at breakthrough vs. NP in both anterior and posterior brain regions. The flux of dextran in anterior and posterior brain regions for NP vs. LP animals was: 113±50 vs. 362±87 for anterior (p<0.05) and 167±58 vs. 545±140 for posterior (p<0.05).

Conclusions: These data demonstrate that pregnancy decreases CVR and causes hyperperfusion of the brain during acute hypertension. Because increases in CVR is a protective function in the brain, impairment of these mechanisms during pregnancy may predispose the brain to edema when blood pressure is elevated, as in eclampsia.

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VEGF Induced Vasodilation and Endocrinology within the Fetoplacental Circulation in the *In Vitro* Dual Perfusion Model Adapted to 'Healthy' and 'Hypoxic' Oxygen Environments. Fatima Soydemir,[1] Elizabeth S Hutchinson,[1] Philip Day,[2] Ian P Crocker,[1] Philip N Baker,[1] Colin P Sibley,[1] Paul Brownbill.[1] *[1]Maternal and Fetal Health Research Group, University of Manchester, United Kingdom; [2]Department of Haematology, Leeds Teaching Hospitals, Leeds, United Kingdom.*

Introduction: This study used the *in vitro* dually perfused human placental lobule to test the hypothesese that placental release of VEGF and the fetoplacental vasodilatory response to exogenous VEGF-165 are altered by tissue oxygenations that mimic healthy and preeclamptic pregancies. **Methods:** Lobules were dually perfused for six hours under one of two oxygenation conditions, representing 'normoxia' and 'hypoxia' (n = 6 each): delivering maternal side inflow perfusate at oxygen concentrations of 15.6 % and 8 %, respectively, distributed via 22 cannulae; and fetal side inflow perfusate oxygen concentration of 2-3 % in both systems. Venous perfusates were sampled and assayed, appropriate to side of release, for erythropoietin (EPO), macrophage inflammatory protein-1 alpha (MIP-1 alpha) (reference oxygen sensitive hormones), free VEGF, sVEGFR-1 and PlGF. In separate perfusions, fetoplacental vasodilation in response to 550 pM VEGF was investigated, following preconstriction of the fetoplacental vasculature to steady state fetal-side inflow hydrostatic pressure (FIHP) with the thromboxane mimetic, U46619, (n = 6 each). **Results:** Maternal-side MIP-1 alpha release was higher in the 'hypoxic' than the 'normoxic' system (680 ± 119 and 531 ± 93 pg/ml, respectively, at 6 hours, mean ± SE; 2-way ANOVA: P<0.01). Maternal-side EPO and fetal-side soluble free VEGF and sVEGFR-1 release were not different between groups. Fetal-side release of PlGF was higher in the 'hypoxic' group than the 'normoxic' group (0.13 ± 0.10 and 0.03 ± 0.01 pM, respectively, at 6 hours, mean ± SE; 2-way ANOVA: P < 0.05). There was no difference in the vasodilatory response to VEGF-165 in the fetoplacental vasculature between the groups (64.8 ± 5.2 and 63.5 ± 6.0 % change in FIHP, mean ± SE). **Discussion:** Differences in MIP-1 alpha and PlGF release provide evidence for metabolic separation of the adapted systems, caused by a changed oxygen environment. Our failure to observe differences in EPO, VEGF and sVEGFR-1 release may be explained by longer lag-times for up regulation of their gene expression. VEGF associated endothelial signalling appears to be unaffected by 'hypoxia' in the placenta over the time course studied here.

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Placental Expression of Angiotensin 3-8 (Ang IV) in Normal Pregnancy (NP) and Pre-Eclampsia (PE). Paula J Williams, Hiten D Mistry, Fiona Broughton Pipkin. *CREL/Division of Obstetrics & Gynaecology, University of Nottingham, Nottingham, United Kingdom.*

Background: There is a placental renin-angiotensin system (RAS) from very early pregnancy. Ang IV mediates various effects by binding to its specific receptor, the AT4R, the active site of which is an insulin-regulated aminopeptidase (IRAP). There is AT4R expression in both endothelial and smooth muscle cells. Ang IV at low concentrations is vasodilatory, increasing blood flow via the AT4Rs; it also stimulates NF-kappa beta and modulates GLUT-4. To date AT4R expression has not been investigated in the placenta. We propose that AT4R plays a part in the placental vascular development necessary for successful pregnancy, and that reduced AT4R expression may be associated with inadequate vascular adaptation contributing to pre-eclampsia (PE).

Aim: To identify and locate AT4R expression in both NP and PE placentae.

Methods: The study had Hospital Ethical approval; written informed consent was obtained from all women. Placental samples were obtained from 4 NP and 4 PE at delivery (Gestational ages: 39 and 38 weeks respectively). Samples were taken from 3 areas, near cord, middle and outer edge of the placenta. Paraffin embedded sections were immunostained for IRAP reactivity using a rabbit polyclonal antibody (gift from Professor David James, Garvan Institute, Australia). Immunoreactivity of trophoblast and uterine cell populations was assessed using a semi-quantitative grading system. Grade 0 = no positive labelling, 1 = 1 – 25%, 2 = 26 – 49%, 3 = 50 – 74% and 4 = 75 – 100% of cells positively labelled. Median (max, min) are shown.

Results: 1) AT4R immunostaining was prominent in the syncytiotrophoblast and Hofbauer cells of all placental villi examined, with no differences in expression between sampling sites. **2)** AT4R positivity was reduced in near cord PE samples (2.2 (2.4,1.2)) compared to NP (3.3 (4.0,2.5) P=0.029).

Conclusion: We have shown for the first time, dense AT4Rs in syncytiotrophoblast and Hofbauer cells in NP placentae, and their down-regulation in PE. Reduced Ang IV/AT4R binding may contribute to increased placental vasoconstriction resulting in increased ischemia/reperfusion. This in turn may stimulate xanthine oxidase, which is itself stimulated by AngII, leading to increased superoxide production. Further work is needed to clearly define the role of this newly identified component of the renin-angiotensin system in normal pregnancy and PE.

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Pre-Eclampsia Is Associated with Lower Percentages of Regulatory T Cells in Maternal Blood. JR Prins,[1] HM Boelens,[1] JJ HM Erwich,[1] J Heimweg,[2] S van der Heide,[2] AJM Van Oosterhout,[2] AEJ Dubois,[3] JG Aarnoudse.[1] *[1]Obstetrics, University Medical Center Groningen, Groningen, Netherlands; [2]Laboratory of Allergology and Pulmonary Disease, University Medical Center Groningen; [3]Pediatric Allergology, Beatrix Children's Clinic, University Medical Center Groningen.*

Pre-eclampsia is a serious disease of human pregnancy and immunological mechanisms play a role in its pathophysiology. Normal pregnancy is associated with an increase in regulatory T (Treg) cells and with a predominant Th2 immune profile. Treg cells are a subpopulation of CD4+ lymphocytes and are

specifically characterized by the lineage specific transcription factor FOXP3. Treg cells seem to induce immunological changes that have a protective role in maintaining normal pregnancy. We hypothesised that percentages of Treg cells are decreased in pregnancies complicated by pre-eclampsia.
Methods
In total, 18 women with pregnancies complicated by pre-eclampsia and 26 healthy pregnant controls were enrolled. To obtain control umbilical cord blood as well, control group I consisted of eighteen healthy pregnant women at term. In addition, since 13 women with pre-eclampsia delivered preterm, control group II (peripheral blood only) consisted of women during normal pregnancy with a gestational age matched for the preterm pre-eclamptic group. Treg cells were measured from whole blood using four-color flow-cytometry.
Results
Women with a pregnancy complicated by pre-eclampsia had a significantly lower percentage of CD4+ FOXP3+ Treg cells (4.5 vs 8.8%; $p<0.05$). In the pre-term group the pregnancies complicated by pre-eclampsia showed a significantly lower percentage of $CD4^+$ $FOXP3^+$ cells in the peripheral blood as compared to the healthy pregnant controls. At term this percentage was also lower but not significantly so. Between pre-term and term pregnancies both complicated by pre-eclampsia no significant difference was found in the percentage of $CD4^+$ $FOXP3^+$ Treg cells. No difference was found in umbilical cord blood (7.2 vs 7.9%).
Conclusions
Our data suggest that pre-eclampsia is associated with a diminished percentage of Treg cells in peripheral blood. We conclude that a deficiency of regulatory T cells may play a role in the pathophysiology of pre-eclampsia.

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Tetrahydrobiopterin (BH4), a Cofactor for eNOS Activity, Decreases Hypertension, and Improves Fetal Weights, in Spontaneous Hypertensive Pregnant Rats. Pandu RR Gangula,[1] Shao Q Shi,[2] Lynette B MacKay,[2] Robert E Garfield.[2] *[1]Department of Internal Medicine, Division of Gastroenterology and Hepatology, The University of Texas Medical Branch, Galveston, TX, USA; [2]Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, TX, USA.*
Background: Preeclampsia and eclampsia are significant causes of maternal and fetal death. However, the pathophysiology of these conditions is unclear. We and others have reported that inhibition of endogenous nitric oxide (NO) synthesis produces symptoms similar to preeclampsia in pregnant rats. Several studies demonstrate that fetoplacental weights are altered in pregnancies of spontaneous hypertensive (SHR) rats. In addition, impaired synthesis of tetrahydrobiopterin (BH4), a major co-factor for endothelial nitric oxide synthase (eNOS) activity and enhanced expression of eNOS has been observed in the pathogenesis of hypertension. In the current study, we examined whether supplementation of BH4 and sepiapterin (SEP, a precursor for BH4 biosynthesis in the salvage pathway) reduces increased blood pressure and improves fetoplacental weights in SHR pregnant rats.
Methods: Groups (4-6) of SHR pregnant rats were either treated with BH4, SEP (20 mg/k.g body weight/day/rat, oral tablets) or vehicle (normal diet) beginning from day 12 of pregnancy until day 20 of gestation. Animals were sacrificed on day 20 of gestation and fetoplacental weights were recorded immediately. Western blot analysis was performed to determine vascular eNOS expression (eNOS/GAPDH).
Results: Significant ($p<0.05$) elevations in blood pressure (BP, mmHg) were observed in SHR (SHR, 141±1.42 vs. WKY, 106 ± 3.04 mmHg) compared to Wistar-Kyoto (WKY) group. Supplementation of either BH4 or SEP (128±2.19), significantly ($p<0.05$) reduced elevated BP beginning from day 14 of pregnancy. Fetal but not placental weights were significantly ($p<0.05$) reduced in SHR (2.39±0.03 grams) compared to WKY (3.10±0.04) rats. BH4 (2.51±0.04) treatment partially ($p<0.05$) increased fetal weights compared to the SHR group. Vascular eNOS expression is significantly ($p<0.05$) elevated in SHR (1.0±0.07) compared to WKY rats (0.56±0.15). Further, treatment with BH4 (0.58±0.1) but not SEP (0.92±0.1) significantly ($p<0.05$) reduced elevated eNOS protein expression in SHR rats.
Conclusions: BH4 may be beneficial treatment of preeclampsia to reduce blood pressure and improve fetal perfusion to increase fetal weights.

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Genetic Risk Factor for Severe Preeclampsia: Significance of Endothelial Nitric Oxide Synthase Gene T-786—>C and Missense Glu298Asp Variants. Toshihiro Yoshimura,[1] Michihiro Yoshimura,[2] Masafumi Nakayama.[3] *[1]Obstetrics and Gynecology, Kumamoto University School of Medicine, Kumamoto, Japan; [2]Cardiovascular Medicine, Jikei University School of Medicine, Tokyo, Japan; [3]Cardiovascular Medicine, Kumamoto University School of Medicine, Kumamoto, Japan.*
Introduction: We recently identified two endothelial nitric oxide synthase (eNOS) gene polymorphisms, a Glu298Asp missense variant in exon 7 and a T-786-->C variant in the 5'-franking region, which are associated with coronary spasm and myocardial infarction in Japanese population. And we also identified a missense Glu298Asp variant is associated with severe preeclampsia and placental abruption. Our objective was to analyze the association between the T-786-->C and severe preeclampsia.
Materials and Methods: The study participants included 62 patients with histories of severe preeclampsia. This is a preliminary study, therefore, the comparisons were made with the 345 general normal population.
Results: The analyses revealed that the frequency of the missense Glu298Asp variant (n=18/62, 29%) was significantly higher than the general population (n=44/345, 13 %), as we previously published. However, the frequency of the T-786-->C variant (n=9/62, 14%) was not different from the general population (n=61/345, 18 %). Interestingly, only one patient had both T-786-->C and missense Glu298Asp variants, and she developed placental abruption.
Conclusion: Although our sample size is small, it is very unlikely that the T-786-->C variant is associated with severe preeclampsia. The T-786-->C variant may not be a genetic susceptibility factor to severe preeclampsia. The T-786-->C may have some reproductive significance in combination with missense Glu298Asp variant, however huge number of patients would be needed to analyze such rare (2.5%) combination of the variance.

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The Association between the Development of Preeclampsia and Methylenetetrahydrofolate Reductase, Angiotensinogen, Vascular Endothelial Growth Factor Single Nucleotide Polymorphism Genotype Combinations. Hyun Soo Park,[1] Jong Kwan Jun,[2] Chan-Wook Park,[2] Joong Shin Park,[2] Bo Hyun Yoon,[2] Hee Chul Syn.[2] *[1]Obstetrics and Gynecology, Dongguk University International Hospital, Goyang-si, Gyeonggi-do, Republic of Korea; [2]Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea.*
Objective This study was conducted to investigate if there exists any genotype combination of multiple single nucleotide polymorphism (SNP)s which is frequently found in preeclampsia patients.
Study Design One hundred sixty two preeclampsia patients and 199 normotensive pregnant women were included in this study between Jan 1998 and Jul 2004. Diagnosis of preeclampsia and assignment of severity were made according to the criteria by National High Blood Pressure Education Working Group and American College of Obstetricians and Gynecologists. The patients were reclassified as early (30 weeks or before) and late-onset (31 weeks or beyond) disease. Genotypes were measured with PCR-RFLP for Methylenetetrahydrofolate reductase (MTHFR) C677T, Angiotensinogen (AGT) M235T, Vascular endothelial growth factor (VEGF) C936T with the DNA extracted from maternal blood. Case-control study for each SNP was done and the frequencies of genotype combination were compared. ANOVA, t-test, chi-square test, Fisher's exact test and logistic regression analysis were used for statistical analysis. A p value of <0.05 was considered statistically significant.
Results Genotypes of MTHFR polymorphism showed significant difference between late onset preeclampsia and control (CC+CT/TT, OR: 1.82, $p<0.05$) but AGT and VEGF polymorphism did not show statistical difference between any case-control combination. Only 20 out of possible 27 genotype combinations were found and there was no statistical difference in the frequencies of genotype combination between case and control group.
Conclusion MTHFR polymorphism might be associated with the development of preeclampsia, but there was no combination of MTHFR, AGT and VEGF polymorphisms which is associated with the development of preeclampsia.

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Enhanced Placental Villus Core IL-1β in Preeclampsia (PE) and Intrauterine Growth Restriction (IUGR): Evidence for Cell Type Specific-Inflammatory Response. Seth Guller, Irina A Buhimschi, Catalin S Buhimschi, Yula Y Ma. *OB/GYN and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Introduction: Although the pathogenesis of severe preeclampsia (PE) and intrauterine growth restriction (IUGR) is poorly defined, inadequate remodeling of uterine spiral arties may promote reperfusion injury leading to focal infarction and reduced nutrient flow between mother and fetus. Our goal was to determine whether an intravillous inflammatory cytokine cascade was associated with PE and IUGR.

Methods: Immunohistochemistry (IHC) examined IL-1β and IL-1α expression in 3 preterm placentas with severe PE+IUGR, and 3 idiopathic preterm controls (PTC) with no evidence of clinical or histological chorioamnionitis. Cultures of fibroblasts (FIBs) and syncytiotrophoblasts (SCTs) from term placentas (n=5) were treated with IL-1β and cytokine levels in conditioned media were determined using ELISA or multiplex array. Results were analyzed by Student's t test or ANOVA.

Results: The gestational age at delivery was not significantly different in PE+IUGR and PTC groups (28.3±2.2 vs 27.6±2.8 wks). IHC of PE+IUGR samples revealed intense villus core staining of IL-1β adjacent to infarcts. Villi distal to infarcts stained with a lower intensity. In contrast, staining was virtually undetectable in PTC. The pattern of IL-1α staining was nearly identical in PE+IUGR and PTC groups, was localized to the syncytium, and did not differ with respect to distance from infarct. To identify cellular targets of IL-1β action, primary cultures of FIBs and SCTs were incubated for 48 h in serum-free medium ± 1 ng/ml IL-1β. By ELISA we observed that IL-1β treatment of FIBs increased IL-8 levels from 4±1 to 2161±346 pg/μg protein ($P<0.001$). Similarly, multiplex array revealed that levels of IL-4, IL-6, and IL-11 were induced 24-, 269-, and 12-fold, respectively in FIBs. The presence of 200 ng/ml IL-1 receptor antagonist reduced the IL-1β-mediated increase in IL-8 levels 95±3% ($P<0.001$), indicating that this response was mediated through IL-1 receptor. In marked contrast, IL-1β treatment did not significantly affect levels of IL-4, IL-6, IL-8 or IL-11 in SCTs, indicating that this response was cell type-specific.

Conclusions: These results indicate that increased expression of IL-1β in the placental villus core in PE and IUGR may promote an inflammatory cascade in FIBs at this site leading to focal infarction and reduced flow of nutrients to the fetus.

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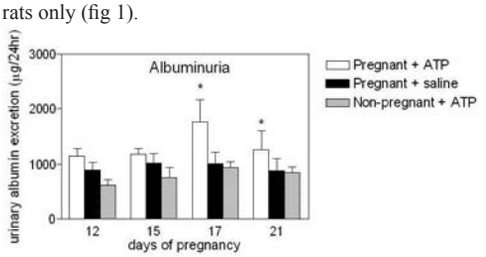
Infusion of Extra Cellular ATP Is Toxic Exclusively in the Pregnant Condition. Marijke M Faas,[1] Jan G Aarnoudse,[2] Gerda van der Schaaf,[1] Rianne M Jongman,[3] Theo Borghuis,[3] Winston W Bakker.[3] *[1]Division of Medical Biology, Dept of Pathol &Lab Medicine; [2]Dept of Obstetrics and Gynecology; [3]Dept of Pathol & Lab Medicine, University Medical Center Groningen, Groningen, Netherlands.*

Introduction: In human pregnancy, decreased responsiveness to angiotensin II (angII) starts in week 10, promoting an expanded vascular bed. At the same time levels of the vasodilatory hemopexin increases[1]. During pre-eclampsia the vascular bed is contracted and the responsiveness upon angII persists. This may be due to the increased levels of extra cellular ATP, a natural inhibitor of hemopexin[1]. In the present study we tested whether extra cellular ATP is toxic in the pregnant condition.

Methods: Pregnant rats were infused with either ATP (750 μg/kg bw; n=9) or saline (n=5) on day 14 of pregnancy (permanent jugular vein cannula/1 hr infusion). Non-pregnant rats (n=8) were infused with ATP identically. One day before and 1, 3, 6 days after infusion, 24 hr urine and blood samples (WBC count and hemopexin activity) were collected. Seven days after the infusion, rats were sacrificed and kidney tissue processed for immunohistology.

Results: Urinary albumin excretion was increased in pregnant ATP infused rats only (fig 1).



WBC were also increased only in pregnant rats infused with ATP (days 15 and 17 vs pre-infusion value). In pregnant ATP infused rats intraglomerular influx of monocytes was increased, which correlated with urinary albumin excretion on the same day ($R^2 = 0.66$). Staining of glomeruli for the angII receptor (AT-1R) showed decreased AT-1R expression in control pregnant rats as compared to non-pregnant rats, while AT-1R expression in ATP infused pregnant rats was increased as compared to control pregnant rats. Hemopexin activity was increased on days 17 and 21 in control pregnant rats as compared to all other groups.

Discussion: These data support the notion that ATP is toxic exclusively in the pregnant condition. It may be suggested that ATP induced an inflammatory response, although the exact mechanism by which ATP induced its effects needs further investigation.

(1): W.W. Bakker et al. Hypert Preg 2007;26:227-239.

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Placental Expression of HGF, c-Met and TGF-β1 in Pregnancies Complicated by Preeclampsia. Elena Vasario,[1] Simona Cardaropoli,[1] Irene Benzonelli,[1] Paolo Accornero,[2] Tullia Todros.[1] *[1]Department of Obstetrics and Gynaecology, University of Turin, Turin, Italy; [2]Department of Veterinary Morphophysiology, University of Turin, Turin, Italy.*

Background: Hepatocyte Growth Factor (HGF) is a multifunctional cytokine that is known to promote division, motility, invasion and morphogenesis of a wide range of cell types and to inhibit apoptosis. The effects of HGF are mediated through its interaction with the tyrosine kinase receptor c-Met. In pregnancy HGF/Met system is involved in the physiologic growth and development of the feto-placental unit. HGF/Met effects are counteracted by Transforming Growth Factor-β1 (TGF-β1), that is known to promote progressive fibrosis in human tissues. Moreover TGF-β1 plays a major role in trophoblast growth and differentiation. TGF-β1 inhibits HGF expression as well as HGF inhibits TGF-β1 expression. An understanding of the mechanisms regulating placental balance of these growth factors may provide insights into the processes that occur in complications of pregnancy, such as preeclampsia (PE) and fetal growth restriction (FGR). **Objective:** To verify the hypothesis that HGF, c-Met and TGF-β1 are differently expressed in tissues of normal and PE placentas. **Methods:** We studied 13 placentas from pregnancies complicated by PE (7 with normal fetal growth and 6 with FGR) and 5 placentas from normal pregnancies. From each placenta random samples were excised and RNA was extracted; quantitative real-time RT-PCR analysis was used to investigate mRNA expression of HGF, c-Met and TGF-β1 in PE (with or without FGR) and in normal placentas. **Results:** Gestational age, neonatal weight and placental weight were, as expected, lower in PE groups. The mRNA expressions of the three molecules are higher in PE than in normal placentas, but the difference is statistically significant only for c-Met. The higher values are mainly due to the increase in the PE group without FGR for c-Met and TGF-β1 and the increase in the PE-FGR group for HGF. **Conclusion:** Increased levels of expression of HGF/Met system in pathological placentas could be explained as an attempt to repair placental damages; nevertheless placental regeneration could be inhibited by the increase of TGF-β1, that promote fibrosis. Placental expression of HGF, c-Met and TGF-β1 is increased in all PE placentas, but particularly in placentas of PE with normal fetal growth, where placental tissue is probably less compromised.

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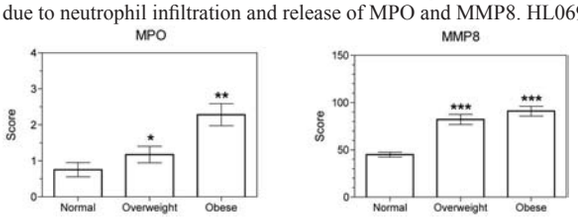
Neutrophil Release of Myeloperoxidase and Matrix Metalloproteinase 8 in Systemic Vasculature of Obese Women May Put Them at Risk for Preeclampsia. Juhi Shukla, Sonya Washington, Scott W Walsh. *Obstetrics and Gynecology, Virginia Commonwealth University, Richmond, VA, USA.*

Obesity is a risk factor for preeclampsia (PE), but the reason for this risk is unknown. Previously, we found that neutrophils infiltrated into the vasculature of PE women released myeloperoxidase (MPO) and matrix metalloproteinase 8 (MMP8), products that can cause oxidative stress and vascular dysfunction. If neutrophils infiltrate the vasculature of obese women and release MPO and/or MMP8, this may help explain why they are at risk of developing PE. HYPOTHESIS: Systemic vascular tissue of obese women will have a significant presence of MPO and MMP8 as a result of neutrophil infiltration. METHODS: Subcutaneous fat, which is highly vascularized, was obtained at abdominal surgery from 5 normal weight, 5 overweight and 5 obese women. Formalin fixed, paraffin embedded 8 μm sections of fat biopsies were stained using immunohistochemistry with specific antibodies for MPO and MMP8. Data were evaluated for intensity of vessel staining by visual score (0-4), density of staining using image analysis software, and % vessels with neutrophil staining,

diffuse staining and vascular smooth muscle (VSM) staining. Resistance-sized vessels (10-200 μm) were evaluated. RESULTS: For MPO, the intensity of staining (Fig) and the % vessels with neutrophil, diffuse and VSM staining was significantly greater for obese than for overweight or normal weight patients: % diffuse staining (64.6 ± 4.2 vs. 40.4 ± 3.9 vs. 21.8 ± 4.7, P<0.001); % VSM staining (30.2 ± 6.1 vs. 20.0 ± 5.3 vs. 4.0 ± 2.6, P<0.01). For MMP8, obese and overweight patients had a greater (P<0.001) % vessels with neutrophil, diffuse and VSM staining than normal weight patients: % diffuse staining (63.0 ± 5.5 vs. 55.2 ± 4.3 vs. 26.0 ± 3.0); % VSM staining (36.8 ± 2.9 vs. 27.4 ± 3.1 vs. 2.8 ± 1.2). CONCLUSIONS: Neutrophils infiltrate the systemic vasculature of obese women and release MPO and MMP8. SPECULATION: Obesity may put women at risk for PE because their vasculature may already be dysfunctional due to neutrophil infiltration and release of MPO and MMP8. HL069851.



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Neutrophils Alter Expression of Vascular Smooth Muscle Collagen Regulating Genes. Guadalupe Estrada,[1,2] Renato Cappello,[1] Jerome F Strauss III,[1] Scott W Walsh.[1] *[1]OB/GYN, Virginia Commonwealth University, Richmond, VA, USA; [2]Direccion de Investigacion, Instituto Nacional de Perinatologia, Mexico City, DF, Mexico.*

Collagen is an important protein that maintains the structural integrity of tissues. Disruption of vascular smooth muscle collagen could result in vascular dysfunction in women with preeclampsia. Recently, neutrophil infiltration of the systemic vasculature was demonstrated in preeclamptic women. Neutrophils produce inflammatory mediators, such as reactive oxygen species (ROS) and TNF-α. We hypothesized that neutrophils, ROS and TNF-α would alter expression of collagen regulating genes. **METHODS:** Primary cultures of human vascular smooth muscle cells (VSMC) were seeded into T-25 flasks (40,000 cells/flask) and grown for 3 days to 70% confluence. The cells were treated for 24 hours with medium control, ROS (HX, 0.05 mM + XO 0.003 U/ml), TNF-α (1 ng/ml); and neutrophils (60,000) activated with arachidonic acid, 50 μM, (1:16 ratio of neutrophils to VSMC). RNA was extracted from cell homogenates and analyzed for gene expression with an RT2 Profiler PCR Array System for Human Extracellular Matrix Genes (SuperArray). To determine the fold-change of gene expression, the results were first normalized to a housekeeping gene and then ΔΔCt was calculated across two RT-PCR arrays where group 1 was the control and group 2 was the experimental treatment. **RESULTS:** Table 1. **CONCLUSIONS:** Neutrophils, ROS and TNFα increased MMP1 expression. Interestingly, genes involved in collagen synthesis (COL1A1) or inhibition of MMP-1 activity (TIMP1) were either not affected or down-regulated. These data suggest that neutrophil infiltration in preeclamptic women could cause vascular dysfunction by creating an imbalance between collagen synthesis and collagen breakdown favoring breakdown. HL069851, Fogarty 5D43TW007692, P60MD002256.

Table 1. Fold Change in Gene Expression

	MMP1	COL1A1	TIMP1
Neutrophils	4.84	-2.15	-3.47
ROS	25.28	-2.66	1.10
TNF-α	13.70	-185	-6.84

MMP1, matrix metalloproteinase-1; COL1A1, collagen, type1, alpha 1; TIMP1, tissue inhibitor of matrix metalloproteinase-1

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The C5B-9 Membrane Attack Complex of the Complement Cascade Coats Microparticles and Cell Debris from Hypoxic Human Trophoblast. Roxane M Rampersad, Aaron Barton, Yoel Sadovsky, D Michael Nelson. *Obstetrics and Gynecology, Washington University, Saint Louis, MO, USA.*

OBJECTIVE: Hypoxia increases membrane attack complex (MAC) binding to cultured human trophoblasts, and MAC enhances apoptosis in trophoblasts exposed to low compared to normal FiO_2. Trophoblast microparticles and cellular fragments released into the maternal circulation *in vivo* may contribute to the systemic pathophysiology of preeclampsia. We tested the hypothesis that hypoxia induced MAC deposition on cultured human trophoblasts yields microparticles and fragments coated with MAC.

STUDY DESIGN: Primary cytotrophoblasts from term human placentas (n=4) were cultured 24 h in 1% and 20% oxygen in DMEM with 10% human serum with active MAC or heat inactivated serum (control). Media were centrifuged to obtain pellets of microparticles and cell debris which were immunostained for MAC or exposed 6-12 h to confluent, phorbol myristate acetate differentiated U937 macrophages. The percentage of macrophages that ingested trophoblast debris was quantified by counting the number of macrophages with immunofluorescence for trophoblast cytokeratin 7 filament staining, as assessed by confocal microscopy.

RESULTS: Cultures exposed to normal human serum, but not heat inactivated control serum, showed MAC immunofluorescence on microparticles and fragments in medium, with the highest level of MAC in cultures exposed to extreme hypoxia. The maximal percentage of macrophages that ingested the trophoblast debris coated with MAC from cultures with 1% oxygen was 56.2%, not different from the 52.6% from cultures exposed a 1% FiO_2 and control serum.

CONCLUSION: Trophoblasts exposed to hypoxia and active complement release microparticles and cellular fragments coated with MAC into the extracellular environment. MAC coating does not influence phagocytic removal of the debris by macrophages suggesting that placental derived, membrane bound MAC could circulate to yield systemic affects on maternal endothelium. Supported by NIH HD29190 and HD045675.

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Is There a Role for Fatty Acids in the Pathogenesis of Pre-Eclampsia? Nicola J Robinson,[1] Laura J Minchell,[1] Jenny E Myers,[1] Philip N Baker,[1] Carl A Hubel,[2] Ian P Crocker.[1] *[1]Maternal and Fetal Health Research Group, The University of Manchester; [2]Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh.*

Objectives: Women with pre-eclampsia (PE) display altered lipid metabolism as characterized by elevated circulating triglycerides and non-esterified fatty acids (NEFA) and these changes are evident before the disease is clinically apparent. We have tested the hypothesis that the increased circulating levels of NEFA contribute to endothelial dysfunction in PE.

Methods: Human umbilical vein endothelial cells were incubated for 24h with pooled plasma (2%) from normal or PE pregnancies, or with palmitic, oleic and linoleic acid in culture media at the concentrations and molar ratios to albumin identified in normal (100, 113, 37μM, ratio 0.9) and PE pregnancies (140, 172, 53μM, ratio 1.6)[1,2]. Lipid droplet accumulation was determined using an oil red O absorbance assay. Endothelial metabolism was measured using the MTT test and mitochondrial membrane potential determined by JC-1 assay as a marker of early apoptosis.

Results: Plasma from PE pregnancies increased endothelial cell lipid droplet accumulation compared to normal plasma (P<0.01, Wilcoxon signed ranks, n=9). This change was replicated following exposure to NEFA at the combined concentrations found in PE compared to normal pregnant controls (P<0.05, n=7). Plasma from women with PE caused a significant decrease in mitochondrial dehydrogenase activity (MTT test; P<0.01, n=8) and a reduction in JC-1 fluorescence (P<0.05, n=7), compared to normal plasma, suggestive of mitochondrial membrane depolarization and increased cellular apoptosis. Again these effects were replicated using NEFA in culture medium at the levels found in PE compared to normal pregnancies (MTT test: P<0.05, n=9; JC-1 assay: P<0.05, n=10).

Conclusions: In endothelial cell cultures, plasma from women with PE caused increased lipid droplet accumulation, decreased cellular metabolism and increased apoptosis. These changes to cellular function were mirrored using NEFA in culture medium at the concentrations and molar ratios to albumin previously reported in PE. These findings provide evidence that the changes in endothelial cell function induced by plasma from women with PE may be due to the increased NEFA circulating levels and that increased palmitic, oleic and linoleic acid, in combination, could play a role in pathogenesis of PE.

[1]Lorentzen (1995) BJOG; [2]Endresen (1992) AJOG.

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Active Soluble Factors from Pre-Eclamptic Placental Villous Explants Induce Changes in Endothelial Cell Function. Nicola J Robinson, Elizabeth E Hutchinson, John D Aplin, Philip N Baker, Ian P Crocker. *Maternal and Fetal Health Research Group, The University of Manchester.*

Background: Skewing of the maternal endothelial phenotype in pre-eclampsia (PE) is attributed to the release of unknown factors from a hypoperfused placenta. We hypothesise that factors secreted from PE placental tissue will impair endothelial cell function. We have tested the effect of soluble factors

liberated from serum-free placental villous explant cultures from normal and PE pregnancies on human endothelial cells, and identified candidate mediators of their differential effects on metabolism and behaviour.
Methods: Term placental villous tissue from normal (n=9) or PE (n=10) pregnancies were explanted for 4 days at 6% oxygen. Conditioned 24h medium (day 3-4) was applied to human umbilical vein endothelial cells (HUVECs). An angiogenesis assay was conducted in which tubule length and number were measured by morphometric imaging following seeding on 80% Matrigel. The effect of explant conditioned medium on endothelial cell metabolism was determined by MTT and bioluminescent ATP assay. The release of vasoactive metabolites (nitrite, endothelin-1, prostacyclin) from HUVECS exposed to this medium was also measured. Finally, a Luminex bead array was used to screen the explant media for a panel of 15 chemokines/cytokines.
Results: In the angiogenesis assay, tubule length ($P<0.05$, Mann-Whitney U test) and number ($P<0.05$) were significantly decreased in PE compared to normal pregnancy medium. There was no change in MTT reduction, but endothelial cellular ATP levels were also significantly reduced following exposure to PE explant medium ($P<0.05$). Both normal and PE-derived medium stimulated HUVECs to produce vasoactive metabolites. The following cytokines were detectable in the explant media: interleukin (IL)-6, IL-8, Gro-α, monocyte chemotactic protein-1 and macrophage inflammatory protein-1β (MIP-1β). Higher levels of both IL-8 and Gro-α were present in the normal medium compared to PE (both $P<0.0001$). MIP-1β was present in PE conditioned media but undetectable in media generated from normal placental explants.
Conclusions: These results suggest that pre-eclampsia stimulates the release of soluble factors from the placenta which have adverse effects on endothelial cell angiogenic potential and metabolism. Altered levels of several cytokines were detected in the explant medium, and the effects of these on endothelial cell function are currently being addressed.

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Placenta-Derived Chymotrypsin-Like Protease (CLP) Disturbs Endothelial Junctional Structure: Potential Role of Placental CLP in Preeclampsia. Yang Gu, Davis F Lewis, Yuping Wang. *Obstetrics and Gynecology, LSUHSC-Shreveport, Shreveport, LA, USA.*
Objective: Placentas from women with preeclampsia (PE) release more chymotrypsin-like protease (CLP). The purpose of this study was to determine if placenta-derived CLP was responsible for altering endothelial (EC) barrier function in PE.
Approaches: Endothelial junctional protein complex (β-catenin/VE-cadherin/p120) expression and junctional protein VE-cadherin distribution were examined in ECs treated with PE placental conditioned medium.
Methods: 1) Confluent ECs were treated with PE placental conditioned medium (CM). The association of EC junctional protein complex VE-cadherin/β-catenin/p120 was examined by a combined immuno-precipitation (IP) and immuno-blotting (IB) assay, in which total cellular protein was immuno-precipitated with monoclonal antibody against β-catenin and then immuno-blotted with antibodies against VE-cadherin or p-120. 2) Confluent ECs grown on cover slips were exposed to PE placental CM with or without depletion of chymotrypsin. EC junction protein VE-cadherin distribution was examined by fluorescent microscopy.
Results: 1) VE-cadherin and p120 are expressed in control ECs but not in ECs exposed to PE CM, which indicate that the junctional protein complex VE-cadherin/β-catenin/ p120 is lost in ECs exposed to PE CM. 2) ECs exposed to PE placental CM showed a discontinuous distribution and reduced expression for VE-cadherin at cell contact areas. The zipper like structure was lost and cleft was formed at cell-cell contacts. These observations indicate that the homotypic cell-cell adhesion and junction protein intracellular partner complex are disrupted. These disruptive phenomena in cells treated with PE conditioned medium were not present in control cells and in cells treated with CM after depletion of chymotrypsin.
Conclusion: CLP released by the placenta could be a candidate agent that is responsible for disrupting EC integrity and inducing endothelial permeability in PE. (Supported by NIH grants HL65997 and HD36822).

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Inflammation in the Uterus Induces pERK and Substance P Immunoreactivity in DRG Neurons Innervating Both Uterus and Colon in Rats. Jichang Li,[1] Paul Micevych,[2] John McDonald,[1,4] Victor Chaban,[3] Andrea Rapkin.[4] *[1]Anesthesiology, Harbor-UCLA Medical Center, Torrence, CA, USA; [2]Neurobiology, UCLA, Los Angeles, CA, USA; [3]Biomedical Sciences, Charles Drew University, Los Angeles, CA, USA; [4]Obstetrics and Gynecology, UCLA, Los Angeles, CA, USA.*
INTRODUCTION: Chronic pelvic pain (CPP) syndromes such as endometriosis, irritable bowel syndrome, and interstitial cystitis are associated with visceral hyperalgesia, and often coexist in the same patient. One possible explanation for this phenomenon is viscero-visceral cross-sensitization in which increased nociceptive input from an inflamed pelvic organ sensitizes neurons that receive convergent input from an unaffected organ to the same dorsal root ganglion (DRG). Nociception induces upregulation of pERK and substance P. The purpose of this study was to determine, in a rodent model, whether uterine inflammation increased the number of pERK- and SP-positive neurons in sensory ganglia innervating both uterus and colon.
METHODS: Colonic and uterine DRGs were retrogradely labeled with fluorescent tracer dyes micro-injected into the colon and uterus. Ganglia were harvested, cryoprotected and cut for fluorescent microscopy.
RESULTS:Approximately 7% neurons were colon-specific and 11% were uterus-specific. Among these uterus- or colon- specific neurons, up to 5% of labeled DRG neurons in the L1-S3 levels innervated both visceral organs. Uterine inflammation increased the number of pERK and SP-immunoreactive neurons in DRG neurons innervating colon, uterus, and those innervating both organs. Furthermore, this effect was specific as non-retrograde labeled DRG neurons did not manifest a significant increase in pERK or SP immunoreactive cells.
CONCLUSIONS: Localized uterine inflammation leads to increased expression of SP and pERK in uterine afferents as well as dichotomizing afferents innervating both uterus and colon, suggesting that viscero-visceral convergence is present at the level of DRG primary afferent cell bodies. This visceral sensory integration may underlie the co-morbidity of female pelvic pain disorders and may provide basic information regarding the etiology of CPP syndromes.

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Autologous Myoblast Injection for Fecal Incontinence Due to Obstetric Trauma – A Pilot Study. Andrea Frudinger,[1] Wolfgang Schwaiger,[3] Johann Pfeifer,[2] Johannes Paede,[4] Dieter Koelle.[3] *[1]Obstetrics & Gynecology, Medical University of Graz, Graz, Styria, Austria; [2]General Surgery, Medical University of Graz, Graz, Styria, Austria; [3]Obstetrics & Gynecology, District Hospital, Schwaz, Tyrolia, Austria; [4]BK Medical, Sandoften, Denmark.*
Purpose: Success rates of medical and surgical modalities for the treatment of severe fecal incontinence due to obstetric trauma are modest. We tested whether the intrasphincteric injection of autologous myoblasts is clinically safe and feasible for postobstetric fecal incontinence.
Methods: Using ultrasound guidance a suspension of autologous myoblasts was injected in the anal sphincter muscle complex in three women with severe postobstetric fecal incontinence. Main outcome measures were safety, feasibility and the Wexner Grading Score. Secondary outcome measures were incontinence episodes, Rockwood Fecal Incontinenece Quality of Life Scale, external anal sphincter morphology and anal pressure values.
Results: All procurement procedures and injections were performed without complications; there were no clinically or laboratory signs of infection or inflammation. Three months post myoblast injection, Wexner Continence Grading scores and Rockwood Incontinence scales were markedly improved and incontinence episodes reduced in all three patients. No change could be observed in sonographic anal sphincter morphology. Mean anal squeeze pressure improved.
Conclusion: Autologous myoblast injection for fecal incontinence is clinically feasible and safe; further studies will evaluate the efficacy of this approach.

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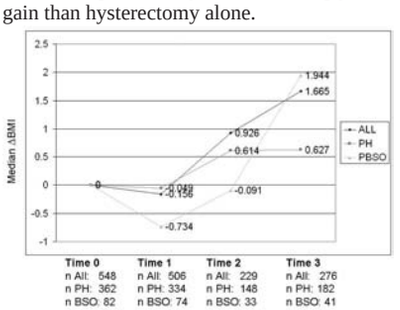
Change in Patient BMI after Hysterectomy. Paul Brezina,[1] C Sage Claydon,[1] Daniel Brezina,[1] Keith H Nelson,[1] Todd Beste,[2] Joshua Woelk,[1] Charles Hodson.[1] *[1]Department of OB/GYN, East Carolina University, Greenville, NC, USA; [2]Department of OB/GYN, Coastal AHEC, Wilmington, NC.*
Objective: To evaluate whether hysterectomy is associated with a change in postoperative BMI. **Methods:** A retrospective cohort study was conducted of 548 hysterectomies performed for benign indications from June 2001 to June 2006. Institutional Review Board approval was obtained. The data were collected by a medical student blinded to the hypothesis. Basic demographic data were recorded. Body Mass Index (BMI) was determined at 4 time points: Time 0 (immediately postoperatively), Time 1 (2 weeks to 5 months postoperatively), Time 2 (5 months to 1 year postoperatively) and Time 3 (1 to 2.5 years postoperatively). One-way ANOVA was performed using NCSS statistical software. A Bonferroni Multiple Comparison Test ($p<0.05$) was used to compare median changes in BMI from baseline. The starting BMI served as the control group. **Results:** There was a statistically significant increase in BMI at Time 3 compared to baseline in all women (31.9 to 33.6). When sorted

by menopausal status, there was a significant increase in BMI in pre- but not in postmenopausal women. In postmenopausal women there was insufficient power to note a statistically significant change in BMI. Results are summarized with the follow-up for each group represented by "n" in the graph below. Premenopausal patients were divided into hysterectomy with oophorectomy (PBSO) versus hysterectomy alone (PH). The PH group showed an increase in BMI that plateaus at Time 3. In the PBSO group the BMI continued to increase over time. Subgroup analysis comparing PH to PBSO demonstrates initial weight loss in PBSO but a significant increase in BMI from baseline at Time 3 compared to PH. **Conclusions:** Hysterectomy appears to be associated with an increase in BMI over time. Subgroup analysis suggests that, in premenopausal women, oophorectomy is more strongly associated with continuing weight gain than hysterectomy alone.



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Waist Circumference, Not BMI, Is a Positive Predictive Factor in Women Who Suffer from Chronic Abdominal and Pelvic Pain. Courtney M O'Donnell,[1] Christopher M Tarnay,[2] Andrea J Rapkin.[3] *[1]Department of Obstetrics and Gynecology, Olive View-UCLA David Geffen School of Medicine, Los Angeles, CA, USA; [2]Department of Obstetrics and Gynecology, Olive View-UCLA David Geffen School of Medicine, Los Angeles, CA, USA; [3]Department of Obstetrics and Gynecology, UCLA David Geffen School of Medicine, Los Angeles, CA, USA.*

PURPOSE: Obesity is implicated as a key risk factor in chronic disease, but no studies have associated central obesity to the presence of chronic abdominal and/or pelvic pain. We set out to identify the prevalence of chronic abdominal/pelvic pain in an underserved, primarily Latina population by a cross-sectional study in the Olive View-UCLA outpatient gynecology clinic. We sought to identify an association between the presence and severity of abdominal/pelvic pain and central obesity.

METHODS: Nonpregnant women presenting to the gynecology clinic were prospectively evaluated and grouped according to the presence of abdominal/pelvic pain ('None-Mild' or 'Moderate-Severe' pain). Body Mass Index (BMI) and abdominal circumference (AC) were measured. Patients with 'Moderate-Severe' pain completed standardized questionnaires for pelvic pain and global health scores.

RESULTS: 207/276 (75%) of patients has 'None-Mild' pain, and 69 (25%) had 'Moderate-Severe' pain. Pain prevalence was not significantly associated with BMI (mean: 'None-Mild' 30.5+7.7 kg/m2; 'Moderate-Severe' 30.6+7.6 kg/m2, p=0.95), nor was pain severity (p=0.58). Pain prevalence was significantly associated with AC (mean: 'None-Mild' 37.1+7.7 inches; 'Moderate-Severe' 40.2+7.0, p=0.004). A borderline positive association exists between AC and pain severity (p=0.077).

CONCLUSIONS: We demonstrate an association between both the presence and severity of chronic abdominal/pelvic pain and central obesity, independent of BMI. AC appears to be a more relevant factor than other traditional measures of habitus in patients with this chronic malady. To improve preventative care in women's health management, further evaluation of the role of central obesity in the pathogenesis of chronic pain is necessary.

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SPHG800 Is Able To Reduce Infective Vulvitis Symptoms. Franco Vicariotto,[1] Silvia Gariboldi,[2] Marco Mastrodonato,[3] Cristiano Rumio.[2] *[1]European Society for Infectious Diseases in Obstetrics and Gynaecology, Trieste, Italy; [2]Department of Human Morphology, Università degli Studi di Milano, Milano, Italy; [3]Sinclair Pharmaceuticals Ltd, Godalming, Surrey, United Kingdom.*

Aims: Vulvitis is one of the most frequently diagnosed gynaecological infections. We aim to assess the efficacy against infective vulvitis of a new topical medical device containing low molecular weight hyaluronic acid (LMW-HA). The ability of this molecule to stimulate β-defensin 2 release in keratinocytes has been recently shown.

Methods: We report preliminary data regarding 20 women suffering from infective vulvitis, as assessed by a gyneacologist: patients were randomly selected to receive SPHG800 (10 patients), a cream containing low-molecular weigh hyaluronic acid, or vehicle (10 patients). Patients were asked to apply the cream to the vulva twice-daily for 7 days. At the end of treatment, evaluation of efficacy, tolerability and acceptability of the cream was assessed by a specific questionnaire.

Results: Preliminary results show that patients receiving SPHG800 report a significative improvement of vulvitis symptoms, in terms of itch, redness of the skin and burning, in comparison to vehicle. SPHG800 also showed a good tolerability, cosmetic acceptability and symptomatic relief perceived by patients.

Conclusion: SPHG800 seems to be efficacious in ameliorating the symptoms of infective vulvitis. This activity may be probably related to the LMW-HA presents in this formulation: in fact, low molecular weight hyaluronic acid has been recently shown to induce β-defensin 2 production by human keratinocytes. Since this peptide exerts antimicrobial and antimicotic activity, the improvement of symptoms assessed in patients receiving SPHG800 might be linked to a reduced infective charge, attributable to the activity of β-defensin 2.

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Labial Fusion: A Rare Complication of Chronic Graft Versus Host Disease. John M Norian,[1,2] Pamela Stratton.[1] *[1]Reproductive Biology and Medicine Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA; [2]Combined Federal Fellowship Program for Reproductive Endocrinology.*

Background:

Allogeneic hematopoietic stem cell transplant (HSCT) is a treatment used for many malignant and nonmalignant diseases of the bone marrow and immune system. HSCT may be complicated by chronic graft-versus-host-disease (cGVHD) in 60 to 70% from matched unrelated donors. Genital cGVHD complicates about 25% of HSCT and may uncommonly result in labial fusion.

Case: 22 year old woman with a history of Ewing's sarcoma and acute myelogenous leukemia, had received chemotherapy and total body irradiation (TBI) followed by a matched unrelated donor HSCT. Menarche occurred at 13 years of age after normal pubertal development. She menstruated regularly until cancer diagnosis. Premature ovarian failure resulted after chemotherapy and TBI and oral contraceptive pills were used for hormone replacement. After transplant, she developed chronic GVHD involving the skin, eyes, mouth and joints, and concomitantly complained of vulvar pruritus. She was presumed to have a yeast infection which was treated with fluconazole without a pelvic exam. She was evaluated by a gynecologist when she was unable to insert a tampon. Pelvic exam revealed dense labia minora adhesions from the clitoris to urethral meatus and posteriorly leaving a 1 cm opening at the urethra. Pelvic MRI revealed a normal uterus and ovaries. After 2 weeks of topical estrogen cream, the adhesions remained dense and were lysed under general anesthesia. Vaginal examination revealed pale, minimally rugated, normal mucosa. Cervical cytology was normal. Post-operatively she used daily topical estrogen and hydrocortisone creams. At 3 months after surgery, her urinary stream was stronger. On pelvic exam, the labial opening was 4 cm, but a small posterior forchette adhesion elicited severe pain. After using dilators coated with topical steroids and estrogen, she was able to insert a tampon.

Conclusion: Genital GVHD should be considered in women with genital tract complaints after HSCT. Labial fusion secondary to chronic GVHD may be treated successfully with surgery and medical therapy.

Support: RBMB/NICHD/NIH.

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Can Endometrial Thickness Predict Rupture of an Ectopic Pregnancy? Dana R Ambler, Mazen E Abdallah, Rahi Victory, Michael P Diamond, Elizabeth E Puscheck, Jay M Berman. *Obstetrics and Gynecology, Wayne State University/Detroit Medical Center, Detroit, MI, USA.*

Objective: To determine which factors are predictive of a ruptured ectopic pregnancy, and whether endometrial stripe thickness can be used as an alternative to such criteria in the diagnosis of an ectopic pregnancy.

Design: Retrospectively collected ectopic pregnancy database.

Setting: Detroit Medical Center, Detroit, Michigan.

Patients or Participants:

413 women with a diagnosis of an ectopic pregnancy were studied, with 90 surgically confirmed tubal rupture cases.
Interventions: Abstracted data included βHCG(IU), gestational age (days), presenting symptoms of pain and/or bleeding, hemoglobin (Hgb), hematocrit (Hct), historical risk factors, ultrasound-determined ectopic size, endometrial stripe thickness (mm), amount of cul de sac fluid (CDS), tubal rupture at time of surgery, and estimated blood loss (EBL)(ml). Covariates included demographics. Results were significant when $p<0.05$.
Results: Chi Square analysis revealed that there is a relationship between endometrial stripe thickness and βHCG levels. Logistic regression models demonstrated that endometrial stripe thickness was not predictive of ectopic rupture, (OR 0.98, p=0.96). However, logistic regression, both forced and forward stepwise analysis, demonstrated that βHCG (OR=6.8, $p<0.001$), a large volume of cul-de-sac fluid (OR=4.2, $p<0.001$), and increasing pain (OR=3.5, p=0.004) were associated with increased risks of rupture. Gestational age, and ectopic related risk factors were also not predictive of rupture.
Conclusions: Endometrial stripe thickness is not a useful predictor for the diagnosis of a ruptured ectopic pregnancy. Serum βHCG measurement, CDS fluid volume and the presence of pain are much stronger diagnostic indicators of ectopic pregnancies.

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Clinical Profile of Migraineurs in a University Hospital Gynecology Department in Japan. Hiroi Kaku,[1] Tadahiro Shoji,[1] Masako Kudo,[2] Yasuo Terayama,[2] Toru Sugiyama.[1] *[1]Obstetrics and Gynecology, Iwate Medical College, Morioka, Iwate, Japan; [2]Neurology, Iwate Medical College, Morioka, Iwate, Japan.*
[Objectives] The changes in hormonal milieu associated with menarche, pregnancy, menopause, and post-menopause are frequently accompanied by changes in the patterns and frequency of migraine. Little is known on the relationship of women's issues of migraine though the balance between estrogen and progesterone is critical in the elimination of migraine. Our aim was to investigate the relations among the prevalence of migraine, the reproductive stage, and gynecologic diseases.
[Materials and Methods]197 female patients (average age: 44.6 years old) who consulted a physician and agreed to answer about the questionnaires during September, 2006 - June, 2007. Migraineurs were diagnosed with the migraine screener by the Japanese Headache Medical Treatment Promotion Committee in 2005. and the patients answered questionnaires that screened about menopausal disorder and abnormal menstruation at once. They were conducted a survey in the form of a questionnaire and sometimes were taken blood samples. [Results] 52 in 197 patients had migraine (26.4% average age: 40.6 years old). Prevalence were 33.3% in one's twenties, higher than another ages. Most patients with migraine was complicated by menstruation disorders (premenstrual syndrome 67%, dysmenorrhea 52.6%), sterility (42.4%), and severe menopausal disorder (80%). When TRH and the LH-RH test were examined for the sterility patient, migraineurs had higher prolactin basal level and LH level after lord but lower FSH level before and after lord than non-migraineurs. On the other hand, the prevalence of migraine for postmenopausal women and women who had gynecology cancer treatment was low, and there was no relation between migraine and pregnancy history.
[Conclusions] This study provides migraine headache is influenced by reproductive stage and that women with migraine are frequently complicated by menstruation disorder. It is thought that migraine account for abnormality of hormone milieu including abnormality of the hypothalamus-pituitary system.

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Efficacy of 3 Doses of Estradiol Gel 0.1% in the Treatment of Vasomotor Symptoms and Vulvar Vaginal Atrophy. William Koltun,[1] Larry J Lambrecht.[2] *[1]Medical Center for Clinical Research, San Diego, CA, USA; [2]Upsher-Smith Laboratories, Inc., Minneapolis, MN, USA.*
OBJECTIVE: This study evaluated the efficacy of 3 doses of Estradiol (E_2) gel 0.1% (Divigel®), a novel formulation of E_2 consisting of 1 mg E_2 per 1 g transdermal gel, to reduce frequency and severity of vasomotor symptoms and signs of vulvar and vaginal atrophy (VVA) associated with menopause.
DESIGN: 488 postmenopausal women were evaluated in a 12-week study comparing placebo to E_2 gel 0.1% at doses of 1.0 g/day, 0.5 g/day, and 0.25 g/day with estimated nominal daily deliveries of 0.027 mg, 0.009 mg, and 0.003 mg of E_2 respectively. Endpoints included mean change from baseline in daily frequency and severity of moderate to severe hot flashes (MSVS). Vaginal pH and % superficial cells were collected at baseline and end of study.
RESULTS: E_2 gel 0.1% showed statistically significant improvements from placebo gel as early as week 2 (Table) that were maintained throughout treatment. Signs of VVA (vaginal pH and % of superficial cells) showed statistically significant improvements from baseline with all 3 doses of E_2 gel 0.1% compared to placebo.
CONCLUSION: E_2 gel 0.1% significantly decreased the frequency and severity of MSVS at all doses evaluated in this trial. E_2 gel 0.1% offers multiple dosing options to individualize patient therapy, including the lowest effective dose that was studied (0.25 mg E_2, delivering 0.003 mg E_2/day), to treat vasomotor symptoms associated with menopause.

Dose	MSVS	Week	CFB	p-value
1.0 g	Frequency	2	-5.30	0.004
	Severity*	2	-0.33	0.043
0.5 g	Frequency	4	-6.17	0.011
	Severity*	3	-0.42	0.008
0.25 g	Frequency	5	-6.51	0.005
	Severity*†	5	-0.49	0.038

*Severity= (2 * # moderate symptoms + 3* # severe symptoms)/total # of MSVS. †Significance was maintained for the 0.25 g dose from week 7-12 for severity.

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Safety and Tolerability of 3 Doses of Estradiol Gel 0.1% in the Treatment of Menopausal Symptoms. William Koltun,[1] Larry J Lambrecht.[2] *[1]Medical Center for Clinical Research, San Diego, CA, USA; [2]Upsher-Smith Laboratories, Inc., Minneapolis, MN, USA.*
OBJECTIVE: Estradiol (E_2) gel 0.1% (Divigel®) is a novel formulation of E_2 consisting of 1 mg E_2 per 1 g transdermal gel for the treatment of vasomotor symptoms associated with menopause. Safety and tolerability of E_2 gel 0.1% were evaluated in a large placebo-controlled trial.
DESIGN: 488 postmenopausal women participated in a 12-week study comparing placebo to 0.25 g/day, 0.5 g/day, and 1.0 g/day of E_2 gel 0.1%. Circulating E_2 and estrone (E_1) concentrations were measured. Safety analyses included the incidence of adverse events (AEs) and clinical laboratory evaluations, including plasma levels of sex hormone binding globulin (SHBG). Application site tolerability was assessed using the Draize scale.
RESULTS: All doses of E_2 gel 0.1% produced physiologic E_2:E_1 ratios similar to those seen in premenopausal women. E_2:E_1 increased from a baseline mean of 0.17 to 0.49, 0.69, and 0.95 with, respectively, the 0.25, 0.5, and 1.0 g/day doses of E_2 gel 0.1%. The most frequently reported AEs were breast tenderness and postmenopausal bleeding that appeared to be dose-related and would be expected with increased circulating estrogen concentrations. There were no remarkable changes in hematology, blood chemistry, urinalysis, lipid, coagulation, and carbohydrate values following treatment with E_2 gel 0.1%. SHBG levels remained unchanged after 12 weeks of treatment at all doses. The vast majority of patients had no evidence of skin irritation throughout the treatment period. Mean Draise scale scores after 4, 8, and 12 weeks of treatment were 0.0 for all treatment groups except for a mean value of 0.1 ± 0.34 for the 0.5 g dose group after 4 weeks of treatment.
CONCLUSION: E_2 gel 0.1% is a safe and well-tolerated therapy for the treatment of menopausal symptoms.

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Comparative Analysis of Non-Patch Transdermal Estradiol for the Treatment of Menopausal Symptoms. David Harari,[1] Michelle Zachman.[2] *[1]Sharp Grossmont Hospital, LaMesa, CA, USA; [2]Upsher-Smith Laboratories, Inc., Minneapolis, MN, USA.*
Objective: Within the last year, 3 new non-patch transdermal estradiol (E_2) therapies for the treatment of menopausal symptoms have been approved by the FDA: Divigel®, Elestrin™, and EvaMist™. The dosing, efficacy, and adverse events associated with these new treatments have been compared to the established transdermal E_2 therapies, EstroGel® and Estrasorb®.
Design: Pharmacokinetic parameters, dosing, efficacy, and safety information for Divigel®, Elestrin™, EvaMist™, EstroGel®, and Estrasorb® were obtained from current prescribing information (obtained from manufacturer websites) and the data were compared.
Results: Together, these new transdermal therapies offer multiple dosing/delivery options and contain a wide range of E_2 (0.25 mg to 4.6 mg), with the lowest systemic daily delivery of E_2 attained by the Divigel® 0.25 g dose. Following 12 weeks of treatment, across the dosing options, each treatment significantly reduced both the frequency and severity of moderate to severe vasomotor symptoms (MSVMS) compared to placebo. Change in frequency

of MSVMS ranged from -6.0 (Divigel® 0.25 g) to -11.1 (Estrasorb®); change in severity scores for MSVMS ranged from -0.3 (Divigel® 0.25 g) to -1.7 (Divigel® 1.0 g). All treatments are safe and well tolerated. Breast tenderness was the only adverse event reported in ≥5% of subjects, occurring with all therapies.
Conclusions: Current treatment guidelines recommend using the lowest effective dose of estrogen for the treatment of menopausal symptoms. This side-by-side comparison of the recently available E_2 non-patch transdermal options to the standard transdermal therapies is meant to assist physicians in individualizing treatment for their patients.

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Effects of a CDB-2914 Intrauterine Device on the Rhesus Macaque Endometrium. Robert M Brenner,[1] Ov D Slayden,[1] Yun-Yen Tsong,[2] Regine L Sitruk-Ware.[2] *[1]Reproductive Sciences, Oregon National Primate Research Center, Beaverton, OR, USA; [2]Population Council, The Rockefeller University, New York, NY, USA.*
Introduction: CDB-2914 (CDB) is a relatively new progesterone receptor modulator being clinically evaluated for contraception and treatment of fibromas. Its use in an intrauterine device/system (IUS) has not been reported. In this study we prepared CDB-filled intrauterine devices (CDB-IUS) and evaluated their effects on endometrial growth and bleeding patterns in rhesus macaques. Methods: Short (1.0-1.5 cm) lengths of Silastic tubing (OD 2.4 mm), either empty (n=3),or filled with silicone rubber matrix containing 50% of micronized CDB (n=5), were inserted into the uterine lumens of 8 ovariectomized rhesus macaques. Animals were induced to cycle by sequential treatment with systemic estradiol and progesterone (P) as reported (Brenner et al, Ann NY Acad, 2002). After 3.5 cycles, at the end of the follicular phase, the uterus was removed and processed. Results: When systemic P treatment was withdrawn at the end of each cycle, animals with empty IUS menstruated normally, while animals with CDB-IUS bled little or not at all. Over the whole 3.5 cycles, animals bearing a blank IUS bled for an average of 11.66 ± 0.88 days while CDB treated animals bled for an average of only 1 ± 0.45 days. At the end of treatment, animals exposed to blank IUS had mean endometrial wet weights of 409 ± 112 mg while the CDB-treated endometria weighed only 188 ± 30 mg. The proliferation markers Ki-67 and Phospho-H3 were substantially lower in the CDB-IUS treated than in the blank treated animals. Histologically, the CDB exposed endometria were atrophied with evidence of glandular degeneration while the blank controls were proliferative and normal. Summary: In cycling rhesus macaques, a CDB-IUS prevented progestational development, blocked menstruation after P withdrawal, and suppressed endometrial proliferation. If these effects are confirmed in women, the CDB-IUS could provide estrogen-free and bleed-free contraception, and could help control heavy menstrual bleeding.
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Long-Term Progestin Contraceptives (LTPOC) Induce aberrant angiogenesis, Oxidative Stress and apoptosis in the Guinea Pig Uterus. Graciela Krikun,[1] Irina A Buhimschi,[1] Martha Hickey,[2] Edward Kuczynski,[1] Lynn Buchwalder,[1] Rebeca Caze,[1] Frederick Schatz,[1] Charles J Lockwood.[1] *[1]Ob/Gyn&Reprod.Sci, Yale University, New Haven, CT, USA; [2]Ob/Gyn, Univ West Australia, Subiaco, WA, Australia.*
Introduction: Irregular uterine bleeding is a major side effect and cause for discontinuation of LTPOC use. While endometria of LTPOC-exposed women display abnormally enlarged, fragile blood vessels (BV), decreased blood flow and evidence of oxidative stress, the mechanisms by which structural and vasomotor endometrial dysfunction occurs remains unknown, in part by the difficulty of manipulating hormone levels in women. The aims of this study were **1)** to validate the guinea pig (GP) as a model to study uterine effects of LTPOC and **2)** to investigate LTPOC-effects on endometrial histology and oxidative stress markers. **Methods:** Oophorectomized GPs were implanted s.c. with time release pellets containing either placebo (CRL,n=6); estradiol (E2,n=3); medroxyprogesterone acetate (MPA,n=6) or E2+MPA (n=3). After 21 days, uterine horns were weighed and frozen or paraffin embedded. Angiogenesis was assessed by quantitative image analysis of vonWillebrand factor staining and included BV density and size. Oxidative stress was detected by 8-isoprostane and 8-OH-deoxyguanosine (8oxoG). Apoptosis was investigated by the TUNEL method. Statistical analysis was by 1-way and 2-way ANOVA.
Results: GP uteri were enlarged by both E2 ($p<0.001$) and MPA ($p=0.025$). Effects of MPA on uterine weight differed significantly depending on E2 levels ($p<0.001$), where MPA opposed the E2 effect in combined treatments. Angiogenesis parameters were similarly impacted upon. Thus, MPA alone increased BV density ($p=0.036$) and BV average area ($p=0.002$). The presence of E2 significantly decreased these parameters (BV density mean± SEM: CRL: 9.4±1.0%, E2: 10.3±1.6%, MPA: 13.6±1.1%, E2+MPA: 6.0±0.7%, $p=0.002$). These changes were associated with highly elevated 8-isoprostane content in E2+MPA-treated uteri compared to all other groups ($p<0.001$). Abnormalities in the E2+MPA group were consistent with chromatin redistribution, nuclear pyknosis, karyolysis and increased nuclear 8oxoG staining and a marked increase in TUNEL labeling. **Conclusions:** LTPOC exposure alters endometrial vascular and tissue morphology consistent with oxidative stress and apoptosis in a complex interplay with endogenous estrogens. The GP is an excellent model for the study of LTPOC effects on the uterus.

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Physiologic and Psychological Symptoms Associated with Injectable and Oral Contraceptive Use. Abbey B Berenson, Susan Odom, Carmen R Breitkopf, Mahbubur Rahman. *Ob/Gyn, UTMB, Galveston, TX, USA.*
Objective: To compare physiologic and psychological symptoms over 24 mo among users of depomedroxyprogesterone acetate (DMPA), an oral contraceptive with 20 micrograms ethinyl estradiol (OC), and non-hormonal (NH) contraception.
Methods: A total of 604 women reported the presence of 16 symptoms prior to initiating contraception (217 DMPA, 216 OC, 171 NH) and every 6 mo thereafter for 24 mo. Longitudinal relationships between symptoms and contraceptives (reference: NH), as well as race/ethnicity (non-Hispanic black, non-Hispanic white, and Hispanic) were assessed by GEE-analysis after adjusting for age, visits and baseline status of symptoms. Persistence, resolution, and new development of symptoms were noted by method in 6 mo increments for 24 mo and compared with that of NH controls.
Results: The GEE analyses showed that OC was protective against mastalgia (OR= 0.7), cramping (OR=0.5), hair loss (OR=0.6), acne (OR=0.4), nervousness (OR=0.5) and mood swings (OR=0.7). When race was considered, OC was protective for all women against acne, for whites against mastalgia and cramping, and for non-whites against nervousness. For whites, it was a risk factor for bleeding between menses. OC use resulted in resolution of acne within 6 mo in nearly 50% of those with it at baseline, but no significant resolution after 6 mo. Also mastalgia (29% at baseline) was less likely to persist at 18 and 24 mo. Bleeding between menses was reported for the first time at 18 mo by 11% of OC users. DMPA users of all races had an increased risk of missed periods (OR=95.1), bleeding between menses (OR=3.6), bleeding >20 d (OR=13.6) and loss of libido (OR=2.2) relative to NH users. It reduced cramping (after 12 mo) and bloating (all 24 mo). Racial differences were observed with DMPA protective against mastalgia and mood swings in whites, cramping in whites and Hispanics, and bloating in non-whites. It was a risk factor for loss of energy in whites only. Neither method affected depressive symptoms.
Conclusion: Side effects of these two methods are mostly related to abnormal bleeding. Very low dose pills can be protective against symptoms (mastalgia, cramping, hair loss, acne, nervousness and mood swings) commonly associated with pills while DMPA protects against cramping and bloating. Knowledge about racial differences will allow physicians to individualize therapy. Counseling should include that some symptoms can develop after 18 mo while resolution often occurs within 6 mo.

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Effect of Oral vs. Transdermal Contraceptives on Ethinyl Estradiol Levels. Julia V Johnson,[1] Peter R Casson,[1] Elizabet Gentzschein,[2] Frank Z Stancyk.[2] *[1]University of Vermont, Obstetrics and Gynecology, Burlington, VT, USA; [2]Obstetrics and Gynecology, University of Southern California, Los Angeles, CA, USA.*
INTRODUCTION: Despite the lack of an hepatic first pass effect with non-oral steroidal contraceptives, an epidemiologic study demonstrated an increased risk of VTE with transdermal contraceptive (TC) compared to oral contraceptives (OCs). While TC has decreased peak ethinyl estradiol (EE) levels, due to the continuous EE absorption, there is an increased area under the curve compared to OCs.
OBJECTIVE: To compare EE levels from a prospective trial on TC compared to OC.
METHODS: Twenty-four women were enrolled in IRB approved prospective, randomized, cross-over trial comparing 2 months of OC (Ortho Cyclen) vs. TC Ortho Evra). The daily OC administers 35 micrograms of EE; the weekly TC contains .75 milligrams of EE. Each treatment was followed by 2 months of washout and 2 months of the alternative contraceptive. Blood was drawn at baseline and final week of treatment for each arm of the study. EE was

quantified by RIA, with preceding organic solvent extraction and Celite column partition chromatography. Data were analyzed by T test with Boferroni's correction, P < .05.
RESULTS: After two months of treatment the mean (+/- SEM) EE levels for TC = 81.9 pg/ml (+/- 12.0) and OC = 94.1 pg/ml (+/- 9.8). The EE level is not significant different for the two medication (p = 0.42).
CONCLUSIONS: There is no difference in EE levels with the use of these oral and transdermal contraceptives. This suggests the transdermal contraceptive, despite the lack of the hepatic first pass effect, has similar levels of ethinyl estradiol compared to OC. The continuous elevation in EE seen with the TC route of administration, versus the episodic increases seen with the OC route, raises concerns of constant exposure to EE. This may explain the increased risk of estrogen-induced thrombotic events with this TC route of administration.

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Impact of Paracervical Block, in Combination with General Anesthesia, on Post-Abortion Pain. Gweneth B Lazenby, Tod Aeby. *Obstetrics and Gynecology, University of Hawaii, Honolulu, HI, USA.*
Objective
To evaluate the impact of paracervical block with a long-acting local anesthetic, in conjunction with general anesthesia, on post-operative pain.
Methods
A power analysis determined 35 patients per arm were needed to demonstrate a significant difference of 2 in mean pain scores. Seventy-two patients were allocated to one of two arms using urn randomization. All patients received standardized anesthesia; intravenous sedation for gestational age under 12 weeks and general anesthesia for over 12 weeks. Thirty-nine patients were randomized to receive a paracervical block with 0.5% bupivacaine and thirty-three were randomized to no local anesthesia prior to surgical abortion. Patients completed visual analog scales for pain and anxiety prior to the procedure, upon awaking, 30 and 60 minutes post-operatively, and prior to discharge. Data were analyzed using an ANOVA and Students T-test

Comparison of Mean Pain Scores

Time	Experimental	Control	p-value
Pre-operative	1.5 (0.8-2.1)	1.2 (0.6-1.9)	0.297
Immediately post-op	1.2 (0.5-1.9)	1.6 (0.8-2.4)	0.224
30 minutes post-op	1.2 (0.5-1.8)	2.0 (1.2-2.9)	0.066
1 hour post-op	0.9 (0.3-1.6)	0.8 (0.3-1.3)	0.376
Discharge	0.5 (0.2-0.9)	0.3 (0.1-0.6)	0.202

Comparison of Mean Anxiety Scores

Time	Experimental	Group	p-value
Pre-operative	3.6 (2.4-4.8)	2.5 (1.5-3.4)	0.073
Immediately post-op	0.5 (0.0-0.9)	0.4 (0.0-0.8)	0.401
30 minutes post-op	0.3 (0.0-0.5)	0.2 (0.1-0.4)	0.300
1 hour post-op	0.6 (0.0-1.2)	0.2 (0.1-0.5)	0.128
Discharge	0.3 (0.2-0.7)	0.2 (0.0-0.1)	0.157

Results
The experimental and control groups were equivelent in age, ethnicity, gravidity, parity, prior abortions, prior vaginal deliveries, prior c-sections, gestational age, number of laminaria, pre-operative and intra-operative dilation, operative time, estimated blood loss, and reported complications. Pain and anxiety were not significantly affected by placement of a paracervical block.
Conclusion
These data do not support the hypothesized benefit of local anesthesia, prior to surgical abortion under general anesthesia, to reduce post-operative pain. We do not recommend the routine use of a paracervical block to decrease post-operative pain.

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Age, Parity, History of Abortion and Contraceptive Choices Affect the Risk of Repeated Abortion. Oskari Heikinheimo,[1] Mika Gissler,[2] Satu Suhonen.[1] *[1]Ob&Gyn, University of Helsinki, Helsinki, Finland; [2]National Research and Development Centre for Welfare and Health, Helsinki, Finland.*
Objective
The rate of repeat abortion varies from 30 to 38% in Northern Europe. However, risk factors for repeat abortion are poorly understood. We characterized risk factors (demographic, as well as those related to abortion and postabortal contraception) of repeat abortion.
Design
A prospective cohort study of 1269 women undergoing medical abortion between August 2000 and December 2002. The subjects were followed by means of Finnish Registry on Induced Abortions until December 2005; the follow-up time (mean ± SD) was 49.2 ± 8.0 months.
Results
Altogether 179 (14.1%) of the subjects requested repeat abortion within the follow-up time. In univariate analysis previous abortion, parity, young age, smoking and failure to attend the follow-up visit were associated with increased risk of repeat abortion. Immediate - in contrast to postponed - initiation of any contraceptive method was linked to lower risk of repeat abortion. In comparison to combined oral contraceptives, use of intrauterine contraception was most efficacious in reducing the risk of another pregnancy termination. In multivariate analysis the effects of young age, parity, previous abortion and type of contraception on the risk of another abortion persisted.
Conclusions
Increased focus on young, parous and those with the history of an abortion may be efficacious in decreasing repeat abortion. Contraceptive choices made at the time of abortion have an important effect on the rate of reabortion. Postabortal use of intrauterine contraception, specifically that of LNG-IUS, might decrease the rate repeat abortion.

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A Review of Failure Associated with the Essure Permanent Birth Control Device at the Detroit Medical Center. Valerie Shavell, Mazen Abdallah, David Kmak, Michael Diamond, Jay Berman. *Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA.*
Introduction. The Essure permanent birth control device is a relatively new form of minimally invasive sterilization for women. Under hysteroscopic guidance, a dynamically expanding micro-insert is introduced into the proximal portion of the fallopian tube. Local fibrosis and occlusion of the tubal lumen result.
Objective. To determine the rate of failure and to analyze factors associated with failure for the Essure permanent birth control device at the Detroit Medical Center (DMC).
Methods. A chart review was conducted on patients who underwent Essure placement at the DMC from January 2003 through June 2007. Patient demographics, past medical and surgical history, anesthesia type, procedure time, intraoperative complications, and procedure failures were noted. Data were analyzed for statistical significance using SPSS.
Results. There were 316 Essure procedures attempted at the DMC from January 2003 through June 2007. Of the 316 attempted procedures, there were 25 failures (7.9%). 13 of the 25 failures were attributed to difficulty visualizing the ostia (52%). Other causes of failure included expulsion of the device (2), tubal spasm (1), uterine perforation (1), and tubal ostia too large for the device (1). There were 3 cases of failed placement for undocumented reasons, one case requiring a laparoscopic tubal ligation secondary to post-procedure tubal patency, and 3 post-procedure pregnancies. Age, race, body mass index, gavidity, parity, history of sexually transmitted infections, medical history, history of cesarean section, tobacco or illicit drug use, anesthesia type, and physician experience with the procedure were not significantly associated with placement failure or difficulty visualizing the ostia. A longer procedure time was significantly associated with failure (40.6 vs 26.6 min, p = 0.004), and history of ectopic pregnancy was significantly associated with difficulty visualizing the ostia (33.3% vs 4.7%, p = 0.003).
Conclusion. The failure rate for placement of the Essure permanent birth control device at the DMC is 7.9% with a pregnancy rate of 0.95%. The majority of failures may be attributed to difficulty visualizing the ostia. A history of ectopic gestation was significantly associated with difficulty visualizing the ostia; thus, it may be reasonable to advise these women that success in Essure placement may be reduced.

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A Review of Follow-Up for the Post-Essure Hysterosalpingogram in a Clinic Population. Valerie Shavell, Mazen Abdallah, Michael Diamond, David Kmak, Jay Berman. *Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA.*
Introduction. The Essure permanent birth control device is a relatively new form of minimally invasive sterilization for women. Under hysteroscopic guidance, a dynamically expanding micro-insert is introduced into the proximal portion of the fallopian tube. The micro-insert induces local fibrosis and ultimately occlusion of the tubal lumen. A hysterosalpingogram (HSG) is performed three months after the procedure to confirm bilateral tubal occlusion.
Objective. To determine the follow-up rate for the post-Essure HSG for a clinic population.
Methods. A retrospective chart review was conducted on University Health Center (UHC) patients who underwent placement of the Essure permanent

birth control device at the Detroit Medical Center from January 2003 through June 2007. Follow-up for the post-Essure HSG as well as the result of the HSG were noted for each patient.

Results. Placement of the Essure permanent birth control device was attempted in 83 UHC patients of which 79 were successfully completed. Of the 79 patients, ten underwent a post-Essure HSG (12.7%). The HSG was performed three to six months after placement of the Essure permanent birth control device. Bilateral tubal occlusion was documented in all ten patients.

Conclusion. Despite counseling patients prior to their procedure that a HSG is needed and providing an information sheet, the follow-up rate for the post-Essure HSG for this clinic population is only 12.7%. For those in whom a HSG was performed three to six months after Essure placement, bilateral tubal occlusion was confirmed in all. Steps and or approaches to improve compliance with post-procedure confirmation of tubal occlusion should be employed to increase follow-up in the future.

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Towards Fibroids Gene Therapy: Adenovirus Mediated Delivery of Herpes Simplex Virus 1 Thymidine Kinase Gene/Ganciclovir Shrinks Uterine Leiomyoma in the Eker Rat Model. Memy Hassan,[1] Dong Zhang,[3] Salama Salama,[1] Cheryl Walker,[2] Hala El-Mazar,[1] Ayman Al-Hendy.[3] *[1]OB/GYN, UTMB; [2]MD anderson; [3]OB/GYN, Meharry Medical College, Nashville, USA.*

Aim: Assessment of the efficacy of gene therapy of uterine leiomyoma in the immune-competent Eker rat model using adenovirus mediated delivery of Herpes simplex-1-thymidine kinase gene followed by Ganciclivir treatment (Ad-TK/GCV). **Method:** Female Eker rats with MRI-confirmed uterine fibroid lesions were randomized to a single treatment with direct intratumor injection of Ad-TK/GCV, Ad-LacZ/GCV, or medium.The tumor volume was evaluated by serial MRI scanning and confirmed with caliper measurement at time of euthanasia. Sample rats were selected randomly and killed at the following time points; 10, 20 and 30 days post treatment. Samples were collected from tumors, other body organs and blood to assess the safety and efficacy of the treatment. **Results:** Ad-TK/GCV treatment produced dramatic shrinkage of the total uterine fibroid volume by 75% ±16, 59% ±6 and 68%±28 of pretreatment volume at days 10, 20 and 30 respectively. The tumor size in negative control animals receiving Ad-LacZ/GCV continued to grow by +26%±10, +66%±23, +102% ±38 while receiving media continue to grow by + 20% ± 6 , +70% ± 8 and +110% ± 15 at same time points. Ad-TK/GCV induced significant increase in caspase 3 activity, Bax expression, decrease in Bcl2 and PARP proteins expression and increased tunnel apoptosis index. Additionally Ad-TK/GCV treatment decreased cyclin D1and PCNA expressions. Ad-TK/GCV did not produce any significant change in liver function tests or relative uterine horns weight to total body weight. The adenovirus transfection did not disseminated significantly to other distal organs except to liver and myometrium in limited number of animals. H&E staining of non targeted organs did not revealed any sign of tissue damage. Ad- transfection increased local CD4 and CD8 expressions as well as serum anti-Ad antibodies. **Conclusion:** Ad-mediated delivery of HSV1TK gene by direct intra-tumor inoculation followed by SC treatment of GCV for ten days effectively shrinks uterine leiomyoma lesions in Eker rats. This effect is mediated via induction of apoptosis and decreasing the proliferation. The treatment regimen is well tolerated. These studies provide essential preclinical data for the development of gene therapy as an alternative non-surgical treatment option for women with symptomatic uterine fibroids.

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Inhibitors of Catechol O-Methyl Transferase Shrinks Uterine Fibroids in the Eker Rat Model. Memy Hassan,[1] Dong Zhang,[3] Hala El-Mazar,[1] Cheryl Walker,[2] Ayman Al-Hendy.[3] *[1]OB/GYN, UTMB; [2]MD Anderson; [3]OB/GYN, Meharry Medical College, Nashville, USA.*

Background : The sex hormone dependent pattern of Uterine leiomyomas and their high content of catechole –O-methy transferase (COMT) raise the possibility for the development of novel treatment option using COMT inhibitors.**Aim :** to assess the potential therapeutic utility of a synthetic COMT inhibitor (Ro 41-0960) in the Eker rat model of uterine leiomyoma. **Methods** Female Eker rat were evaluated by MRI to confirm the prescence uterine fibroid lesion, then randomized for SC treatment with Ro 41-0960 150 mg /kg ,twice/ day for 28 days versus vehicle injection.Fibroid tumor burden was evaluated by serial MRI measurement and confirmed by direct caliper measurement at time of euthanasia. Sample animals were euthanized at 2 and 4 weeks. At that time tumor tissue, blood and most of animal organs including long bones were collected and subjected for further evaluations. 24 hours urine samples were collected for evaluation of estrogen (E2) metabolites and bone resorption marker. **Results:**Animals treated with Ro 41-0960 exhibited significantly lower uterine fibroid tumor burden (86% and 105%) of pretreatment volume at 2 and 4 weeks post treatment respectively. Conversely, the tumor size in control animals continued to grow and reached 300 %, and 300 % of pretreatment size at the same time points. Ro 41-0960 treatment resulted in an increase in urinary 2/16 hydroxy E2 metabolite ratio. In addition Ro 41-0960 Increased BAX expression and decreased PARP , PCNA and cyclinD1 experssions . All Ro 41-0960 treated animals tolerated the treatment protocol with no signs of toxicity. H&E staining of different body organs did not reveal any signs of tissue damage. Furthermore, there was no significant change in both liver function tests (ALT, AST, billirubin) and bone resorption marker , DeoxyPyridinoline croslinks, between treated and control rats. **Conclusion** Ro 41-0960, a synthetic selective COMT inhibitor, caused immediate arrest of the growth of Eker rat uterine leiomyoma. This effect might be in part due to modulation of various estrogen dependent genes regulating leiomyoma apoptosis (PARP, BAX) and proliferation (PCNA, cyclin D1). This anti-estrogenic effect is due to the accumulation of the the antiestrogenic metabolite 2 hydroxy estrogen secondary to COMT inhibition.COMT inhibitors might present an alternative non-surgical option for the treatment of women with symptomatic uterine fibroids.

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Novel Therapeutic Interventions for Uterine Leiomyomas: Opposing Roles for Retinoic Acid and TGF-β on Leiomyoma Development. Minnie Malik,[1] John M Norian,[1,2] Joy Britten,[1] Doina Joseph,[1] William H Catherino.[1,2] *[1]Obstetrics and Gynecology, Uniformed Services University, Bethesda, MD, USA; [2]Reproductive Biology and Medicine Branch, NICHD, NIH, Bethesda, MD, USA.*

Background

Development of uterine leiomyomas (fibroids) is the most common pathological feature in the female reproductive tract. They negatively impact patients of virtually every gynecologist. Despite such morbidity, leiomyoma development is poorly understood. We have recently demonstrated that leiomyomas have a genomic expression pattern that limits retinoic acid (RA) exposure. Our group and others have demonstrated that TGF-beta regulation is altered as well. These two pathways likely play central roles in leiomyoma development.

Hypothesis

The central feature of uterine leiomyomas, the extracellular matrix (ECM), is regulated by both all-trans retinoic acid and TGF-β3, focusing on versican as a critical ECM component.

Methods

Human uterine leiomyoma and patient-matched myometrium were obtained from surgical specimens under an IRB-approved protocol. These tissues were immortalized and treated with either all-trans retinoic acid, TGF-β3, or anti-TGF-β3 antibody. RNA was isolated for qRT-PCR.

Results

Human immortalized leiomyoma cells demonstrated the same increased template expression of TGF-β3 (2.96±0.71 fold), retinoic acid metabolizing protein (CYP26A1; 16.81±4.50), and versican variant V0 (7.75±2.2 fold) as was found in the progenitor tissue. When treated with all-trans RA, expression of versican variant V0 decreased to levels found in myometrial cells (0.96±0.04 fold). Conversely, when treated with TGF-β3, expression of versican variant V0 increased 2.98±0.22 fold. To confirm that TGF-β3 was central to the overexpression of V0, we treated leiomyoma cells with anti-TGF-β3 antibody, and found that baseline over-expression of V0 template was decreased to expression levels similar to untreated myometrial cells. Finally, we elucidated a link between the RA and TGF-β pathways by assessing the impact of RA treatment of TGF-β3 expression, demonstrating that TGF-β3 template decreased to levels comparable to myometrial cell expression (0.84±0.12 fold).

Conclusion

The disrupted leiomyoma ECM, of which versican is a central component, defines the leiomyoma phenotype. In this study, the leiomyoma fibrotic phenotype regressed when treated with RA and increased when treated with TGF-β3, providing the basis for novel therapeutic interventions directed at cell differentiation and ECM formation.

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Uterine Fibroids: Development of a Cell Model of Early Fibroid Growth. Liping Feng, Millie Behera, Friederike Jayes, Phyllis Leppert. *OBGYN, Duke University School of Medicine, Durham, NC, USA.*

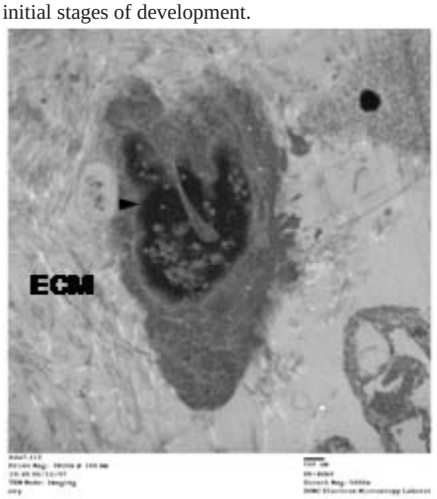
OBJECTIVES: Uterine fibroids are the leading cause for hysterectomies in the US. The lack of an appropriate *in vitro cell* model for the initiation of fibroid growth has hindered advancement in understanding the cellular and molecular

basis for the development and progression of uterine fibroids. Fibrosis is the underlying mechanism of uterine fibroid formation and myofibroblasts cells are the principal fibrogenic cell type in the uterus. We sought to develop a myofibroblast *in vitro* cell model for analyzing the initiating molecular events of uterine fibrosis.

METHODS: Smooth muscle cells (SMCs) were enzymatically isolated from the myometrium of non-pregnant women and cultured in the presence of 10% serum until 70% confluent. For the next 72 h cells were cultured in serum-free media followed by replacement with serum containing media. Cells were fixed at 0, 10, 20, 30, 60 m later. Cell fine structure and cytoskeletal organization were evaluated by transmission electron microscopy. Smooth muscle specific alpha-actin (α-SMA) and progesterone receptors (PR) were detected by western blot.

RESULTS: We observed SMC differentiation into myofibroblasts, marked by the presence of notched nuclei (Figure) and the increased expression of α-SMA 20 m after serum replacement. PR-A and PR-B were detectable at 10, 20 and 30 m.

CONCLUSIONS: The development of myofibroblasts is important in wound healing and fibrosis. We show for the first time that uterine myofibroblasts can be derived in culture from myometrial SMCs. Thus, these cells will be utilized as a model for developing "*in vitro* fibroids". This model will enable the study of myofibroblast activation, cytokine signaling, intracellular regulation of uterine fibrogenesis, production of extracellular matrix proteins and development of antifibrotic drugs. The presence of PRs in our model enables us to evaluate PR mediated events in fibroid pathogenesis and treatment. This model will be more useful in determining the molecular biology of fibroid initiation than cell models derived from established fibroids that are already well past their initial stages of development.



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Novel Approach to Genome-Wide Expression Profiling Analysis. Liping Feng, Morgan Walls, Insuk Sohn, Millie Behera, Sin-Ho Jung, Phyllis Leppert. *OBGYN; Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC, USA.*

BACKGROUND: 10 microarray studies have examined the differential gene expression between uterine fibroid and normal myometrium. All previous studies considered the fibroid as a whole and analyzed only fold changes. We have developed a novel statistical approach to genome-wide expression analysis comparing two fibroid tissue sites to myometrium.

METHODS: Using Affymetrix™ U133A GeneChip, we have compared the gene expression between C and E and matched adjacent M. Data has been analyzed by considering the 3 specimens per subject and 5 subjects as individuals. We used a block one-way ANOVA method to test if each gene was differentially expressed among the three sites. The p-value is calculated using a permutation method accounting for possible dependency among three lesions. The multiple testing issue was addressed by controlling the false discovery rate. Expression values were calculated using the Robust Multichip Average (RMA) method. RMA estimates are based upon a robust average of background corrected perfect/mismatch (PM) intensities. Normalization was done using quantile normalization. Expression values were then transformed by taking Logarithm base 2. Confirmatory RT-PCR was performed.

RESULTS: We applied a hierarchical clustering analysis to all raw data sets and then displayed a dendrogram, where the height of each branch point indicates the similarity level at each generated cluster. Identical gene expression among sites clusters together. Due to a strong site effect, M tissues clustered separately from E and C combined. 45 genes were differentially expressed when we used a 0.1 q-value cut off. Expression data revealed concordant changes in genes regulating cholesterol biosynthesis, gene transcription, estrogen and extracellular matrix formation when both E and C were examined. CYP19 was detected and we report for the first time that SCC-112 (a cell cycle-regulated molecule) folliculin and L-selectin are differentially expressed suggesting that they may be involved in the regulation of cell growth and proliferation of uterine fibroids.

CONCLUSIONS: Our novel robust analysis of gene expression provides new clues to the relevant pathways of fibroid development. This new statistical approach that can be used in clinical and/or translational studies to identify differentially expressed genes comparing treatment regimens, cells or tissues.

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Thrombospondin-1 Expression Is Increased in Serum-Deprived Differentiated Myometrial Cell Model of Uterine Fibroids. Liping Feng, Friederike Jayes, David Schomberg, Phyllis Leppert. *OBGYN, Duke University School of Medicine, Durham, NC, USA.*

OBJECTIVES: Thrombospondin-1 (TSP-1) is a large matricellular glycoprotein secreted by many cell types. Matricellular proteins modulate interactions between cells and their environment, regulate cell adhesion and are expressed during tissue formative processes. They are especially important in fibrosis. TSP-1 plays an important role in angiogenesis and is an activator of TGFβ-3. In a previous study, we found that differential expression of TSP-1 in uterine fibroids may contribute to an altered healing process leading to fibrosis. This alteration in tissue response to injury initiates the development of abundant, nonaligned collagen fibrils and changes in other components of the ECM.

METHODS: We measureed the pattern of mRNA and protein expression of TSP-1 by RT-PCR and western blot in an *in vitro* serum-deprived differentiated myometrial cell (myofibroblast) model. Specifically, smooth muscle cells (SMCs) were enzymatically isolated from the myometrium of non-pregnant women and grown in primary culture to 70% confluence. Then SMC were serum deprived for 72 h and treated back with 10% serum for 10, 20, 30, and 60 m. Cells were collected for RNA and protein, and TSP-1 expression was evaluated. In addition, cells were stained using the combination of anti-CD45 and anti-smooth muscle α-actin or combination of anti-CD42d and anti-smooth muscle α-actin as well as the appropriate single and double negative controls. Stained sections were analyzed using Zeiss Axio Imager widefield fluorescence confocal microscopy.

RESULTS: TSP-1 mRNA and protein was present in cells in this serum-starvation model after the addition of serum and the expression level remained elevated for 60 m following the addition of serum. Fluorescence staining analysis indicated that these cells were positive for human smooth muscle α-actin, but negative for leukocyte antigen CD45 and platelet marker CD42d suggesting that the myofibroblasts cells themselves were the source of the TSP-1.

CONCLUSIONS: Unlike skin wounds, where TSP-1 is derived from the blood macrophages, monocytes and platelets, differentiated myometrial cells appear to produce TSP-1. Elucidating the roles of TSP-1 in myometrium physiology and pathobiology will increase our understanding of the etiology of uterine fibroids and may lead to improved therapies. Further studies utilizing this cell model to determine the role of TSP-1 in the activation of TGFβ-3 are indicated.

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Invasion of Ovarian Cancer Cells Is Modulated by the Bioactive Phospholipid S1P Via Gi, Rac and Rho Pathways. Yoel Smicun, Armando Wu Pimentel, Jennifer Gilman, David A Fishman. *Obstetrics & Gynecology, New York University School of Medicine, New York, NY, USA.*

Objectives: Sphingosine-1-phosphate (S1P) levels are elevated in serum and ascites of ovarian cancer patients. We have demonstrated that low concentration S1P enhances while high dose S1P inhibits invasion of epithelial ovarian carcinoma (EOC) cells in a dose and attachment mode dependent manner. We sought to further dissect the pathways by which S1P affects invasion, using specific inhibitors.

Methods: Dov13 EOC cells were pretreated for 4-hrs with vehicle, 0.5μM or 20μM S1P and with inhibitors for Gi, p38-MAPK, Rac and ROCK, thereafter cells were detached and tested for invasion towards 40μM LPA chemoattractant in Matrigel-coated chambers. Conditioned media from pretreated cells and invading cells were quantified for uPA activity using colorimetric assays. The significance of results was calculated by student's t-test.

Results: Inhibition of Gi mildly increased invasion of both control (p=0.019) and 20μM S1P treated cells (p=0.0002). Inhibition of both p38-MAPK and Rac did not affect 20μM S1P treated cells, in contrast invasion of control cells was mildly increased (p=0.025, 0.034). Inhibition of ROCK, a protein effector downstream of Rho, highly elevated invasion of both control and 20μM S1P treated cells (8 fold, p=0.0035, 23 fold, p=0.00037). Both uPA and gelatinase activities were higher in conditioned media of invading cells than of attached cells. Gelatinase activity was enhanced by both concentrations of S1P (p=0.002, 0.001). PTX fully inhibited gelatinase activity of control and 0.5μM S1P treated cells, and partially of the 20μM S1P treated cells (p<0.00007). 0.5μM S1P significantly increased uPA activity of attached (P=0.011) but not of invading cells. This increase was sensitive to PTX and Rac inhibitor. 20μM S1P inhibited uPA in both attached and invading cells (P=0.0004), this inhibition was ROCK dependent.
Conclusions: These findings suggest a strong inhibition of invasion and uPA by the Rho pathway, of both control and 20μM S1P treated cells. This inhibition is induced partially by upstream Gi protein. Increased invasion by 0.5μM S1P is associated with elevation of gelatinase activity through Gi, Rac and Rock pathways. This suggests that attached cells and invading cells affect uPA activity through different pathways.

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S1P Signaling Via the Gi and Rac Pathways Affects Differently Invading Than Attached Ovarian Cancer Cells. Yoel Smicun, Armando Wu Pimentel, Jennifer Gilman, David A Fishman. *Obstetrics & Gynecology, NYU School of Medicine, New York, NY, USA.*
Objectives: Sphingosine-1-phosphate (S1P) levels are elevated in serum and ascites of epithelial ovarian cancer (EOC) patients. We have demonstrated that invasion of attached EOC cells differentially react to S1P as compared to invading cells. We examined the impact of the inhibitors for Gi and Rac on attached and invading EOC.
Methods: Dov13 EOC cells were pretreated for 4-hrs with vehicle, 0.5μM or 20μM S1P and with inhibitors for Gi (pertussis toxin (PTX)), and Rac (NSC23766, (Rac-I)), and cells were detached and assayed for invasion towards 40μM LPA in Matrigel-coated chambers. To distinguish the response of attached from invading cells, inhibition of cells pretreated with inhibitors was either continued or not in the invasion chambers. Conditioned media (CM) of invading cells were quantified for uPA and gelatinase activity by fluorometric and colorimetric assays. Significance of results was calculated by student's t-test.
Results: The significant (p=0.004) increase of invasion by 0.5μM S1P was inhibited by both PTX and Rac-I, either by pretreatment alone or by continuous treatment (p=0.0024-0.015). However, the invasion was higher in cells inhibited continuously than cells inhibited only in dishes. Both inhibitors did not affect cells treated with 20μM S1P. Control cells invasion was increased (2.3 fold, p=0.0019) by continuous Rac-I treatment. 0.5μM S1P increased gelatinolysis in CM of invading cells. This and control cells activity was inhibited by PTX pretreatment. Continuous treatment with PTX or Rac-I elevated 3 and 2 fold gelatinase activity of control and 20μM S1P treated cells. In contrast uPA activity was inhibited by both 0.5μM and 20μM S1P. Activity of control cells was inhibited by both pretreatment and continuous treatment with both PTX and RacI. uPA activity of cells treated with 0.5μM S1P was increased only by pretreatment with PTX and RacI. In contrast, in cells treated with 20μM S1P uPA was inhibited only by continuous inhibition with PTX and RacI.
Conclusions: Invasion of S1P induced EOC cells correlated with the gelatinase and uPA activities in their microenvironment. PTX and Rac-I affect attached and invading cells in different manner, inhibition of invasion and gelatinolysis of attached and increased invasion of invading cells. This suggests a dual effect of the Gi-Rac pathway, inhibiting attached and stimulating invasion via gelatinolysis of invading cells.

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EGFR Kinase Activity Is Required for LPA Stimulation of Ovarian Cancer Cell Dissemination. Thuy Vy Do,[1] Orlando D Gil,[2] Madhavi Tady,[1] Jay Symoviwcz,[1] David Fishman.[2] *[1]OB/GYN, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [2]OB/GYN, New York University School of Medicine, New York, NY, USA.*
Lysophosphatidic acid (LPA) levels are elevated in the ascites and plasma of early- and late-stage ovarian cancer patients, underscoring the unique role this bioactive lipid plays in the pathobiology of epithelial ovarian cancer (EOC). LPA binding to its cognate G-protein-coupled receptor can transactivate the receptor tyrosine kinase, EGFR, which is often overexpressed in ovarian tumors. In the current study, we investigated the role that LPA activation of EGFR plays in the processing of the metalloproteinase, MMP-2, and EOC dissemination. LPA stimulates tyrosine phosphorylation of EGFR in ovarian cancer cells, and EGFR kinase activity is required for optimal LPA induction of pro-MMP-2 activation. LPA-dependent pro-MMP-2 activation does not require the EGFR ligands, amphiregulin, b-cellulin, EGF, HB-EGF, and TGF-a. We previously reported that when cells are cultured at high density, LPA represses RhoA activity to induce loss of stress fibers, and these changes in actin microfilament organization contribute to pro-MMP-2 activation. In the current study, inhibition of Rho-kinase/ROCK with Y-27632 reversed the repression of LPA-stimulated pro-MMP-2 processing observed with treatment of the EGFR kinase inhibitor, AG1478. Correspondingly, LPA induced the loss of stress fibers, while inhibition of EGFR kinase restored stress fiber formation in LPA-treated cells. This suggests that LPA acts through EGFR to modulate microfilament organization and pro-MMP-2 processing. Finally, LPA-induced cellular haptotactic migration and invasion are abrogated when EGFR kinase activity is blocked. Taken together, these results suggest that EGFR signaling plays a critical role in LPA regulation of metastatic pathways by mediating changes in the cytoskeleton which impact protease activity.

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Heterotypic Signaling by Membrane Derived Vesicles from Mesothelioma, Breast and Ovarian Cancer Induces Inhibition of Cellular Invasion. Edgardo V Ariztia, Maria Bartellino, Jill Whyte, Joanie Mayer Hope, David A Fishman. *OB/GYN, NYU School of Medicine, New York, NY, USA.*
Objectives: Membrane-derived vesicles are active modulators of tumor dissemination; they promote and contribute to extracellular matrix degradation and tumor cell invasion. We have isolated vesicles from ascites of ovarian cancer patients and from ovarian cancer cells *in vitro*. Here we analyzed the functional consequences of exposure of cancer cells to vesicles derived from a different type of malignancy in order to evaluate their proinvasive properties.
Methods: Ovarian cancer (DOV13), breast cancer (MDA MB 231) and mesothelioma (HP-1) cells were grown in media supplemented with 10% FBS. After serum deprivation, 10% vesicle-free FBS was added for 4 hr to induce vesicle release. Vesicles were isolated from media by differential centrifugation and quantified with a Bradford assay. Fusion to cells was followed by fluorescence of the lipophilic tracer DiI. Each cell line was stimulated with 1 and 10 μg/ml of each type of vesicles and tested in a Matrigel invasion assay. Changes in proliferation were evaluated in an MTS assay. Gelatin zymography was used to assess matrix metalloproteinase activity of vesicles.
Results: Zymographic analysis showed that vesicles contained MMP-2 and 7. Fusion experiments showed that all vesicles fused to all cell types. All vesicles induced the invasion of their respective cell types in a dose-dependent manner. When vesicles were used at 1 μg/ml they induced significantly high levels of invasion in all cell types tested. At 10 μg/ml they significantly inhibited invasion in different cell types. Both concentrations of vesicles stimulated cell proliferation in all cell types tested.
Conclusions: Membrane derived vesicles are potent mediators of invasion for mesothelioma, breast and ovarian cancer cells *in vitro*. This effect occurs in a dose-dependent manner when vesicles induce invasion of the same cell type from which they were isolated. When vesicles are used to induce a different cell type, low concentrations induce invasion while high concentrations inhibit invasion, this effect was independent of cellular proliferation. These results suggest that a novel mechanism may be at play where activation of recognition of self and non-self specific pathways may determine the invasive potential of the tumor cell upon fusion to microvesicles. The identification of signaling pathways responsible for this heterotypic signaling is a current effort.

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VEGFR-2 as a Potential Target To Inhibit LPA-Induced Epithelial Ovarian Cancer (EOC) Invasion. Sonia Dutta, Fengqiang Wang, Elaine Barfield, David A Fishman. *Obstetrics and Gynecology, New York University School of Medicine, New York, NY, USA.*
Objective: VEGF and VEGF receptors (VEGFRs) play important roles in ovarian cancer metastasis. In this study, we examined the expression profiles of VEGF and VEGFRs (VEGFR1, VEGFR2, co-receptor NRP1 and NRP2) in established EOC cells lines (DOV13, R182, OVCA429, SKOV3) and an immortalized normal ovarian epithelium (IOSE-29). The effect of lysophosphatidic acid (LPA) on VEGF and VEGFRs expression and the effect of VEGFR-2 silencing by RNAi on LPA-induced invasion were also evaluated.
Methods: VEGF and VEGFRs expression in ovarian cancer cell lines and normal ovarian epithelium was quantified by real time PCR. Cell invasion

was measured by matrigel invasion assay. Small interference RNA targeting VEGFR-2 were predesigned by Ambion Inc. and cells were transfected using Ambion siPORT NeoFX according to the optimized protocol.
Results: Of the four receptors (VEGFR-1, VEGFR-2, NRP-1 and NRP-2), VEGFR-2 was the predominant receptor that expressed in the more invasive cell lines (DOV13, SKOV3 and R182). LPA, at 10-20 μM, significantly induced VEGFR-2 expression in DOV13 and SKOV3 cells (P<0.05), without significantly affecting VEGFR-1 expression. LPA (1-20 μM) also significantly induced the expression of $VEGF_{121}$ and $VEGF_{165}$ (P<0.05) in DOV13 and SKOV3 cells. By small interference RNA (siRNA) transfection, we demonstrated that inhibition of VEGFR-2 expression could significantly decrease DOV13 cells' invasiveness (P<0.001) and moderately decrease SKOV3 cells' invasiveness. Moreover, silencing of VEGFR-2 by siRNA significantly suppressed LPA-induced DOV13 and SKOV3 invasion.
Conclusion: These results suggest that knocking down VEGFR-2 expression by RNAi may be an effective strategy to inhibit LPA-induced ovarian cancer metastasis.

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MMP-1, MMP-7, and Osteopontin Over-Expressed in Epithelial Ovarian Cancer. Fengqiang Wang, David Fishman. *Obstetrics and Gynecology, New York University School of Medicine, New York, NY, USA.*
Objectives: Expression of matrix metalloproteinases, such as MMP-1 and MMP-7, has implicated in epithelial ovarian cancer (EOC) invasion and metastasis. Osteopontin (OPN) was also expressed in various human cancers and associated with tumor progression, invasion and metastasis. In the present study, we examined the correlation of MMP-1, MMP-7 and osteopontin expression with tumor stage in 41 ovarian tumor tissues and 7 normal ovaries using a commercial tissue scan array.
Methods: Complimentary DNA (cDNA) from normal ovaries (n = 7) and ovarian tumors (n = 41) of different stages (stage I, n = 16; stage II, n = 3; stage III, n = 19; stage IV, n = 3) was amplified by real time PCR using gene specific primer pairs for MMP-1, MMP-7, and osteopontin. GAPDH was also amplified as a reference control. The expression level of MMP-1, MMP-7 and osteopontin was calculated as relative expression normalized to that of GAPDH in each tissue sample, and the expression in sample that has the lowest target gene expression was arbitrarily set as 1.
Results: The average expression of MMP-7 in ovarian tumor tissues (14630 ± 30606) is 49 fold of that in normal ovaries (301 ± 641, P = 0.0047). Using an arbitrarily set standard, 1 of 7 normal ovaries (14.3%) has elevated MMP-7 expression; in ovarian tumor tissues, the percentage is 82.9% (34 of 41), significantly higher than in normal tissues (P = 0.0008). In early stage tumors (stage I/II, n = 19), 17 of them have elevated MMP-7 expression (89.4%, P = 0.0008 vs. normal ovaries), whereh the average expression of MMP-7 is 63 fold of that in normal ovaries (P = 0.069) and 1.8 fold of that in late stage tumors (stage III/IV, n = 22). The MMP-1 expression in ovarian tumors (n = 41, 154 ± 632) is 28 fold of that in normal ovaries (n = 7), however, the difference is not significant (P > 0.05) due to the extremely high expression of MMP-1 in two tumor tissues. Osteopontin expression in ovarian tumor tissues is 11.2 fold of that in normal ovarian tissues (11 ± 12.51, n=7 vs. 123.6 ± 160.0, n = 41, P<0.0001). In early stage (I/II) ovarian tumors, osteopontin expression is 8.1 fold of that in normal ovaries, while in late stage (III/IV) ovarian tumors, its expression is 13.9 fold of that in normal ovaries (P<0.05), suggesting an increase trend associated with disease stages.
Conclusions: Our results suggest that MMP-1, MMP-7 and osteopontin alone or combined may have clinical value for ovarian cancer detection.

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Microtubule-Interacting Agents Increase α-Tubulin Acetylation and Cell Surface Expression of Epithelial Cell Adhesion Molecules in Ovarian Cancer Cells. Shohreh Shahabi,[1] Pascal Verdier-Pinard,[2] Mohamed A Borghan,[1] Gary L Goldberg,[1] Susan B Horwitz.[2] *[1]Obstetrics & Gynecology and Women's Health, Albert Einstein College of Medicine, Bronx, NY, USA; [2]Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA.*
Objectives: α-Tubulin acetylation has been proposed to control the dynamic nature of microtubule stability. Acetylated α-tubulin plays a role in regulating microtubule functions in mitosis and cell migration. Here, we sought to identify the relationship between post-translational α-tubulin acetylation and the expression of epithelial cell adhesion molecules (Ep-CAM) after exposure to microtubule interacting agents in ovarian cancer cells.
Methods: Epithelial ovarian cancer cell line, Hey was treated with microtubule stabilizing agents (Taxol, Epothilone B and Discodermolide), microtubule-destabilizing agent (Vinblastine), HDAC6 inhibitor Trichostatin A (TSA), anti-metabolite Fluorouracil (5FU), or alkalyting agents (Cisplatin and Carboplatin). Cells were separately treated with either IC_{50} or 10-fold IC_{50} of each agent, and incubated at 37°C for 1 h, 24 h and 48 h. Acetylation of α-tubulin and pan- α-tubulin were evaluated by Western blot analysis followed by protein quantification. Cell surface Ep-CAM expression was examined by flow cytometry.
Results: Increased acetylation of α-tubulin was seen with Taxol, Epothilone B, Discodermolide, Vinblastine and TSA. α-tubulin acetylation was time and dose dependent. The highest level of α-tubulin acetylation (2.5-fold) was observed with Vinblastine at 10-fold IC_{50} after 48 h. Exposure to Microtubule interacting agents and TSA resulted in increased cell surface expression of Ep-CAM in a time and dose dependent manner. The highest level of cell surface Ep-CAM expression (8.5 fold) was observed with 10-fold IC_{50} of Vinblastine at 48 h. The increase in acetylated α-tubulin and Ep-CAM expression was clearly detectable after 1 h treatment. This data reveals a similar dose and time dependent increases between the acetylation of α-tubulin and the increase of Ep-CAM expression.
Conclusions: These data demonstrate the promotion of α-tubulin acetylation and cell surface Ep-CAM expression by a microtubule destabilizing agent and by microtubule stabilizing agents. Interestingly, Vinblastine induces the highest α-tubulin acetylation and Ep-CAM expression. Acetylation of alpha-tubulin may be associated with redistribution of cell surface antigens in ovarian cancer cells.

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Role of Phosphorylated p38 Mitogen-Activated Protein Kinase in Epothilone B-Induced p53 Activation and Apoptosis. Shohreh Shahabi,[1] Chia-Ping H Yang,[2] Mohamed A Borghan,[1] Susan B Horwitz,[2] Gary L Goldberg.[1] *[1]Department of Obstetrics & Gynecology and Women's Health, Division of Gynecologic Oncology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY, USA; [2]and Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA.*
OBJECTIVE: Epothilone B (EpoB) is similar to Taxol in its ability to enhance tubulin polymerization and to stabilize microtubules. EpoB is currently being evaluated as an antitumor agent and is in phase III clinical trials.
The tumor suppressor gene, p53 plays an important role in the induction of apoptosis by a variety of anticancer drugs. P38 Mitogen-Activated Protein Kinase is activated by a wide array of stress stimuli including chemotherapeutic agents and promotes apoptosis. Since both p38 and p53 activation induces apoptosis, we hoped to evaluate the relationship between p38, p-p53 and PARP cleavage, an early indicator of apoptosis, in ovarian cancer cells after treatment with EpoB.
METHODS: Hey cells were treated with EpoB (5 to 200nM) for 16h. PARP cleavage product p85 as well as p-p38, p53, phospho-p53 (Ser-15), p21 and survivin were determined by Western blot analysis. A wild type ovarian cancer cell line Hey, was treated with or without 5μM SB203580, a specific inhibitor of p-p38, followed by treatment with EpoB (20 or 50 nM) for 16h. Lysates were prepared and Western blot analysis was performed with polyclonal phospho-p38 and anti-phospho-p53 antibodies.
RESULTS: EpoB induces p53 activation and apoptosis demonstrated by increased PARP cleavage product. Time course studies indicated that phosphorylation of p53 precedes phosphorylation of p53 in Hey cells. The expression of p53 targeted gene, p21 (Survivin) were differentially expressed depending on the dose of EpoB and the duration of drug exposure. Pretreatment with specific inhibitor of p38 markedly inhibited the p53 phosphorylation at serine 15.
CONCLUSIONS: 50 nM EpoB (a concentration that triggered mitotic arrest) causes a decrease in p21 expression and an increase in Survivin expression. EpoB induces PARP cleavage. P38 inhibitor SB203580 inhibits Epo-B -induced p53 phosphorylation. These results suggest that phosphorylation of p38 may lead to p53 activation and these signaling events may be related to Epo-B induced cell death in ovarian cells.

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A Functional TLR-4-MyD88-NF-κB Pathway in Epithelial Ovarian Cancer Cells Induces a Pro-Tumor Phenotype in THP-1 Monocytic Cells. Ayesha B Alvero, Michele K Montagna, Paulomi B Aldo, Gil Mor. *Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*
Background: How tumor cells interact with the immune system will determine the nature of immune response. Monocytes/macrophages have the capacity to

differentiate into either a pro-tumor or anti-tumor phenotype depending on the specific tumor microenvironment. Previously, we described a subgroup of epithelial ovarian cancer (EOC) cells with a functional TLR-4-MyD88-NF-κB pathway (Type I EOC cells). These cells have constitutive NF-κB activity and constitutively secrete IL-6, IL-8, MCP-1, and GROα. We hypothesize that Type I EOC cells, but not Type II, can promote macrophage differentiation into a tumor-supporting immune cell.
Methods: EOC cell conditioned media (CM) was prepared by incubating EOC cells in log-phase growth in OptiMem for 48h. Migration assay was done using an in vitro cell culture insert with 8 μM-size pore and PKH26 Red Fluorescent-labeled THP-1. Cytokine profile of THP-1 cells co-cultured with EOC cell CM was determined using xMAP technology. Modulation of response to apoptotic bodies was determined by "pre-educating" THP-1 cells with EOC cell CM for 24 h prior to exposure to apoptotic bodies (1:1 ratio with THP-1 cells).
Results:
Monocytes migrate toward EOC cells. However, migration is significantly higher towards Type I EOC cells. Type I, but not Type II, EOC cells alter monocytes' cytokine profile by inducing the secretion of high levels of pro-growth and angiogenic cytokines IL-6, IL-8, MCP-1, and GROα. Furthermore, Type I EOC cells modify monocytes response to apoptotic bodies by inducing a significant increase in the secretion of IL-6, IL-8, MIP-1α, MIP-1β, and GROα (18-fold, 2.5-fold, 2-fold, 3-fold, and 11-fold respectively).
Conclusion: We demonstrate for the first time a differential interaction between two subtypes of EOC cells and monocytes. We showed that the microenvironment created by Type I EOC cells is able to modify the function and differentiation of immune cells towards a tumor supporting phenotype. Understanding the molecular mechanisms mediating this tumor-immune interaction will help to design appropriate immune therapies that will take into consideration the tumor microenvironment.

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Multiplex Serum Tumor Markers for the Prediction of Early Relapse in Ovarian Cancer Patients. Karina D Steffensen,[1] Marianne Waldstrom,[2] Irene Visintin,[4] Ivan Brandslund,[3] Anders Jakobsen,[1] Gil Mor.[4] *[1]Oncology, Vejle Hospital, Vejle, Denmark; [2]Pathology, Vejle Hospital, Denmark; [3]Biochemistry, Vejle Hospital, Denmark; [4]OB/GYN/Reproductive Sciences, Yale University School of Medicine, New Haven, USA.*
Objectives: The standard of treatment for patients with ovarian cancer (OC) is intravenous combination chemotherapy (CT) after primary cytoreductive surgery. Although initial response is above 80%, most of these patients experience recurrence. The only approach for these patients is salvage CT which may prolong their lives for months. Early detection of patients who are not responding to current therapy or are at risk of experiencing an early relapse of disease might improve response rates and survival if alternative therapy is possible. No single predictive marker has yet been proven sufficiently sensitive or specific to find a place in the daily clinic. In the present study we use a newly described biomarker test for the detection of OC (Visintin et al 2007 Clinical Cancer Research) and evaluated the ability of the test to monitor CT response
M**ethods:** 55 patients with OC, FIGO stage I-IV, were included in this study. All patients recieved postsurgery first line combination CT (paclitaxel/carboplatin). Samples were evaluated at 1) baseline (mean 35 days after surgery), prior to the first cycle of CT, and 2) after 3 cycles of CT. 20 μL serum samples were analyzed by multiplex assay (Beadlyte® Cancer Biomarker Panel Kit) for six markers. Changes during CT and differences in markers between patients with short time to progression (TTP) and patients with long TTP were determined.
Results: Positive test for OC was observed in 96 % of the patients evaluated at baseline. All patients had residual disease after surgery. From patients with long TTP, 15/20 patients (specificity 75 %) had a negative test after 3 cycles. From the patients with short TTP, 13/14 had a positive test (sensitivity 93 %) P=0.000096 (χ^2). The risk of experiencing a TTP shorter than 6 months when having a positive test after 3 cycles of CT was 72 %.
Conclusion: We describe for the first time the use of a panel of biomarkers for OC that might have an application for monitoring CT response and risk of relapse. The test detects the presence of residual disease following debulking surgery, and differentiates between long term and short term progression. A longitudinal study is performed to determine how early during CT the test can identify responder versus non responder.

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KSP Inhibitor (ARRY-520) as an Alternative for Paclitaxel in MyD88-Positive Epithelial Ovarian Cancer Cells. Ki H Kim,[1] Ayesha B Alvero,[1] Yanhua Xie,[1] David Trollinger,[2] Gil Mor.[1] *[1]Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA; [2]Array BioPharma Inc., Boulder, CO, USA.*
Background: We previously described that epithelial ovarian cancer (EOC) cells ubiquitously express TLR-4, but not the adaptor protein MyD88. When treated with Paclitaxel, a known TLR-4 ligand, MyD88-positive EOC cells exhibited NF-κB activation, increased secretion of IL-6, IL-8, MCP-1, and GROα, and increased p-ERK levels . Since majority of EOC patients are given Paclitaxel in combination with a platinum drug, it is not only important to distinguish those patients that should not be given Paclitaxel; it is also important to identify alternative chemotherapy agents that would benefit this sub-group of patients. The objective of this study is to determine if the KSP inhibitor, ARRY-520, can be an alternative for Paclitaxel in MyD88-positive ovarian cancer patients.
Methods: MyD88-positive and MyD88-negative EOC cell lines isolated from either ascites or tumor tissue were treated with increasing concentrations of ARRY-520 (3 to 3000 nM) or Paclitaxel (0.2 to 20 μM) for 24, 48, and 72 hours and cell viability was determined using the CellTiter 96 AQueous One Solution Cell Proliferation Assay. Cytokine profiling was performed from supernatants using xMAP technology. NF-κB activity was determined using a Luciferase reporter system. p-ERK levels were measured by Western blot analysis.
Results: ARRY-520 and Paclitaxel exhibited the same cytotoxic effect on MyD88-negative and positive EOC cells. The IC_{50} at 48h for MyD88-negative EOC cells was ≤0.003 μM and ≤0.2 uM for ARRY-520 and Paclitaxel, respectively. For MyD88-positive EOC cells, the IC_{50} at 48h was ≥3 μM and ≥20 μM for ARRY-520 and Paclitaxel, respectively. However, unlike Paclitaxel, ARRY-520 did not induce NF-κB activation or enhance the secretion of IL-6, IL-8, MCP-1, and GROα , and did not induce ERK phosphorylation on MyD88 positive cells.
Conclusions: Administration of paclitaxel to patients with a MyD88-positive tumor could have detrimental effects due to the paclitaxel-induced enhancement of cytokine production which promotes chemoresistance and tumor growth. ARRY-520 has similar anti-tumor activity in EOC cells as that of Paclitaxel. However, unlike Paclitaxel, it does not induce cytokine production in MyD88-positive EOC cells, and therefore, the KSP inhibitor ARRY-520 may represent an alternative to Paclitaxel in this subgroup of EOC patients.

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Eriocalyxin B Induces Apoptosis of Chemoresistant Ovarian Cancer Cells through NF-κB Inhibition. Aliza Leiser,[1] Han-Hsuan Fu,[1] Rui Chen,[1] Ayesha Alvero,[1] Yung-Chi Cheng,[2] Gil Mor.[1] *[1]Department of Obstetrics, Gynecology and Reproductive Science, Yale University School of Medicine, New Haven, CT, USA; [2]Department of Pharmacology, Yale University School of Medicine, New Haven, CT, USA.*
Introduction:
NF-κB activation has been associated with cell proliferation, angiogenesis, metastasis and suppression of apoptosis in ovarian cancer. In addition, NF-κB activity induces the production of pro-inflammatory cytokines which may contribute to chemoresistance. Inhibition of NF-κB may represent a new approach to prevent tumor growth and reverse chemoresistance. In the present study we described the characterization of a novel NF-κB inhibitor, Eriocalyxin B (EriB) that is able to re-establish the apoptotic cascade in chemoresistant ovarian cancer cells by suppressing pro-inflammatory cytokines and anti-apoptotic proteins.
Materials and Methods:
EOC cell lines were isolated from malignant ovarian cancer ascites. Caspase activity was determined by Caspase-GloTM assay. Cytokine production and secretion were determined using Multiplex assay. EriB effect on cancer cells was evaluated in a time and dose dependent manner using CellTiter 96 Cell Proliferation Assay. Protein expression was determined by Western blot analysis. Combination studies were done with paclitaxel and carboplatin in addition to EriB treatment. NF-κB activity was determined by monitoring the expression of a NF-κB luciferase reporter.
Results:
EriB decreased cell viability of ovarian cancer cells with an IC50 of 0.5-1μM in 48 hours and was associated to increasing levels of caspases 8,9 and 3 activity. Intracellular changes induced by EriB included: 1) inhibition of NF-κB activity; 2) decrease in cytokine production; 3) down regulation of anti-apoptotic proteins XIAP and FLIP, and reversal of resistance to TNF-α and FasL-mediated cell death; and 4) chemosensitization to carboplatin and paclitaxel.

Conclusions:
NF-κB has been shown to control the expression and function of anti-apoptotic proteins and pro-inflammatory cytokines. In the present study we demonstrated that specific inhibition of NF-κB by EriB reversed the anti-apoptotic state of chemoresistant ovarian cancer cells, therefore may provide a new potential venue for the treatment of ovarian cancer patients.

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Expression of Intermedin (IMD) in Rat and Human Cervix: Increased Expression in Cervical Cancer. Madhu Chauhan, Chandra Yallampalli. *Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX, USA.*

BACKGROUND: Intermedin (IMD)/Adrenomedullin2 is a CT/CGRP family peptide. IMD is expressed in a variety of tissues such as pituitary, stomach, placenta, uterus, and ovary .We have shown that IMD plays a role in a variety of physiological functions, including vasodilatation and fetoplacental growth regulation. Further we have data indicating an angiogenic activity of IMD in first trimester trophoblast cells. We hypothesize that IMD is expressed in cervix and may have a role in the pathology of cervical cancer

OBJECTIVE: 1) to analyze expression of IMD mRNA in human cervix, rat cervix from non-pregnant and pregnant rats; 2) to assess the differences in the expression of IMD in normal human cervix and cervical carcinoma tissues and 3) to analyze the effect of IMD on the expression of TNF-alpha and IL-1beta in human epithelial cervical carcinoma cells (HELA).

METHODS: Groups of 4 Sprague-Dawley, non-pregnant and pregnant rats on day 18 of gestation were used in this study. Cervical tissues were collected from the non-pregnant and pregnant rats, women undergoing hysterectomy and from women diagnosed with cervical carcinoma. Total RNA was extracted using Trizol method. HELA cells were grown to 80% confluency in RPMI medium supplemented with 10% FBS. The cells were starved in 1%FBS for 4 hrs followed by treatment with IMD (10^{-8}M) in presence or absence of IMD antagonist, IMDA (10^{-5}M). Cells were further incubated for 24hrs and total RNA was extracted using Trizol reagent. RNA was treated with DNase1 before performing the reverse transcriptase polymerase chain reaction (RT-PCR). The RT-PCR data was normalized to that of 18S.

RESULTS: 1) IMD mRNA is expressed in rat and human cervix and in HELA cells. 2) Expression of IMD is elevated in pregnant rat cervix as compared to the non-pregnant. 3) IMD has no effect on TNF-alpha expression in HELA cells treated with IMD but caused a decline in the expression of IL-1beta mRNA and, 4) expression of IMD mRNA is significantly elevated ($p< 0.05$) in cervical carcinoma as compared to the normal cervix.

CONCLUSION: IMD is expressed in both rat and human cervix and is elevated in pregnant rat suggesting that it may have a role in cervical function during pregnancy in rat. In addition we demonstrate that IMD is involved in the pathology of cervical carcinoma and thus may have a clinical significance in the pathology of cervical cancer.

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Evaluation of the Impact of Treatment Delays and Dose Reductions on Tumor Response in Human Ovarian Cancer Xenograft Models. Jing Hong,[1] Jenifer M Saucier,[2] Anjali Gaikwad,[2] Jubilee Brown,[2] Judith A Smith.[1,2,3] *[1]Division of Pharmacy, UT MD Anderson Cancer Center, Houston, TX, USA; [2]Gynecologic Oncology, UT MD Anderson Cancer Center, Houston, TX, USA; [3]Obstetrics, Gynecology and Reproductive Sciences, UT Health Sciences Center at Houston Medical School, Houston, TX, USA.*

Objective: There is minimal information about the impact of treatment delays or dose reductions on chemotherapeutic treatment responses and overall outcomes in ovarian cancer patients. The primary objective of this study was to quantify the impact of treatment delays and dose reductions in an *in vivo* xenograft ovarian cancer model and to evaluate if the growth factors could improve overall survival.

Methods: HeyA-8 and SKOV3-IP Xenograft mouse models to determine the effect of treatment delays or dose reductions on tumor response.

Results: The results indicated that full dose of paclitaxel (20 mg/kg) and carboplatin (50 mg/kg) delivered on timeevery 21 daysachieved better tumor response in both aggressive (HeyA-8) and metastatic (SKOV3-IP1) human ovarian tumors compared to the non-treatment and vehicle groups. In HeyA-8 mice, paclitaxel/carboplatin alone and paclitaxel/carboplatin followed by growth factor support agents including pegfilgrastim (1000 μg/kg) and darbepoetin (10μg/kg) improved overall survival rate. Growth factors also improved the tolerability in HeyA-8 model. For SKOV3-IP1 mice, the treatment delays resulted in a significant reduce in overall survival time compared to full dose, on time treatment ($P< 0.01$). For the Dose reduction group, there was a significant different survival comparing to full dose, on time treatment ($P< 0.05$).

Conclusion: Treatment delays had a negative impact on tumor response and overall survival compare with treatment controls. In addition, use of growth factor agents also improved treatment response and tolerability of chemotherapy and ultimately overall survival.

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Elevated hCG Levels in Placental Site Tumors Decreasing Risk of Breast Cancer. Giuseppe Del-Priore,[1] Mitchell R Sierecki.[2] *[1]Obstetrics and Gynecology, New York Downtown Hospital, New York, NY, USA; [2]Medicine, New York Downtown Hospital, New York, NY, USA.*

Purpose
We hypothesize that women diagnosed with choriocarcinoma have a decreased risk of breast cancer as a result of exposure to high levels of hCG.
Materials and Methods
We utilized Surveillance, Epidemiology, and End Results (SEER) cancer registry to prove our hypothesis. Using SEER limited-use database 1973-2004, a query on placental site tumors (PSTs) (C 58, ICD-10 category) was done by using SEER*Stat case listing sessions. The following demographics were obtained: patient ID, primary site, year of diagnosis, sex, race, DOB, age group and survival months. In a new case listing session, patient ID's diagnosed with PSTs were compared to women diagnosed with subsequent breast cancer. Cohort with diagnosis of PSTs and subsequent breast cancers are compared to general population-based rate of breast cancers. Woman Years (WYs) = total observation months of cohort/12.
Results
A query on PSTs yielded 646 women between the ages15-54 years. Of the 646 women, 422 were white, 129 were African American, and 95 were 'Other'. Total WYs of survival were 7,165.3. Two patients developed breast cancer in 7,165.3 WYs of observation; yielding breast cancer rate of 27.91/100,000 WYs. The incidence rate ratio (IRR) of PST cohort to general population was 0.21 (27.9/128.2; 95% CI (0.145-0.327; $P< 0.05$). Of 642, 485 were under the age of 35 years. Two patients developed breast cancer in 5,862 WYs of observation; yielding breast cancer rate of 34.1/100,000 WYs. The IRR was 0.27(34.1/128.2; 95%CI (0.182-0.386); $P<0.01$).
Conclusion
Women with PSTs had 80% and 73% reduction in breast cancer risk compared to general population and population under 35yrs, respectively. Given the high level of hCG present and decreased rate of breast cancer among women with PSTs, the hypothesis that hCG is protective seems plausible.

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Outcomes of Patients with Recurrent Vulval Squamous Cell Cancer. John B Butler,[1,2] Jane E Bridges,[1] Thomas EJ Ind,[1] John H Shepherd,[1] Suzanne A Eccles,[2] Desmond PJ Barton.[1] *[1]Gynaecology Oncology, Royal Marsden Hospital, London, United Kingdom; [2]Tumour Biology and Metastasis, Institute of Cancer Research, London, United Kingdom.*

Background and objectives:
Vulval Squamous Cell Cancer (VSCC) is a rare cancer and most studies are from single centres with small numbers. This study evaluates the outcomes in a large cohort of patients with recurrent VSCC.
Methods
Case note and histology review was conducted on 512 patients with primary or recurrent disease managed at three cancer centres. Patients with recurrent disease were identified and a dataset created.
Results
144 patients were identified with their first episode of recurrent disease diagnosed between 1988 and 2006. 25% of these patients originally had Stage 1 disease, 36% Stage 2, 26% Stage 3, and 12% Stage 4. Mean length of follow up was 47 months (1-415). Median age at recurrence was 73 (33-104), and median interval to recurrence 13.5 months (1-415). 81 (56%) patients died of recurrent disease and 10 (7%) of other causes. Site of recurrence and was local in 92, groin in 30 and distant in 22. 58 of the local recurrences were managed with wide local excision (WLE) 12 radical surgery (6 radical excisions with skin flaps, 4 posterior exenterations, 1 anterior exenteration, 1 total exenteration), 10 WLE and radiotherapy (RT)/chemoRT, 6 chemo/chemoRT/RT, and 6 palliation. 12 groin recurrences were managed surgically (5 adjuvant RT), 3 RT alone, 1 chemotherapy alone, and 1 palliation. 2 patients with distant metastases were managed surgically, 10 with RT/chemo/chemoRT, and 9 palliation. Overall median survival after recurrence was 17 months. Median survival (months)

by site of recurrence was: local 56, groin 12, distant 4, groin or distant 6. There was a significant difference in survival in local vs groin node recurrence (p<0.0001), local vs groin and distant (p<0.0001), and groin node vs distant (P 0.0075). 3 and 5 years after recurrence survival rates were: local 56% and 46%, groin 13% and 13% distant 0,0.

Conclusion

Patients with VSCC remain at risk of recurrent disease therefore long term follow up these patients is essential. Patients with local recurrence often have a good prognosis. Outcomes for groin and distant recurrences are poor, but a proportion of those with groin recurrences can be salvaged. Therefore early identification of local and groin recurrences is essential for improving outcomes in recurrent VSCC particularly in cases with more conservative management of primary disease.

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BPA and DES Exposure *In Vitro* and *In Utero* Increases EZH2, a Histone Methyltransferase Associated with Uterine and Breast Cancer. Jason G Bromer, Jie Wu, Hugh S Taylor. *Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Introduction: Endocrine disrupting chemicals (EDCs) are environmental agents possessing hormone-like activity. Exposure to EDCs during differentiation can interfere with hormonal signaling, resulting in predisposition to some cancers. It has been suggested that some of these effects may be epigenetic, mediated by changes in DNA and histone methylation. We hypothesized that 2 EDCs, diethylstilbestrol (DES), and bisphenol-A (BPA), may act by altering the expression of DNA and histone methyltransferases (DNMTs and HMTs).

Methods: Ishikawa (endometrial) and MCF7 (breast) cells were cultured in steroid-free, phenol-free media with BPA (25 μM), DES (5 x 10-8 M) or vehicle for 48 hours. Pregnant CD-1 mice were treated with intraperitoneal injections of DES (10 mg/kg) or vehicle on days 9-16 of gestation. 2 weeks after birth, offspring were sacrificed and tissue obtained. mRNA was extracted, cDNA was generated and quantitative real time RT-PCR was performed and normalized to β-actin. All experiments were conducted in triplicate, repeated three times and compared using ANOVA.

Results: 3 DNMTs (DNMT1, 3a, and 3b) and 2 HMTs (MLL and EZH2) were screened after *in vitro* exposure to EDCs (Table 1). EZH2 mRNA expression was significantly increased in Ishikawa cells after treatment with DES or BPA. A similar response in EZH2 expression was seen in MCF7 cells treated with DES or BPA. Due to the consistent induction of EZH2 in all cells with either treatment, we examined the effect of DES exposure on EZH2 expression *in vivo*. In adult mice exposed to DES *in utero*, EZH2 expression was persistently increased (3.0±0.66 fold, p<0.05).

Conclusions: Breast and uterine cell lines show increased expression of EZH2 in response to BPA or DES exposure. This differential expression persists in the adult offspring of mice exposed to DES *in utero*. EZH2 has been identified as a risk for neoplastic progression in the breast and for increased proliferation in uterine cancers. DES induced EZH2 expression may be a mechanism for the increased incidence of breast and reproductive tract cancers seen in DES exposed women.

Fold Induction

GENE	MCF7/DES	MCF7/BPA	Ish/DES	Ish/BPA
DNMT1	0.69*	0.73*	0.78*	0.90
DNMT3a	0.92	0.86	1.26	1.08
DNMT3b	1.08	1.04	0.96	1.07
MLL	1.19	0.95	1.62*	1.17
EZH2	2.67*	2.04*	2.00*	1.48*

* = p<0.05

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Curcumin Exhibits Anti-Tumor Activity in an In Vitro Model of Ovarian Cancer through Suppression of Proliferation and Angiogenesis and Induction of Apoptosis. Xin Tan, Neil Sidell, Ruo-Pan Huang, Robert N Taylor, Ira Horowitz, Fritz Wieser. *Gynecology and Obstetrics, Emory Universty School of Medicine, Atlanta, GA, USA.*

Introduction

Defects in cellular programs that control apoptosis lead to an imbalance of cell proliferation and cell death in ovarian cancer. Recent evidence suggests that the use of some anti-inflammatory drugs decreases risk of ovarian cancer. Natural curcumin-based anti-inflammatory therapies were shown to be beneficial in pre-clinical models of ovarian cancer (Lin et al., 2007). In this study, we examined the effects of plant derived curcumin on cell proliferation, apoptosis, and VEGF expression in cultivated ovarian cancer cells.

Material and Methods

Ovarian cancer IGROV1 cells were cultured under standard conditions to study the effects of curcumin on cell kinetics and on VEGF expression. Cell proliferation was measured by SRB and MTT assays. Apoptosis was determined by measuring cytoplasmic histone-associated-DNA-fragments (Cell Death Detection Elisa, Roche, Germany). VEGF gene promoter-reporter activation and real-time quantitative reverse transcription polymerase chain reaction were used. Conditioned media concentrations of VEGF were measured with a commercially available enzyme-linked immunosorbent assay (Quantikine, R&D Systems, Minneapolis, MN).

Results

We observed that curcumin dose-dependently suppressed cell growth in IGROV1 cancer cells (IC_{50}=10uM). Treated cells showed a 2-3 fold increase in DNA fragmentation compared to controls. Curcumin also resulted in a significant decrease of VEGFmRNA expression and VEGF protein secretion into the conditioned media in a dose-dependent manner.

Conclusions

In this study, we have demonstrated that curcumin induces apoptosis, suppresses growth, and inhibits VEGF gene and protein expression in an ovarian cancer cell line. Experiments are underway to identify specific mechanism of curcumin action. Curcumin may act as a chemosensitizing drug by potentiating the anti-tumor effects of standard treatments including taxols and platins in ovarian cancer.

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ST6Gal I Expression in an Ovarian Cancer Cell Line Alters Function. Daniel R Christie,[1] Faheem M Shaikh,[2] John A Lucas III,[1] Susan L Bellis.[2] *[1]Obsterics and Gynecology, University of Alabama at Birmingham, Birmingham, AL, USA; [2]Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL, USA.*

Introduction: Previous work has shown that an upregulation of the enzyme ST6Gal I, which is responsible for the α2,6 sialylation of β1 integrins, confers a more tumorigenic/metastatic phenotype to colon carcinoma cell lines. It is not known, however, if this is unique to colon cancer, or if it is more broadly applicable to other forms of cancers. Quantitatively increased expression of ST6Gal I has been reported in ovarian cancers versus controls, but no biochemical or functional assays have been described to date.

Objective: Because β1 integrins are involved in cell adhesion and migration, and because metastasis of epithelial ovarian cancers is largely an intraperitoneal dissemination, we hypothesized that upregulation in the expression of ST6Gal I in an ovarian cancer cell line would enhance binding with the extracellular matrix, increase invasiveness, and alter migration.

Methods: In the present study we forced a stable transduction of ST6Gal I into the OV4 ovarian tumor cell line, which we found to be lacking the ST6Gal I enzyme, establishing a parental (Par), an empty lentiviral vector (EV), and an ST6Gal I expressing (ST6) cell line. A collagen I cell adhesion assay was performed and quantified by staining adherent cells and measuring absorbance. A haptotactic collagen I cell migration assay was performed by seeding cells in Boyden chambers lined with collagen I concentration gradient, and quantified with cell staining and absorbance measurement. Cell invasion through a reconstituted basement membrane (Matrigel) was quantified as previously described for the cell migration assay.

Results: We were able to demonstrate successful creation of the OV4-ST6Gal I cell line by Western blot analysis. Functional assays demonstrated increased adhesion to collagen I ($p < 0.05$), increased haptotactic collagen I cell migration ($p < 0.01$), and increased invasiveness ($p < 0.05$) in the ST6 cell line as compared to Par and EV when analyzed by one-way ANOVA.

Conclusion: This initial study into ST6Gal I in ovarian cancer may have future therapeutic implications, and, in addition, lend greater insight into the intraperitoneal dissemination of disease.

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Defining the Gene Signature of Ovarian Tumor-Initiating Cells. Michael D Curley,[1] Christine L Cummings,[2] Petra A Sergent,[1] Surinder K Batra,[3] John S Davis,[4] David T Scadden,[2] Bo R Rueda,[1] Rosemary Foster.[5] *[1]Vincent Center for Reproductive Biology, Massachusetts General Hospital, Boston, MA, USA; [2]Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, USA; [3]Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA; [4]Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, NE, USA; [5]Division of Hematology/Oncology, Massachusetts General Hospital, Boston, MA, USA.*

At present, evidence is accumulating regarding the existence of unique populations of specialized tumor-initiating, stem-like cells within various tumor types of distinct origins. These cancer stem cells (CSC), with characteristics reminiscent of normal stem cells, are thought to be responsible for driving tumor growth. We propose that ovarian cancers arise from CSC, and are using microarray-based technology to identify specific genes/cell surface markers associated with ovarian CSC that will permit distinction of these rare cells from the remaining tumor bulk. To identify unique gene signatures associated with an ovarian tumor-initiating cell population, we have utilized an *in vivo* serial transplantation model. This model selects for primary human ovarian tumor cells with increased tumorigenic capacity, given that time to tumor formation decreases with successive serial transplant despite fewer cells injected. Our initial studies used cells derived from a human ovarian clear cell carcinoma, serially transplanted for three passages in NOD/SCID mice. RNA derived from these transplanted tumors was analyzed on human genome microarrays. From these analyses, several differentially expressed genes were identified. The differential expression noted for potential genes of interest is currently being validated by RT-PCR. Of particular interest, expression of the transmembrane glycoprotein MUC4 was found to increase both at the RNA and protein level with successive transplant of this clear cell carcinoma. Further studies are ongoing to determine the functional significance of MUC4 and other identified differentially expressed genes in ovarian clear cell cancer. In addition, we are carrying out parallel microarray analyses in other ovarian tumor subtypes.

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Ritonavir Inhibits Polycomb Group Protein – EZH2 Mediated Suppression of DNA Repair in Ovarian Cancer Cell Lines. Sanjeev Kumar,[2] Christopher S Bryant,[2] Aamer Qazi,[1] Robert T Morris,[2] Chris Steffes,[1] Madhu Prasad,[1] Donald Weaver,[1] Masood A Shammas,[1] Ramesh B Batchu.[1] *[1]Divison of Surgical Oncology & Developmental Therapeutics; [2]Divison of Gynecologic Oncology, Wayne State University/Karmanos Cancer Institute, Detroit, MI, USA.*

Background

Recent observations suggest a decreased incidence of neoplastic lesions in HIV infected individuals treated with Highly Active Anti-Retroviral Therapy comprised of Protease Inhibitors, such as Ritonavir. The polycomb group protein EZH2 is associated with aggressive human malignancies via transcriptional suppression of DNA repair proteins.

Objective

Objective of the present study was to assess the antineoplastic impact of Ritonavir on epithelial ovarian cancer (EOC) cell lines.

Methods

EOC cell lines (MDAH 2774 and SKOV-3) were treated with serial dilutions of Ritonavir (1-10 mM) dissolved in DMSO. Normal diploid human fibroblasts served as controls. Growth curves, apoptosis and cell cycle analysis were performed with Cell Counting Kit-8, Annexin V and flow cytometry. Signal transduction was studied with western blotting. DNA double strand breaks (DSB) were induced with 0.5 mM Etoposide treatment for 16 hours. Homologous recombination (HR) repair of DNA damage was measured by assessment of Rad51 foci formation in the nucleus of the cells as visualized by fluorescence microscopy according to previously published criteria.

Results

Untreated EOC cells expressed higher levels of EZH2 and lower levels of Rad51 and XRCC2 as compared to controls and in turn, had lower prevalence of Rad51 repair foci formation in response to DSB. Serial treatments of EOC cells with Ritonavir resulted in a decrease in expression of EZH2 and an increase in expression of Rad51 and XRCC2 when compared to untreated EOC cells. After induction of DSB, the Rad51 repair foci formation was significantly more prevalent in EOC cells treated with Ritonavir as compared to untreated EOC cells. In addition, Ritonavir induced apoptosis in ovarian cancer cell lines by down-regulation of Akt pathway and caused G1 cell cycle arrest mediated by down modulating levels of pRB phosphorylation and depleting the cyclin-dependent kinase 4 and 6.

Conclusion

Ritonavir effectively induces apoptosis, cell cycle arrest and improves repair of DNA damage by HR in ovarian cancer cell lines. As impaired HR is a key event in causation and progression of neoplastic lesions, Ritonavir may have a role in chemoprophylaxis and treatment of human malignancies.

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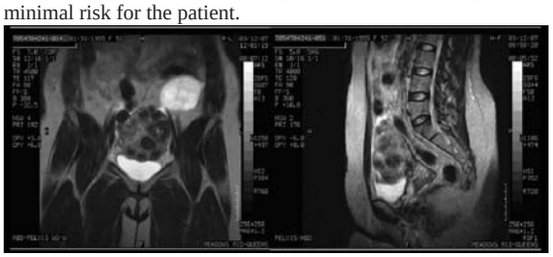
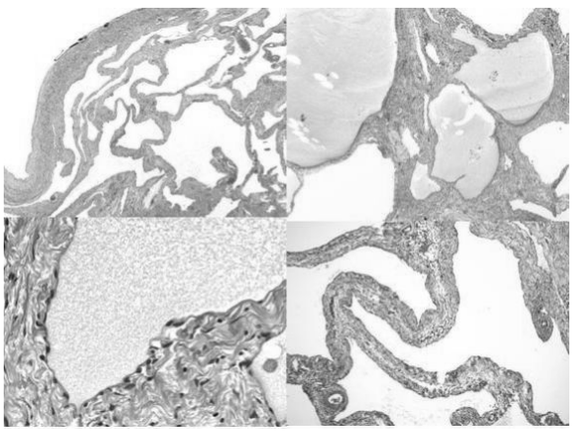
A Rare Case of Ovarian Cystic Lymphangioma. Tomer Singer,[1] Tamer Seckin,[1] Noa Feldman,[1] Susan Jormark,[2] Michael Divon.[1] *[1]Department of Obstetrics and Gynecology, Lenox Hill Hospital, N.Y., NY, USA; [2]Department of Pathology, Lenox Hill Hospital, N.Y., NY, USA.*

CYSTIC LYMPHANGIOMA (CL) is a rare, benign malformation of the lymphatic system whose exact etiology remains uncertain. CL may arise in different sites: the spleen, the mediastinum, the axillary region, the retroperitoneum, and the mesentery. Retroperitoneal CL is extremely rare and its true incidence is unknown. The majority of cases are symptomatic during childhood. Clinical presentation of adult CL is variable and may be misleading. Typically, this is a slow-growing tumor and it remains asymptomatic for a long period of time. It is most often found incidentally during abdominal or pelvic imaging studies, surgeries or autopsies. Total surgical removal of the lesions with microscopically clear margins is the best approach when it is possible. We report, for the first time, a case of cystic lymphangioma arising from the ovary in a post-menopausal woman and present the feasibility and the advantages of laparoscopic surgery, allowing accurate diagnosis, optimal treatment and minimal risk for the patient.





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Significant Genes Involved in Low Grade Ovarian Carcinogenesis. Taymaa May,[1] Monika Sharma,[2] Igor Jurisica,[2] Barry Rosen,[3] Joan Murphy,[3] Theodore Brown,[1] Patricia Shaw.[4] *[1]Department of Ob/Gyn, University of Toronto, Toronto, ON, Canada; [2]Division of Signaling Biology, Ontario Cancer Institute, Toronto, ON, Canada; [3]Division of Gyn Oncology, Princess Margaret Hospital, Toronto, ON, Canada; [4]Department of Laboratory Medicine, University Health Network, Toronto, ON, Canada.*

Introduction: Low Grade Serous Carcinoma (LGSC) is a chemoresistant ovarian neoplasm that has been molecularly linked to Low Malignant Potential Tumor (LMP), which often behaves in a non-invasive fashion. Micropapillary features within LMP (LMP-MP) are associated with increased invasive behavior. The aim of this study was to determine the differential gene expression of LMP, LMP-MP, and LGSC to identify genes involved in malignant transformation and carcinogenesis.

Methods: Laser Capture Microdissection was used to isolate epithelial cells from snap-frozen LMP (n=18), LMP-MP (n=9) and LGSC (n=11). RNA was extracted, amplified, reverse transcribed to cDNA and hybridized to Affymetrix U133 Plus 2 arrays. Data was analyzed by Significance Analysis

of Microarrays (SAM), ArrayAssist and Binary Tree Structured Vector Quantization. Differentially expressed genes were integrated with a database of known & predicted protein-protein interactions (OPHID) and key genes are being validated with real-time PCR and immunohistochemistry.

Results: Unsupervised hierarchical clustering revealed collective clustering of all tumors, irrespective of their pathological classification. SAM analysis has highlighted 47 probe sets as differentially expressed between LMP and LMP-MP, 134 probes between LMP and LGSC, and 180 probes between LMP and LMP-MP+LGSC. No differential gene expression was detected between LMP-MP and LGSC. OPHID analysis demonstrated gene members of the EGFR and MAPK1/3 pathways to be differentially regulated between the non-invasive and the invasive tumors. To date, we have successfully validated 2 members of the MAPK1/3 pathway- Poly ADP ribose polymerase1 (PPOL) and TRAF Family Associated NF Kappa B Activator (TANK)- using real-time PCR.

Conclusion: Our data demonstrate that although the 3 tumors have related genetic profiles, LMP-MP and LGSC are similar to each other and different from LMP, in keeping with their clinical behavior. Members of the MAPK1/3 and EGFR pathways appear to play a key role in low grade serous cancer. Identification of novel genes associated with malignant transformation, may lead to development of more effective targeted therapy for LGSC.

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Significantly Improved Positive Predictive Value for High Grade Cervical Dysplasia Provided by Immunostaining ASC-H Pap Smears for ProEx™C. Andrew Schreiner,[1] Andy Veyliotti,[1] Sarah Wachs,[1] Gayle Countryman,[1] Robert Krum,[2] Terry Morgan.[1] *[1]Pathology, OHSU; [2]Pathology, Kaiser Permanente, Portland, OR, USA.*

Background: Approximately 50% of pap smears with the ambiguous diagnosis of atypical squamous cells, cannot exclude high grade (ASC-H), are negative for dysplasia in follow up colposcopic examination and biopsy. Although HPV testing provides excellent negative predictive value (NPV) (95%), the prevalence of high risk HPV infection is high in young women and the positive predictive value (PPV) in ASC-H pap smears is no better than cytologic diagnosis alone (50%). Recent studies have shown that immunostaining for $p16^{INK4A}$, or ProEx™C, supports a diagnosis of dysplasia in surgical biopsies. Our objective was to determine whether staining for p16 or ProExC provides sufficient predictive value to reliably distinguish high grade dysplasia in ASC-H pap smears.

Design: We retrospectively collected samples from liquid based pap smears diagnosed as ASC-H at either OHSU or Portland Oregon Kaiser Permanente (2005-07). Known HSIL and negative pap cases were included as immunostaining controls. ASC-H cases with followup cervical biopsies (n=90) were included for subsequent immunostaining according to manufacture's instructions. Samples were also sequestered for HPV testing (pending). Immunostained slides were scored as positive or negative by two independent cytopathologists (AS and TM) while blinded to pap diagnoses and surgical biopsy outcomes.

Results: We observed excellent agreement between pathologists' scores (pairwise kappa statistic 0.85). The correlation between p16 and ProExC scores was moderate (kappa 0.40). Chi-square analysis comparing staining to biopsy outcome revealed a significant association between ProEx C positivity and cervical dysplasia (*** P<0.001). Sensitivity and predictive value of ProExC exceeded p16 in ASC-H cases (**p16:** SN 75, PPV 66, NPV 50; **ProExC:** SN 82, PPV 74, NPV 66).

Diagnosis	p16 Negative	p16 Positive	ProExC Negative	ProExC Positive
Negative pap (n=8)	100%	0	75%	25%
ASC-H: Negative Biopsy (n=35)	40%	60%	54%	46%
ASC-H: Dysplastic Biopsy (n=55)	25%	75%	18%	82%***
HSIL pap (n=10)	0	100%***	0	100%***

Comment: Given the known limited sensitivity of colposcopic biopsy (66%), and the poor PPV of HPV testing, immunostaining liquid based pap smears may have an increased role in managing patients with ASC-H diagnoses.

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LH/hCG Dependent Expression of Uterine Matrix Metalloproteinases. Jing Lin, Zhenmin Lei, Ch V Rao. *OB/GYN & Women Health, University of Louisville Health Sciences Center, Louisville, KY, USA.*

Introduction: Matrix metalloproteinases (MMPs) are a family of highly homologous zinc-dependent endopeptidases, which degrade all kinds of extracellular matrix proteins. The degradation process is required for tissue growth and remodelling. These enzymes are probably involved in uterine growth and devolopment and its differentiated functions. Ovarian steroid hormones, estradiol (E_2) and progesterone (P_4), are known to regulate some of the uterine MMPs. Since it is now known that LH/hCG can directly regulate uterine growth and devolopment and its functions, we questioned whether these gonadotropins could also regulate MMPs in the uterus.

Methods: Sixty day old wild type (WT) and LH receptor knockout (LHRKO) mice and 21-day E_2 and P_4 treated 30-day old animals were used. In addition, primary cultures of uterine epithelial cells from WT animals were treated for 24 hrs either with no hormone or with a single or a combination of 100 pg/ml of E_2 or P_4, 100 ng/ml of hCG. Then MMP-2, -7 and -10 mRNA levels were quantified by the semiquantitative RT-PCR.

Results: While the uterine MMP-2 mRNA levels were unaffected, MMP-7 mRNA levels significantly decreased and MMP-10 mRNA levels significantly increased in LHRKO animals as compared with WT siblings. E_2 and P_4 treatment reversed MMP-7 and MMP-10 changes in LHRKO animals. Treatment of WT type primary endometrial epithelial cell cultures with hCG had no effect on MMP-10 mRNA levels, but it did increase MMP-7 mRNA levels. This increase was synergistic with both E_2 or P_4.

Conclusion: While LH/hCG do not regulate uterine MMP-2 and MMP-10, they seem to co-regulate MMP-7 with ovarian steroid hormones.

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Correlation between Androgen Receptor Expression and Response in Patients Conservatively Managed with Endometrial Hyperplasia or Cancer. Radhika Gogoi, Christine Ardalan, Dorota Popiolek, Leslie Gold, John Curtin, Stephanie V Blank, Bhavana Pothuri. *New York University, New York, NY, USA.*

Objective:

Megesterol, a synthetic progestin with strong androgenic properties, is used in the medical management of patients (pts) with atypical endometrial hyperplasia (AEH) or endometrial carcinoma (EmCA). Our hypothesis was that androgen receptor (AR) expression in the endometrium of AEH and EmCA pts would correlate with degree of response to treatment.

Methods:

Pre- and post-treatment endometrial biopsy specimens were obtained from 8 pts treated with megesterol for AEH or EmCA. AR expression was investigated by immunohistochemical (IHC) analysis with appropriate positive and negative controls.

IHC staining was scored for intensity (1-4) and percentage of positive cells (1-4) in the glandular and stromal compartments by a pathologist, blinded to clinical response data. A composite score utilizing both intensity and percentage of positive cells was calculated. We evaluated pre- and post-treatment AR expression in responders and nonresponders as well as AR expression in EmCA, AEH and normal endometrium. The Mann Whitney U and the Wilxocon Signed rank tests were utilized for statistical analysis.

Results:

Eight pts' pre- and post-treatment samples were obtained; 4 with EmCA and 4 with AEH. Three pts had no response, 1, a partial response and 4, complete responses. AR expression in EmCA samples when compared to the normal endometrium was significantly lower in both the glands (mean 2.8 vs. 8; $p<0.01$) and the stroma (mean 2.3 vs. 5; $p<0.05$). Although there was no statistically significant difference in glandular AR expression in the pretreatment biopsies of responders compared to nonresponders (mean 4.6 vs. 2.7; $p=0.14$), there was a significantly higher level of glandular AR expression in the post treatment biopsies of responders compared to non-responders (mean 7.4 vs. 3; $p<0.05$). Furthermore, we noted a trend towards higher levels of glandular AR expression in the post-treatment versus the pre-treatment biopsies in the responders (mean 7.4 vs. 4.6; $p=0.07$).

Conclusion:

We noted a significant decrease in AR expression in EmCA compared to the normal endometrium. Increased AR expression after treatment in responders suggests an important role of the AR in pts treated conservatively with progestational therapy, and needs further prospective validation as a means to predict treatment response in these pts.

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Adenomyosis; Non-Surgical Risk Factors. Radwan Asaad, Waseem Khoder, Soubhi Zitouni, Mohummed R Khani, Gene Mcneeley, Elizabeth E Puscheck, Michael P Diamond. *OB/GYN, WSU, Detroit, MI, USA.*

Objectives: Study the correlation between adenomyosis and some potential non-surgical risk factors.

Methods: Medical records were reviewed for patients who underwent hysterectomy for all indications at WSU hospitals from 1/1/05 – 4/30/06. Pathology reports were reviewed to identify the incidence of adenomyosis. Data obtained from medial records included: age, race, insurance, BMI, social history, medical history, and presenting symptoms. The correlation between adenomyosis and all the above factors was tested using the appropriate statistical methods.

Results: 850 patients were included. Adenomyosis was confirmed by pathology in 375 patients, an incidence of 44.1%. Incidence was not significantly different among races after controlling for parity. 46.3% in African Americans, 35.3% in Caucasians, and 40.9% in others. Incidence was not statistically different between uninsured(28.0%), privately insured (47.7%), and Medicaid (41.9%) patients. P=.065. Incidence of adenomyosis was not different between smokers (38.9%) and nonsmokers (44.6%). P= .386.

Table I shows the correlation between adenomyosis and different risk factors.

Conclusion: Adenomyosis was diagnosed in 44.1% of hysterectomy specimens. Race, socioeconomic status or social habits didn't affect its incidence. Diabetes and endometrial cancer were negative risk factors for adenomyosis, whereas HTN, hypothyroid, breast cancer, fibroids, polyps and endometriosis didn't affect its incidence. Menorrhagia, dysmenorrhea, dyspareunia, and chronic pelvic pain but not metrorrhagia had a positive correlation with adenomyosis.

Table I: Risk Factors

Risk Factor	Incidence of adenomyosis with the risk factor (%)	Incidence of adenomyosis without the risk factor (%)	P value
ETOH	28/72(38.9)	347/778(44.6)	.386
Smoking	125/280(44.6)	250/570(43.9)	.883
DM	15/53(28.3)	360/797(45.2)	.022
HTN	129/302(42.7)	246/548(44.9)	.564
Hypothyroid	11/22(50)	364/828 (44.0)	.665
Breast Ca	2/11(18.2)	373/839(44.5)	.125
Dysmenorrhea	73/137(53.3)	302/713(42.4)	.019
Dyspareunia	15/22(68.2)	359/827(43.4)	.028
CPP	152/302(50.3)	223/548(40.7)	.008
Menorrhagia	282/574(49.1)	63/276(33.7)	.001
Metrorrhahia	119/244(48.8)	256/606(42.2)	.093
Fibroids	310/677(45.8)	65/173(37.6)	.059
Endometriosis	19/42(45.2)	356/808(44.1)	.875
Polyps	23/60(38.3)	352/790(44.6)	.419
Uterine Ca	19/78(24)	356/772(46.1)	.001

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Absence of MGMT Promoter Methylation in Endometrial Cancer. BJ Rimel,[1] Phyllis C Huettner,[2] Matthew A Powell,[3] David G Mutch,[4] Paul J Goodfellow.[5] *[1]Gynecologic Oncology, Washington University in St. Louis, St. Louis, MO, USA; [2]Anatomic and Molecular Pathology, Washington University in St. Louis, St. Louis, MO, USA; [3]Gynecologic Oncology, Washington University in St. Louis, St. Louis, MO, USA; [4]Gynecologic Oncology, Washington University in St. Louis, St. Louis, MO, USA; [5]Gynecologic Oncology and General Surgery, Washington University in St. Louis, St. Louis, MO, USA.*

Objective: O^6 –methylguanine-DNA methyltransferase (MGMT) acts to repair DNA damaged by alkylation of guanine residues. MGMT promoter methylation and gene silencing is seen in a variety of cancers and pre-cancerous changes. The loss of MGMT activity is associated with increased sensitivity to alkylating agents and is a favorable prognostic indicator in gliomas. We sought to determine if MGMT promoter methylation plays a role in endometrial cancer.

Methods: One hundred and twenty primary endometrial cancers were analyzed for MGMT promoter methylation by combined bisulfite restriction analysis (COBRA). The cohort included 77 endometrioid endometrial cancers, 43 endometrial tumors of adverse histologic type, and 6 endometrial cancer cell lines. Twenty one endometrioid and mixed endometrioid ovarian cancers were also analyzed. A subset of the primary tumors was analyzed for MGMT expression by immunohistochemistry.

Results: No MGMT promoter methylation was seen in the 120 endometrial cancers evaluated or the 6 endometrial cancer cell lines. One of the 21 ovarian cancers showed methylation. Immunohistochemistry for MGMT expression is ongoing.

Conclusion: MGMT promoter methylation is an infrequent event in endometrial cancer. MGMT expression in tumor cells and repair of alkylguanine residues could explain in part the limited response of endometrial tumors to alkylating chemotherapy.

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Establishment and Characterization of a Human Uterine Sarcoma Cell Line Sensitive to TNF-Related Apoptosis Inducing Ligand Plus Doxorubicin. Mauricio A Cuello,[1] Sumie Kato,[1] Anil Sadarangani,[2] Jorge A Carvajal,[1] Gareth I Owen.[2] *[1]Obstetrics and Gynecology, Pontificia Universidad Catolica de Chile, Santiago, RM, Chile; [2]Physiological Sciences, Pontificia Universidad Catolica de Chile, Santiago, RM, Chile.*

Objective: Uterine sarcomas (1% of gynecological malignancies) originate from uterine mesenchyma. Patients with high-risk disease (i.e. high grade) or at advanced stages have poor 5-years overall survival (< 10-30%). Pelvic radiation and/or chemotherapy have not demonstrated to improve survival. Identification of new therapies for this malignancy is a major goal. Few in vitro models have been established to test therapeutic agents for uterine sarcoma. Here we sought to establish a new human uterine sarcoma cell line and to test the effects of chemotherapeutic drugs: TNF-related apoptosis inducing ligand (TRAIL) used alone or in combination.

Methods: Tissue sample was obtained from a woman with uterine sarcoma undergoing hysterectomy. Sarcoma cell lines were established using a published protocol for endometrial cancer. Phenotypic characterization was made through the different passages (1 to 13) by Western Blot. Levels of estrogen (ER), progesterone (PR) and TRAIL receptors were also studied (RT-PCR, W-B). Cells were incubated with chemotherapy agents (cisplatin, paclitaxel and doxorubicin and TRAIL) and cytotoxicity (MTS assays) and apoptosis (flow cytometry, PARP cleavage by W-B, and DNA laddering) measured.

Results: Human uterine sarcoma cell line was established from a high-grade uterine sarcoma. Through the different passages the cell line remains expressing cytokeratin, vimentin, tissue factor, caveolin-1 and -actin. This cell line expresses low ER and PR levels. TRAIL receptors (R1 and R2) were also detectable (RT-PCR, W-B). Cisplatin, paclitaxel and doxorubicin (5uM for 24 h) produced low cell cytotoxicity (< 20%). TRAIL (200 ng/ml for 18 h) induced about 30% cell cytotoxicity. Apoptosis was confirmed by PARP cleavage. Doxorubicin significantly enhances TRAIL mediated cytotoxicity (up to 80%), this was demonstrated by a significant increase in the sub G0/G1 region in the DNA histogram.

Conclusions: We establish a human uterine sarcoma cell lines using protocols for endometrial cancer. More importantly, we demonstrated that doxorubicin enhances TRAIL effect on this uterine sarcomas cell line. Thus, this combination might be considered as a treatment for high-risk uterine sarcomas. (Financial support FONDECYT 1050744).

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Defining the Tumor Initiating Capacity of $CD133^+$ Human Endometrial Cancer Cells. Anne M Friel,[1] Petra A Sergent,[1] Christine L Cummings,[2] Rosemary Foster,[3] David T Scadden,[2] Bo R Rueda.[1] *[1]Vincent Center for Reproductive Biology, Massachusetts General Hospital, Boston, MA, USA; [2]Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, USA; [3]Division of Hematology/Oncology, Massachusetts General Hospital, Boston, MA, USA.*

Current data suggest rare subpopulations of cells with tumor initiating capabilities are a common feature of solid tumors. Several investigators have recently identified CD133, a cell surface marker expressed on primitive cells of neural, hematopoietic, endothelial and epithelial lineages, as a potential tumor initiating cell (TIC) marker in solid tumors of the brain, colon and pancreas. In our efforts to investigate such a population in human endometrial cancers (EnCa), we developed an *in vivo* model that is based on serial transplants of TICs from endometrial tumor explants. Serial transplant experiments were initiated in NOD/SCID mice that were injected with primary human EnCa cells. A subset of cells derived from the generated tumor was subsequently transplanted into new recipients. Tumors formed following serial transplantation retained the original histological phenotype of the primary EnCa, and the number of cells required to initiate tumor formation was reduced at each successive transplant stage suggesting an increase in the ratio of TICs to non-TICs. We exploited this model in our initial efforts to investigate the tumor initiating potential of CD133-expressing EnCa cells. To evaluate the tumorigenic potential of $CD133^+$ cells, the tumor initiation capacity of xenograft derived $CD133^+$ and $CD133^-$ cells were compared following subcutaneous injection of each population into NOD/SCID mice. Only the $CD133^+$ fraction was tumorigenic consistent with the hypothesis that tumors are generated and maintained by a subpopulation of cells with phenotypically distinct profiles. We are further investigating the functional significance and characteristics of this fraction *in vitro* and *in vivo*.

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Role of Proteoglycans in Endometrial Benign and Malignant Neoplasia. Elisabetta Trabucco, Sara D'Avino, Maria Teresa Schettino, Nicola Colacurci, Eugenio De Lucia, Luigi Cobellis. *Department of Gynaecology, Obstetrics and Reproductive Medicine, Second University of Naples, Naples, Italy.*

Objectives. Central issues in tumor biology are the understanding of factors that control tumor cell proliferation and the identification of extracellular matrix cues controlling the signaling transducing repertoire that make cancer cells proliferate and invade the host tissues. Among these factors, small leucine-rich proteoglycans (SRLPs):decorin, fibromodulin, lumican, biglycan, are emerging as powerful modulators of angiogenesis and cell growth, by affecting several key elements including matrix assembly, growth factor binding, and receptor tyrosine kinase activity. Recently has been demonstrated that SRLPs can act as a pan-ErbB ligand and, in doing so, down-regulate the activity of one of the most potent oncogenic proteins, ErbB2, whose overexpression is linked to poor prognosis and increased cancer mortality in breast, ovary, and prostate. Since SRLPs have not been previously investigated in the endometrial cancer biology, we investigated their role in the benign and malignant endometrial neoplasia.

Method. The sampling has been obtained in women (n=60; mean age 50± 2.7) with endometrial hyperplasia (n=20), polyps(n=20), and cancer(n=20), during therapeutic and diagnostic procedures (hysterectomy, colpohysterectomy, hysteroscopy). Physiological endometrial samples (n=20) were obtained from menopausal women (n=20; mean age 51± 2.5), during procedures for others gynaecologic indications. Immunohistochemestry was the biology technique used for the detection of SRLPs.

Results. In the physiological endometrial samples immunoreactivity for Decorin, Fibromodulin and Biglycan was intense (+++), while it was weak in polyps and hyperplasia (+) and absent (-) in cancer. No significant difference in the staining of lumican between the physiological and pathological samples.

Conclusions. These results could provide a mechanism by which naturally occurring proteins normally synthesized by fibroblasts and smooth muscle cells, the two key components of the tumor stroma, may play a protective role in the genesis and progression of endometrial neoplasia counteracting the growth of neoplastic cells and suppressing tumor cell-mediated angiogenesis. Although further studies are necessary to understand mechanisms whereby SRLPs might influence endometrial cell growth and survival, these molecules may represent potential target for pharmacological cancer therapy.

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Presence of Myostatin in Uterus: Its Action on Myometrial Cells. Pasquapina Ciarmela,[1,3] Ezra Wiater,[1] Sean Smith,[1] Felice Petraglia,[2] Mario Castellucci,[3] Wylie Vale.[1] *[1]Peptide Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA, USA; [2]Department of Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Siena, SI, Italy; [3]Institute of Normal Human Morphology, Polytechnic University of Marche, Ancona, AN, Italy.*

Myostatin is a member of the TGF-beta superfamily of protein and a well-known inhibitor of skeletal muscle proliferation. The muscular component of the uterus is the myometrium, a tissue that regulates its mass in response to different physiologic conditions under the influence of steroids.

We determined the expression of myostatin mRNA in immortalized pregnant human myometrial (PHM1) cells and we verified its biological activity. Functional assays showed myostatin induced phosphorylation of smad-2 and reduction of proliferation of PHM1 cells in a time and dose-dependent manner. To investigate the physiological relevance of our *in vitro* findings, the expression of myostatin in rat uterus was examined at various phases of the estrous cycle. For the first time we report that myostatin is expressed in rat uterus and that levels of myostatin mRNA change during distinct phases of the estrus cycle. Uterine levels of myostatin peaked during late estrous and were the lowest at pro-estrous. To further examine the role of steroids in myostatin regulation, we examined the effects of gonadal steroid administration in ovariectomized (OVX) rats. Ovaryectomy increased myostatin expression compared to normal cycling controls. Estrogen treatment strong decreased myostatin levels while progesterone produced less robust effects on myostatin expression. These findings taken together suggest an important role for myostatin in the regulation of myometrial functionality.

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Her2neu Over-Expression and PI3kinase/Akt Pathway Activation in Paget's Disease of the Vulva. Amy Stenson,[1] Bita Behjatnia,[1] Jaime Shamonki,[1] Jianyu Rao,[1] Amer Karam,[1] Jonathan Berek,[2] Oliver Dorigo.[1] *[1]OB/GYN and Pathology, UCLA, Los Angeles, CA, USA; [2]OB/GYN, Stanford University, Palo Alto, CA, USA.*

Background: Paget's disease of the vulva is rare with high recurrence rates. Treatment of recurrent disease is challenging due to its extent and location. Non-surgical approaches have limited clinical efficacy, obviating the need for novel therapies. In contrast to Paget's of the breast with well-described overexpression of HER2neu, molecular features of vulvar Paget's are poorly characterized. Our objective was to study therapeutic targets in vulvar Paget's, including the HER2neu protein and the phosphorylated oncoprotein AKT (pAKT). In addition, detailed clinical characteristics were correlated with molecular expression.

Methods: Specimens with vulvar Paget's disease were retrieved from UCLA's Department of Pathology. Protein expression was evaluated by immunohistochemistry on slides from paraffin embedded tissue using the Hercep Test (Dako) for Her2neu expression and a standard protocol to assess expression of activated pAKT. Slides were scored by two independent pathologists based on a nominal scale of 0 (negative) to 3 (strongly positive). Clinical data was retrieved via chart review.

Results: Between 1995 and 2007, 25 patients with vulvar Paget's were identified. Median age was 65 yrs (36-83 yrs). A family history of cancer was found in 14/25 (54%), 7/25 (28%) were smokers and 13/18 (72%) had a history of hormone use. Intraepithelial lesions accounted for the majority (15/25, 60%), while 6/25 (24%) demonstrated invasion and 4/25 (16%) were associated with underlying GI malignancy. 16/25 (64%) had at least one recurrence, with median time to recurrence 24 months. So far, 13 specimens were stained for HER2neu and pAKT. Overexpression of Her2Neu was found in 10/13 (77%.) Positive staining for pAKT was evident in 10/13 (77%.) Statistical analysis suggested a correlation between HER2neu and pAKT expression.

Conclusions: Our study demonstrates that overexpression of Her2Neu and activation of the PI3kinase/pAKT pathway are important features of vulvar Paget's disease. To the best of our knowledge, this is the largest series evaluating these molecular pathways in vulvar Paget's. Our data suggest that HER2neu and pAKT may be important molecular targets for novel therapies using for example the monoclonal antibody trastuzumab directed against Her2neu, or a PI3kinase pathway inhibitor like rapamycin.

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Green Tea Extract (EGCG) Inhibitis Proliferation of Human Leiomyoma Cells. Dong Zhang, Mohamed Al-Hendy, Ayman Al-Hendy. *Center for Women Health Research, Dept of Obstetrics and Gynecology, Meharry Medical College, Nashville, TN, USA.*

Introduction: Uterine leiomyomas are the most frequent tumor of the female reproductive tract and are the primary indication for hysterectomy in women worldwide. These tumors occur in up to 70% of adult women, and their prevalence is especially high in Africa-American women. Currently there is no effective and safe medical treatment for uterine fibroids and surgery is the main stay. Epigallocatechin gallate (EGCG) constitutes the main solid extract of green tea and is believed to contributes most of the antioxidant and anti-inflammation capacity of green tea. EGCG has been shown to have beneficial effects on prostate cancer and breast cancer by inducing apoptosis and inhibiting proliferation of cancer cells. In this study, we investigated the ability of EGCG to inhibit proliferation of human leiomyoma cells (HLM) in vitro.

Methods: Human immortalized leiomyoma cells were cultured at 37ºC in a humidified atmosphere of 5% CO_2-95% air in SmBM medium supplied with 5%FBS, 0.1% insulin, 0.2% hFGF-B, 0.1% GA-1000 and 0.1% hEGF(Lonza). The cells were plated at density of 2×10^5cells/well in 6-well plates and grown under the same conditions above. The monolayer cultures at approximately 70% confluence were treated with various concentrations (0, 0.1, 1.0, 10, 50, 100 and 200μm) of EGCG (Sigma) for 7 days. A fluorometric assay using Hoechst 33258 dye (Sigma) for DNA quantitation was conducted at the following designed time points, day 1, 3, 5, and 7 post EGCG treatment. The cells were lysed and DNA content was determined using Hoechst dye solution (1μg/ml). Fluorescence was measured after excitation at 355nm and emission at 458nm.

Results: EGCG exhibited marked anti-proliferation effect on the growth of HLM cells. Compared with untreated control, the inhibitory effect of EGCG on HLM cells was observed at 10 μM and peaked at 100 μM concentration. The difference was statistically significant ($p = 0.002$). Evaluation of the mechanism of action of EGCG is cuurently under investigation in the lab.

Conclusion: EGCG significantly inhibited the proliferation of human leiomyoma cells in a dose-depended pattern. EGCG may present a potential effective and safe medicinal treatment for uterine fibroids.

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A Novel Recombinant hCG-Antagonist Inhibits Pro-Invasive Gene Expression in the JEG-3 Choriocarcinoma Cell Line. Vuk P Jovanovic,[1] Drew V Tortoriello,[2,3] Prati A Vardhana,[1] Martin A Julius,[1] Susan V Pollak,[1] Joyce W Lustbader.[1] *[1]Department of Obstetrics and Gynecology, Columbia University, College of Physicians and Surgeons, New York, NY, USA; [2]Sher Institute for Reproductive Medicine, New York, NY, USA; [3]Diabetes and Endocrinology Research Center, Columbia University, College of Physicians and Surgeons, New York, NY, USA.*

Objective: Choriocarcinoma is an aggressive form of germ cell tumor that exhibits rapid growth with early metastases. Choriocarcinomas autonomously secrete hCG which acts as an autocrine/paracrine growth factor in these cancers. We hypothesize that a novel hCG antagonist (hCG-Ant) can limit tumor expansion by blocking hCG-induced expression of pro-invasive genes. We investigated if hCG-Ant could alter RNA expression of matrix metalloproteinases (MMP-1 and MMP-7), which facilitate basement membrane degradation and hence invasion, and metastin (kisspeptin), a strong suppressant of metastasis, in the choriocarcinoma cell line JEG-3.

Design: *In vitro* experiments using the JEG-3 cell line.

Materials and Methods: After plating JEG-3 cells in a 24 well tray overnight in RPMI media containing 10% FBS, cells were washed twice with PBS and then cultured in 500 uL of serum-free RPMI media containing one of four treatments: 1) saline; 2) hCG (50 IU); 3) hCG (50 IU) plus hCG-Ant (100 IU); or 4) hCG-Ant (100 IU). RNA was extracted from each well using Trizol and reverse transcribed using Sensiscript (Qiagen). The relative expression of MMP-1, MMP-7, and metastin mRNA was quantified using Sybr-green based real time PCR. The expression of the housekeeping gene HPRT was used to normalize expression data.

Results: Treatment of JEG-3 cells with hCG-Ant *vs*. hCG alone reduced expression of MMP-1 (51.95 ±15.51 *vs*. 85.99 ±10.56) and MMP-7 (66.27 ±13.01 *vs*. 116.50 ±16.48). HCG-Ant reduced MMP-1 and MMP-7 expression by 40% and 43%, respectively ($p<0.05$). Treatment with hCG-Ant *vs*. hCG increased metastin expression (5.44 ±2.29 *vs*. 4.63 ±1.32). Metastin expression was increased by 17% following hCG-Ant treatment.

Conclusion: Treatment of the JEG-3 choriocarcinoma cell line with hCG-Ant reversed the increased expression of MMP-1 and MMP-7 following treatment with hCG. Metastin expression was increased by hCG-Ant. This data suggests that hCG antagonism is capable of altering gene expression thereby inhibiting invasion in a choriocarcinoma cell line. The role of hCG-Ant as an adjuvant therapy in hCG sensitive tumors offers promise.

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Retinoids, but Not Progesterone, Directly Induce Differentiation and Apoptosis of Endometrial Cancer Cells. You-Hong Cheng, Serdar E Bulun. *OB/GYN, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.*

Objectives: The opposing actions of estrogen and progesterone during the menstrual cycle regulate endometrial proliferation, differentiation and secretion. The unopposed action of estrogen contributes to the development of type I endometrial cancer. However, the mechanisms for progesterone protection of estrogen-induced carcinogenesis in endometrium remain unclear.

Methods and results: In the current study, we demonstrated that retinoids (9-cis retinoic acid (RA) or all-trans RA (ATRA)) significantly inhibited basal and hormone-stimulated Ishikawa cell proliferation by over 60% using MTT assay. The same experiment indicated that estrogen had no significant effect, whereas progesterone slightly induced, on cell proliferation. Cell cycle analysis indicated that ATRA significantly increased the G1/G0 cell population by 20% and decreased S phase cells by 20%, suggesting that RA induces cell cycle arrest at the S phase. Knock-down of RARα alone or both RARα and RXRα significantly increased proliferating cell nuclear antigen (PCNA) levels in epithelial Ishikawa cells, suggesting that RA signaling via RAR/RXR activation is critical for normal endometrial growth and differentiation. To determine whether retinoids are naturally secreted by the endometrial stromal cells, we cultured primary stromal cells and analyzed the culture media using HPLC. We found that retinol is the predominant retinoid form secreted by stromal cells. The average concentration of retinol in the cultured media of eutopic endometrial stromal cells was approximately 4 to 6 ng/ml/10^5 cells (n=5). Although there was less than 0.25 ng/ml/10^5 cells of all-trans retinal or ATRA in the cultured media, we did find a small peak for all-trans retinal and ATRA in the media using HPLC analysis. Furthermore, progesterone significantly increased secreted retinol levels from eutopic endometrial stromal cells, but decreased retinol levels secreted from endometriotic stromal cells. Retinol is a precursor for RA that is converted to retinal and then to RA.

Conclusions: We demonstrated that progesterone signaling via PR induces endometrial stromal cells to secrete paracrine retionids that in turn control the phenotype of adjacent epithelial cells. Conversely, this interaction is disrupted in diseased endometrial tissues, such as endometrial cancer and endometriosis.

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The Effects of Hormonal Contraceptives on Antimullerian Hormone by Obesity Status. Anne Z Steiner,[1] Frank Z Stanczyk,[2] Stan Patel,[2] Alison Edelman.[3] *[1]OB/GYN, University of North Carolina, Chapel Hill, NC, USA; [2]OB/GYN, USC Keck School of Medicine, Los Angeles, CA, USA; [3]OB/GYN, Oregon Health and Sciences University, Portland, OR, USA.*

Background: Antimullerian hormone (AMH) is emerging as a predictor of reproductive potential. Serum levels of FSH, a commonly used measure of ovarian reserve, are suppressed with the use of oral contraceptives (OCs) thereby limiting its use. The impact of OCs and on serum AMH levels in normal and obese women is unknown.

Objective: To examine the impact of OCs on serum AMH levels by obesity status in reproductive-age women.

Materials and Methods: Ovulatory women, ages 18-35 years, of normal (< 25 kg/m^2; n = 10) and obese (> 30 kg/m^2; n = 9) body mass index (BMI) received a low dose OC (20 mcg ethinyl estradiol/100 mcg levonorgestrel) for two cycles. Serum was obtained at three time points: after 21 days of active pills (cycle 1, day 21), at the end of the 7-day hormone-free interval (cycle 1, day 28), and during the first week of active pills in Cycle 2 (cycle 2, day 5,6, or 7). AMH levels were quantified by ELISA; FSH and LH levels were determined by chemiluminescent immunoassay. AMH at the three time points was compared using repeated measures ANOVA. Models were used to assess the relationship between AMH and cycle day by obesity status. AMH and gonadotropin levels were compared using Spearman's correlation.

Results: AMH levels did not differ by OC cycle day (p_{ANOVA} = 0.90) or by active versus placebo pill use (2.79 ng/mL ± 1.3 vs. 2.85 ng/mL ± 1.4, p=0.93) among normal BMI women. Among obese women, AMH levels differed by OC cycle day (p_{ANOVA} = 0.04), but not by use of active or placebo pill (1.63 ng/mL ± 1.2 vs.1.71 ng/mL ± 1.2, p = 0.88). Across the cycle, CV(standard deviation/mean) averaged 20.2%± 3.1 in the obese and 8.8%±1.3 in the normal BMI women (p=0.003). Modeling to determine differences in AMH throughout the cycles based on obesity status showed a significant interaction (p = 0.02) and lower AMH levels in the obese group (p<0.001). Among both BMI groups, serum AMH and FSH levels did not correlate during active pills or after 7 days of placebo pills.

Conclusions: In young, normal BMI women serum AMH levels do not appear to fluctuate during OC use. Among obese women, AMH levels are lower and fluctuate significantly. These fluctuations do not appear to mirror changes in gonadotropins and may complicate clinical interpretation of AMH.

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Comprehension of Risks and Benefits of Hormone Replacement Therapy: Responses to Informed Consent Questionnaires from the Kronos Early Estrogen Prevention Study (KEEPS). Rajeevi Madankumar,[1] Lubna Pal,[2] Ruth Freeman,[2] Hussein Amin,[2] Barbara Isaac,[2] Maureen Magnani,[2] Alicia Figueroa,[2] Lisa Alemany,[2] Nanette Santoro.[2] *[1]Ob/Gyn & Women's Health, Jacobi Medical Center, Bronx, NY, USA; [2]Ob/Gyn & Women's Health, Albert Einstein College of Medicine, Bronx, NY, USA.*

Background: Menopausal hormone therapy (HT) is a confusing topic for many clinicians and patients.

Objective: To assess comprehension of basic clinical trial features among prospective participants for the KEEPS trial, designed to study the effects of HT initiated within 3 years of menopause on CHD markers.

Methods: Screening materials were provided giving an overview of study purpose, duration, medications, likelihood of receiving drug vs. placebo, HT related risks and side effects. At a subsequent interview, a 10-item questionnaire assessed the participant's level of comprehension. A score of ≥80% was adequate to complete the informed consent process. Those scoring <80 % were re-counseled and retested. Demographic variables determining the likelihood of an initial score ≥80% were evaluated by univariate and multivariable analyses.

Results: 32% (24/76) scored ≥80% on initial testing. All women scored ≥80% after re-counseling and retesting. Likelihood of an initial response ≥80% correct was unrelated to age or time since menopause. Ability to correctly respond was influenced by highest educational level attained. None of 8 women whose furthest educational level was high school scored ≥80% on initial testing, significantly less than those with a college education (p=0.04). A higher proportion of college graduates (13/32) scored ≥80% compared to those attaining further education (11/35) (p=0.43). Comprehension was greatest for study purpose and duration (64/76 and 70/76 correct responses respectively) and least for questions related to results of the WHI hormone trial breast cancer and CHD. That E alone was not associated with an increased risk of CHD (41%) or breast cancer (50%) was poorly understood.
Conclusion: Comprehension of the risks and benefits of HT by potential research subjects is low despite provision of reading materials prior to the informed consent process. (supported by the Montefiore Medical Center/Albert Einstein College of Medicine site for the Kronos Longevity Research Institute and K24-HD41978 to NS).

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Variation in Menopausal Symptoms within a Sample of Hispanic Women: SWAN, the Study of Women's Health Across the Nation. Robin R Green,[1] Alex J Polotsky,[1] Aileen P McGinn,[1] Carol A Derby,[1] Rachel P Wildman,[1] Lhasa Ray,[1] Kavitha T Ram,[1] Gerson Weiss,[2] Nanette F Santoro.[1] *[1]Albert Einstein Coll of Med, Bronx, NY; [2]Univ of Med and Dent of New Jersey, Newark, NJ.*
Background: Menopausal symptoms are experienced by over 75% of women.
Purpose: To describe symptom frequency in a sample of midlife Hispanic women from different countries of origin.
Methods: The Study of Women's Health Across the Nation (SWAN) recruited 277 women at baseline who self-identified as Hispanic. Their baseline responses to validated questionnaires regarding common menopausal symptomatology were examined. Symptoms were reported over the previous two weeks and scored on a frequency scale ranging from 1 (not at all) to 5 (every day). For all analyses, symptoms were dichotomized into "absent" vs. "present" variables. Responses were correlated with acculturation (4-item scale: Marin, Hisp J Behav Sci 2:183, 1987) and analyzed by sub-ethnicities: Central/South American (C/S Am), Puerto Rican (PR), Dominican (DR), and Cuban (Cu). Associations between symptoms and sub-ethnicity were tested by chi-square. Logistic regression was used to test for associations with acculturation and being US-born.
Results: (selected symptoms shown, data are presented as percentages of participants reporting the symptom)

Symptom	C/S Am	PR	DR	Cu	p value
Hot Flushes,%	32	36	21	20	0.22
Night Sweats,%	44	39	31	30	0.26
Irritability,%	73	77	69	66	0.65
Headaches,%	48	71	57	53	0.032
Trouble Sleeping,%	47	66	64	36	0.005
Vaginal Dryness,%	37	18	38	25	0.035

There was significant variation in several menopausal symptoms. While Puerto-Rican women had the highest likelihood of reporting trouble sleeping (OR=2.2, 95%CI: 1.1-4.3) and headaches (OR=2.7, 95%CI:1.4-5.3), Dominican women were most likely to report vaginal dryness (OR=2.8, 95%CI: 1.1-7.1) Acculturation and being US-born did not explain the variation between sub-ethnicities in any of the models tested
Conclusion: There appear to be significant differences among Hispanic women with respect to menopausal symptomatology. These differences were not readily explained by measures of acculturation.
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Relative Effects of Tamoxifen, Raloxifene, and Estrogen Therapy on Cognition: Results from the Women's Health Initiative Memory Study (WHIMS) and the Cognition in the Study of Tamoxifen and Raloxifene (CoSTAR) Clinical Trials. David H Barad,[1] Therese B Bevers,[2] Laura H Coker,[3] Mark A Espeland,[3] Sarah A Jaramillo,[3] Marian Limacher,[4] Dorothy S Lane,[5] Stephen Rapp,[3] Pauline Maki,[6,7] Susan Resnick,[7] Sally A Shumaker,[3] Marcia L Stefanick.[8] *[1]Albert Einstein College of Medicine, Bronx, NY, USA; [2]University of Texas, Houston, TX, USA; [3]Wake Forest University School of Medicine, Winston-Salem, NC, USA; [4]University of Florida, Gainesville, FL, USA; [5]Stony Brook University School of Medicine, Stony Brook, NY, USA; [6]University of Illinois at Chicago, Chicago, IL, USA; [7]Gerontology Research Center, NIA, NIH, Baltimore, MD, USA; [8]Stanford University, Palo Alto, CA, USA.*
Purpose: To compare the relative effects of conjugated equine estrogen, raloxifene, and tamoxifen therapies on cognition among women aged 65 years or older. **Participants and Methods:** Annual Modified Mini Mental State (3MS) examinations were used to assess global cognitive function among the 7,212 women enrolled in the two randomized placebo-controlled clinical trials of the Women s Health Initiative Memory Study (WHIMS) and 300 women enrolled in Co-STAR, the cognitive substudy of the NSABP's Study of Tamoxifen and Raloxifen (STAR) trial who had baseline 3MS testing. Associations between baseline cognitive risk factors common to both trials and baseline 3MS scores were assessed and interactions used to examine whether risk factor relationships differed between cohorts. Factors for which relationships were similar were used as covariates in analyses comparing on-trial 3MS scores. Factors for which relationships did not appear to be similar were used to stratify analyses. **Results:** Compared to placebo, each of the active therapies was associated with a small mean relative deficit in 3MS scores of 0.5 units or less, which was fairly consistent between women with and without prior hysterectomy. Overall, relative deficits appeared to be most marked for tamoxifen (unadjusted p=0.003) but were also evident for raloxifene (p=0.06) and CEE (p=0.02). **Conclusions:** While unmeasured differences between trials may have confounded our analysis, these findings suggest that both tamoxifen and raloxifene may adversely affect cognitive function in older women. *Supported in part by N01-AG-1-2106 and the Intramural Research Program of the National Institute on Aging, NIH.*

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Effects of Fat Distribution and Quantity on Adipocytokine Levels and Bone Mineral Density (BMD) in Normal Weight Early Postmenopausal Women. Enrico Carmina,[1] N Douglas,[2] Rogerio A Lobo.[2] *[1]Department of Medicine, Univeristy of Palermo, Palermo, Italy; [2]Department of Obstetrics and Gynecology, Columbia University, New York, NY, USA.*
Weight gain and increased abdominal fat have been found in women after menopause and is associated with higher levels of leptin, and decreased levels of the cardioprotective adipocytokine adiponectin. At the same time, BMD characteristically decreases. In an effort to determine the evolution and correlates of these changes, we studied 25 postmenopausal women (PM) within 4 years of menopause (age 58.4±1 yrs) of normal weight (BMI 26.3±1) and compared them to 20 weight matched (BMI 27.4±1) premenopausal (PRE) controls (age 26.4±1yrs.) All subjects had BMD and body composition studies by DEXA and measurements of leptin, adiponectin, visfatin and retinol-binding protein 4 (RBP4.) While total fat was similar in the 2 groups, PM had more trunk and abdominal fat (10516±800; 818±67g) compared to PRE (8626±493g $p<0.05$; 577±33g $p<0.01$) PM also had greater %trunk fat and %central abdominal fat compared to PRE, $p<0.01$.Serum leptin (31.8±0.5 vs 28±5 pg/ml) and visfatin (10.4±1 vs. 9.4±0.5 ng/ml) were similar but adiponectin (17±1 vs. 13.3±1 μg/ml) and RBP4 (28.7±2 vs. 22.3±1 ng/ml) were higher, $p<0.05$ in PM. While in PRE, abdominal fat correlated negatively with adiponectin ($p<0.01$) in PM only leptin correlated with various parameters of fat mass, $p<0.02$, and adiponectin did not correlate but correlated positively with age (r 0.47, $p<0.05$.) As expected, PM had reduced BMD at the lumbar spine and hip (0.84±0.025 vs. 0.99±0.025 g/cm2; 0.72±0.025 vs. 0.87±0.025 g/cm2, $p<0.01$) but there was a correlation between total and trunk fat in PM and lumbar BMD (r 0.69, 0.66, $p<0.01$) but not with hip BMD; or any correlations in PRE. There was a correlation between leptin and lumbar BMD in PM (r 0.47, $p<0.02$) but not in PRE.
In summary, in normal weight early PM, abdominal fat is increased, but only adiponectin and RBP4 are altered with an increase in the former correlating with age. Lumbar BMD correlated with fat mass in PM and is partially explained by increases in leptin. It is suggested that in spite of increasing abdominal fat in normal weight early PM, (which correlates with a higher lumbar BMD)

there is a protective adipocytokine profile. We hypothesize that this finding may precede some ill-defined threshold of fat mass and/or insulin resistance after which adiponectin decreases.

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The Relationship of Bleeding Patterns to Daily Reproductive Hormones in Women Approaching Menopause. Bradley J Van Voorhis,[1] Nanette Santoro,[2] Sioban Harlow,[3] Sybil L Crawford,[4] John F Randolph.[3] [1]*Department of Ob-Gyn, University of Iowa College of Medicine, Iowa City, IA, USA;* [2]*Department of Ob-Gyn, Albert Einstein College of Medicine, New York, NY, USA;* [3]*Department of Ob-Gyn, University of Michigan College of Medicine, Ann Arbor, MI, USA;* [4]*New England Research Institutes, University of Massachusetts, Worcester, MA, USA.*

Objective: To correlate reproductive hormone production with menstrual bleeding patterns among women in the menopause transition.

Methods: A sub-cohort of the SWAN study consisting of 848 women age 42-52 was studied. Each woman collected daily first void urine samples for one complete menstrual cycle or 50 days (whichever came first) once a year for 3 years. Urine was assayed for excreted levels of FSH, LH, estrogen metabolites and progesterone metabolites which were normalized for creatinine concentration. Ovulation was detected by a validated algorithm. Menstrual bleeding parameters were derived from daily calendars. Correlations between bleeding characteristics, hormone concentrations, and other potential clinical predictors were analyzed using multivariate logistic regression models.

Results: The cohort was ethnically diverse with a median age of 47 and with 73% in the early perimenopause at the start of the study. 21% of all cycles were anovulatory. Short cycle intervals (< 21 days) were associated with the early perimenopause (OR 5.2, CI 2.1, 12.9) and with anovulation (OR 20.3, CI 7.2, 56.8). Long cycle intervals (36+ days) were associated with late perimenopause (OR 8.7, CI 1.0, 79.1)and with anovulatory cycles (OR 2.6, CI 1.9, 3.5). Both short (1-3 days) and long (8+ days) duration of menstrual bleeding were significantly associated with anovulation (OR 2.4 and 2.6, respectively). Women with anovulatory cycles were less likely to report heavy menstrual bleeding than women with ovulatory cycles. Menorrhagia was not associated with steroid hormone concentrations but was associated with obesity (OR 4.7, CI 2.6, 8.5) and with the self-reported presence of fibroids (OR 4.1, CI 1.8, 9.5).

Conclusions: Among women in the menopause transition, abnormalities in timing of menstrual bleeding (cycle intervals or bleeding duration) have a hormonal basis and are frequently associated with anovulation. In contrast, abnormally heavy periods do not have a hormonal basis and are less likely following anovulatory cycles. Heavy periods are associated with obesity and fibroids.

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Effective Treatment of Vaginal Atrophy with Topical Testosterone for Women on Aromatase Inhibitors. Sabrina M Withbery,[4] Julia V Johnson,[1] Peter R Casson,[1] Sharon L Mount,[3] Hyman B Muss.[2] [1]*Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA;* [2]*Hematology Oncology, University of Vermont, Burlington, VT, USA;* [3]*Pathology, University of Vermont, Burlington, VT, USA;* [4]*Hematology Oncology, Warren Alpert School of Medicine at Brown University, Providence, RI, USA.*

INTRODUCTION: Twenty-five to fifty percent of postmenopausal women are reported to have symptomatic atrophic vaginitis (AV). Breast cancer survivors are placed on aromatase inhibitors (AI) lower serum estradiol (E) to undetectable levels. The use is vaginal E is contraindicated, although these women have even higher rates of AV. Topical testosterone (TT) has successfully treated vulvar atrophy; testosterone receptors are also present in the vaginal epithelium.

OBJECTIVE: Assess the effect of TT on vaginal maturation index (MI) and relief of AV symptoms.

METHODS: 10 postmenopausal women on aromatase inhibitor with symptomatic atrophic vaginitis were enrolled in prospective, IRB approved study. Estradiol (E) and testosterone (T) levels, AV questionnaires (score 0-3; none to most severe symptoms of dryness, irritation, and dyspareunia), gynecologic exam, and vaginal smears (for pH and vaginal maturation index, MI, by Meisels criteria) were performed at baseline and after 1 month of daily treatment with 300 mcg of TT. Data was assessed by T test and Fischer's exact test; significant $p < .05$.

RESULTS: E levels were undetectable at baseline and following treatment. T levels increased (mean +/- SEM) from baseline (16 +/- 0.8) to treatment (38 +/- 10.9); the difference was not significant; $p = .8$, although one patient had an appreciable rise in serum T level. Two AV symptoms improved significantly with TT use; comparing baseline to treatment scores: vaginal dryness (2.2 vs. 0.75; $p = .03$) and dyspareunia (2.3 vs. 1; $p = .02$). There was no difference in vaginal itching/irritation (1 vs. 0.8; $p = 0.9$). Vaginal pH decreased significantly by 0.5 (+/- 0.22); $p = .008$. Vaginal MI (range 0 -100 based on number of parabasal, intermediate, and superficial vaginal epithelium), was significantly improved from mean score (+/- SEM) of 15.7 (+/- 1.3) at baseline to 50.7 (+/- 8.1) following therapy.

CONCLUSIONS: This pilot study demonstrated improvement in AV symptoms, vaginal pH, and maturation index, with undetectable E level, with the administration of vaginal TT. This suggests a potential treatment for AV for breast cancer survivors on aromatase inhibitors.

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Extraction Prior to Enzyme Immunoassay Gives Reliable Salivary Estradiol Monitoring during Estrogen Therapy. Mark S Newman,[1] Frank Z Stanczyk,[2] David T Zava.[1] [1]*ZRT Laboratory, Beaverton, OR, USA;* [2]*Reproductive Endocrinology Laboratory, University of Southern California, Los Angeles, CA, USA.*

Saliva analysis is a convenient, non-invasive and rapid method for assessing estradiol (E2) levels. However, particularly in postmenopausal women, the low salivary E2 levels are often near or below the sensitivity of available assays, compromising both accuracy and precision. We present results using an extraction step prior to E2 assay, which concentrates the sample to increase sensitivity and removes potentially interfering substances.

Morning saliva samples were obtained from premenopausal (mid-luteal phase, n=4,651) and postmenopausal women (n=1,770) not taking hormones, and from postmenopausal women receiving oral conjugated equine estrogens (Cenestin, n=119; Premarin, n=439), oral micronized E2 (Estrace, n=145; compounded E2, n=1618), transdermal E2 patches (Climara, n=623; Vivelle, n=1619); or topical E2 cream (compounded E2, n=107). E2 levels were determined by an automated enzyme immunoassay (EIA) after solid phase extraction.

The functional sensitivity of the assay was determined to be 0.8 pg/ml, compared with >2 pg/ml without extraction.

Results are shown in the tables below:

Median (Mean) Salivary E2 without Estrogen Therapy

Premenopausal	1.9 (2.3) pg/ml
Postmenopausal	1.0 (1.3) pg/ml

Median Salivary E2 During Estrogen Therapy

Therapy	Dosage (mg/day)	Median Salivary E2 (pg/ml)	Therapy	Dosage (mg/day)	Median Salivary E2 (pg/ml)
Cenestin[1]	0.3	1.6	Premarin[1]	0.3	1.4
	0.625	2.0		0.625	1.9
	1.25	3.0		1.25	2.8
Estrace[2]	n/a	n/a	Compounded E2[2]	0.25	2.8
	0.5	3.4		0.5	3.3
	1.0	4.2		1.0	4.8
	2.0	8.3		2.0	8.8
Climara[3]	0.025	1.2	Vivelle[3]	0.025	1.2
	0.05	1.4		0.05	1.2
	0.1	1.7		0.1	1.5
Compounded E2[4]	0.3	3.9			
	0.5	5.7			
	1.0	9.6			

[1]Oral Conjugated Equine Estrogens; [2]Oral E2; [3]E2 Patch; [4]Topical cream

Salivary E2 levels corresponded with the hormone dosage, suggesting a reliable assessment of unbound E2 levels with each formulation, dosage and type of estrogen therapy.

Extraction prior to EIA in an automated assay dramatically increased precision and accuracy at low concentrations. Omitting the extraction step may have contributed to poor serum versus saliva correlations in other studies. This method may therefore allow reliable monitoring of estrogen therapy without the need for expensive and inconvenient blood tests.

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The Role of Fibulin-3 in Pelvic Organ Prolapse. David D Rahn,[1] Jesus F Acevedo,[1] Patrick W Keller,[1] Lihua Marmorstein,[2] R Ann Word.[1] [1]*Ob-Gyn, UT Southwestern, Dallas, TX, USA;* [2]*Visual Sciences, Univ Arizona, Tuscon, AZ, USA.*

OBJECTIVES: Fibulin-5 (Fib-5) is crucial for normal elastic fibers in the

vaginal wall, and >90% of Fib-5 knockout (KO) mice develop pelvic organ prolapse by 20 weeks of age. In contrast, Fib-1 and -2 deficiencies do not result in prolapse, and Fib-4 KOs die shortly after birth. Herein, we evaluated the role of Fib-3 in pelvic organ support. **METHODS**: Two observers serially measured the degree of vaginal, perineal, and anal prolapse in 227 Fib-3 KO (*Fib3*$^{-/-}$) and 16 mature virginal wild-type (WT) mice of the same strain weekly for 7 months. Matrix metalloprotease (MMP-2 and 9) activity was determined using gelatin zymography. Relative amounts of Fib-5 and tropoelastin were determined by immunoblot analysis. **RESULTS**: Diameter of perineal bulge (6.9 vs. 4.2 mm) and perineal body length (7.1 vs. 5.0 mm were increased significantly in *Fib3*$^{-/-}$ mice, P<0.001. Vaginal weight was also increased (76.6 vs. 58.7 mg, P=0.03). In *Fib3*$^{-/-}$ mice >10 wks, 27% developed overt prolapse, 34% anal prolapse, and 17% abdominal or inguinal hernias. Severity of prolapse was significantly related to age, but not parity, and the average age at diagnosis was 37 wks. *Fib3*$^{-/-}$ animals with advanced prolapse had attenuated uterosacral ligaments and descent of the bladder and uterus caudal to the symphysis. Pro-MMP2 (2-fold, P=0.03), active MMP2 (2-fold, P=0.06), and proMMP9 (3-fold, P=0.05) were increased in vaginal tissues from mice with gross prolapse compared with age-, strain-, and cycle-matched controls. This increase in protease activity, however, was also accompanied by increased expression of Fib-5 and tropoelastin (1.7-fold, P<0.001). Regardless of prolapse, elastic fibers were few in number, blunted, and thickened in the *Fib3*$^{-/-}$ vagina. **CONCLUSIONS:** This is the third KO mouse model of pelvic organ prolapse. As in women, frequency of prolapse increased with age, suggesting that Fib-3 is necessary to maintain support during aging. Upregulation of Fib-5 and tropoelastin may compensate for the lack of Fib-3 until the effects of aging alter the balance in favor of prolapse. Overall, the results indicate that both Fib-3 and Fib-5 are important in maintaining pelvic organ support in mice. Increased vaginal protease activity, together with compromised synthesis of elastic fibers, are important mechanisms in the pathogenesis of pelvic organ prolapse during aging in mice.

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Role of Vaginal Distention in the Pathogenesis of Pelvic Organ Prolapse. David D Rahn, Jesus F Acevedo, R Ann Word. *Ob-Gyn, UT Southwestern, Dallas, TX, USA.*

OBJECTIVES: Matrix metalloprotease (MMP) activity is increased in the postpartum vagina of wild type (WT) animals, and this activity is accompanied by a burst in elastic fiber synthesis. The mechanisms that precipitate these changes are unclear. The goals of this study were to determine how vaginal distention (such as in parturition) affects elastic fiber homeostasis in the vaginal wall and the potential significance of these changes in the pathogenesis of pelvic organ prolapse. **METHODS**: Nonpregnant (18) and 20 pregnant (d14) WT mice underwent either vaginal distention with a 750µL balloon x 40 min (to simulate parturition) or sham procedure. Tissues were obtained at 24 and 48 h for immunoblot analysis, zymography, and histology. Distention was also performed in 30 young *Fbln5*$^{-/-}$, *Fbln5*$^{+/-}$, and WTs, and prolapse was quantified for 8 weeks. **RESULTS**: Distention resulted in marked increases in MMP activity in nonpregnant animals (proMMP9, 5.6-fold; active MMP9, 3.5-fold; proMMP2, 2.4-fold; active MMP2, 3.7-fold; all P < 0.05 compared with sham) which was accompanied by fragmented and disrupted elastic fibers in the vaginal wall. Abundant amounts of tropoelastin and fibulin-5 in the nonpregnant vagina were not increased further by distention. In pregnant animals (which normally have suppressed vaginal wall fibulin-5 and tropoelastin), however, distention resulted in 3-fold upregulation of both proteins (P<0.05). Distention also induced increased MMPs in pregnant animals (MMP-9, 1.7-fold; proMMP-2, 1.7-fold; active MMP-2, 2.3-fold; all P < 0.05). Thirteen young *Fbln5*$^{-/-}$ (4-6 wks prior to age of prolapse development), 11 het siblings, and 6 age-matched WTs underwent serial exams after ballooning. Distention induced rapid increases in perineal bulge and vaginal diameter (within 3 d) in *Fbln5*$^{-/-}$ mice which never recovered. **CONCLUSIONS**: In pregnant mice, vaginal distention results in increased protease activity in the vaginal wall but also increased synthesis of proteins important for elastic fiber assembly. Distention may thereby contribute to the burst of elastic fiber synthesis in the postpartum vagina. The finding that distention results in accelerated pelvic organ prolapse in *Fbln5*$^{-/-}$ animals, but not WT, indicates that distention results in loss of pelvic organ support if elastic fiber synthesis is compromised. Further, the data suggest that elastic fiber synthesis is crucial for recovery of the vaginal wall from parturition/distention-induced increases in vaginal protease activity.

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Extracellular Matrix Remodelling Is Increased in Vaginal Tissue of Premenopausal Patients with Pelvic Organ Prolapse. May Alarab,[1,2,4] Oksana Shynlova,[1] Harold Dutz,[2,4] Stephen Lye.[1,2,3] *[1]Samuel Lunefeld Res Institute, Mt Sinai Hospital; [2]Ob/Gyn; [3]Physiology; [4]Div of Urogynecology, Mt Sinai Hospital, Toronto, ON, Canada.*

Objectives:
The molecular etiology of pelvic organ prolapse (POP) is complex and not yet well understood. Defects in the connective tissue, such as a decrease in extracellular matrix (ECM) protein content may predispose women to POP. Our objective was to study the expression and the enzymatic activity of matrix metalloproteinases (MMPs) and their tissue inhibitor (TIMPs) in vaginal tissue of patients with advanced POP and controls.

Methods:
After informed consent, pre-menopausal Caucasian patients affected by POP (≥grade 3 by POP-Q), and control patients (no POP) matched for age and BMI, undergoing vaginal and abdominal hysterectomy respectively were recruited. Full thickness anterior vaginal epithelial tissue was obtained from the surgical cuff of patients and controls in the proliferative phase of the menstrual cycle. Total protein was extracted using RIPA lyses buffer. Western immunoblot analysis was performed (patients: n=7, controls: n=6) to study the protein expression of MMP-1, -12, TIMP-1, -2, -4. Gelatin zymography was used to quantify the activity of MMP-2 and -9. Immunohistochemical analysis for TIMP-2 and -4 was performed on PFA-fixed vaginal biopsy tissue (n=3) in each group.

Results:
Both patient and control vaginal biopsy samples expressed latent and active forms of MMP-12, and MMP-1. The protein expression of the 45 kDa active form of MMP-12 was significantly increased in patients with POP compared to controls (P=0.03). Zymography detected the enzymatic activity of the pro-form and active form of both MMP-2 and MMP-9. We found a significant increase in gelatinolytic activity of both 68 kDa pro-form and 62 kDa active forms of MMP-2 (P=0.01 and P<0.001 respectively) as well as 87 kDa active form of MMP-9 (P<0.001) in patients with POP compared to controls. TIMP-1 protein expression in vaginal tissue showed a significant reduction in POP patients compared to controls (P=0.002). TIMP-2 and -4 immunostaining were mainly localized in the smooth muscle cells at the muscularis layer of the vaginal biopsies.

Conclusions:
In vaginal tissue of patients with POP, we have shown a decrease in TIMP protein expression paralleled by an increase in MMP protein expression and activity. These findings reflect an active ECM remodelling that may compromise the structural integrity of the pelvic floor leading to POP.

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Reduced Lysyl Oxidase Family Protein Expression in Vaginal Tissue of Premenopausal Women with Pelvic Organ Prolapse. May Alarab,[1,2,4] Oksana Shynlova,[1] Harold Drutz,[2,4] Stephen Lye.[1,2,3] *[1]Samuel Lunenfeld Research Institute, Mt Sinai Hospital, Toronto, ON, Canada; [2]Ob/Gyn; [3]Physiology; [4]Division of Urogynecology, University of Toronto, ON, Canada.*

Objectives:
Aberrant elastin and collagen synthesis may play a role in the pathogenesis of pelvic organ prolapse (POP). The lysyl oxidase (LOX) family of enzymes direct cross linking of collagen and elastin polymers, however to-date no information is available on the expression and localization of these proteins in human vaginal tissue. Our objectives were to study the expression and *in situ* localization of LOX, LOXL1, LOXL3 and LOXL4 proteins in the vaginal tissue of patients with advanced POP and asymptomatic controls.

Methods:
Pre-menopausal Caucasian patients affected by POP (≥grade 3 by POP-Q) and control patients (no POP) matched for age and BMI, undergoing vaginal and abdominal hysterectomy respectively were recruited. Full thickness anterior vaginal epithelial tissue was obtained from the surgical cuff of patients and controls in the proliferative phase of the menstrual cycle. Total protein was extracted using RIPA lyses buffer and Western immunoblot analysis was performed (patients: n=7, controls: n=6). PFA-fixed vaginal biopsy tissues (n=3 for each group) were used for immunohistochemical study.

Results:
Vaginal biopsy samples from both patient and control groups expressed all four members of LOX family proteins: LOX (47 kDa pro-form and 35 kDa active form), LOXL1 (47 kDa pro-form and 35 kDa active form), LOXL3 (83 kDa) and LOXL4 (84 kDa). The expression of all LOX family proteins was reduced in patients with POP compared to controls; however only the pro-form of LOX

protein showed a statistically significant reduction in its expression (P= 0.01). LOX, LOXL1, 3 and 4 proteins were detected by immunohistochemistry in all three layers of vaginal skin biopsies: (1) stratified squamous epithelium; (2) the lamina propria and (3) the muscularis layer from both patients with POP and controls. Significantly, in both groups we detected a numerous macrophages scattered throughout the vaginal thickness which displayed a very strong immunostaining to LOXL1.

Conclusions:

Patients with severe POP showed reduced expression of proteins regulating collagen and elastin biogenesis. Our finding raises the possibility that failure of ECM homeostasis could underlie the etiology of POP in women.

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Fibroblast Proliferation Is Regulated by HOXA11: Molecular Implications for Pelvic Organ Prolapse. Kathleen A Connell, Marsha K Guess, Richard Bercik, Hugh S Taylor. *Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objectives: The integrity of the extracellular matrix (ECM) is maintained by a delicate balance between collagen synthesis and degradation. Previously, we have demonstrated that Hoxa11 is essential for the development of the uterosacral ligament in mice and regulates the expression of collagen type III and MMP2. We have also shown that HOXA11 is deficient in the uterosacral ligament of women with pelvic organ prolapse (POP) compared to women with normal support. The exact mechanisms by which HOXA11 regulates pelvic organ integrity and repair remains to be elucidated. The aim of this study was to determine the effect of Hoxa11 expression on fibroblast proliferation, and its potential role in POP.

Methods: NIH 3T3 cells, a murine fibroblast cell line, were seeded onto a six well plate (1x 10 5 cells/well) and transfected with either a vector carrying a Hoxa11 cDNA or empty vector alone as a control. Immunohistochemistry using bromodeoxyuridine (BrdU) was performed to evaluate cell proliferation. Cell division in the uterosacral ligament (USL) was also compared in women with and without POP. USL specimens were obtained at the time of hysterectomy for benign disease. Immunohistochemistry was performed on paraffin embedded sections of the USL to evaluate expression of two mitotic markers, cyclin B1 and phospho-histone 3.

Results: Constitutive expression of HOXA11 in murine fibroblasts resulted in significantly higher proliferation. Cells transfected with Hoxa11 had a mean BrdU incorporation of 40.8$\pm$ 8.8 cells/ 100 cells compared with 32.2 $\pm$ 7.5 cells/ 100 cells in controls (P=0.03). In the USL obtained from women with and without POP, cell proliferation as determined by cyclin b1 and phospho-histone3 expression was not significantly different. Cyclin B1 and phosphor-histone 3 were expressed in comparable numbers of cells in both groups.

Conclusion: Hoxa11 is necessary for USL development and promotes proliferation of adult fibroblasts. HOXA11 may have a similar function in vivo in USL, and may regulate fibroblast proliferation during growth and the acute phase response following trauma when fibroblasts are activated to proliferate and remodel the ECM. It is likely that HOXA11 mediated proliferation of USL fibroblasts contributes to the tensile strength and resilience of these structures and thereby prevents POP.

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Connective Tissue Composition, in Term of Collagen I, III and Proteoglycans Content, in Support and Nonsupport Structures of Women with Uterine Prolapse. Elisabetta Trabucco,[1] Gennaro Acone,[1] Sara D'Avino,[1] Marco Torella,[1] Gennaro Trezza,[2] Annamaria Marenna,[1] Nicola Colacurci,[1] Luigi Cobellis.[1] *[1]Department of Obstetrics and Gynecology, Second University of Naples, Naples, Italy; [2]Loreto Mare Hospital, Naples, Italy.*

Objective. Connective tissue consists mainly of collagen and elastic fibers, glycoproteins and proteoglycans (PGs) and is considered an important factor of the support and nonsupport structures of the genitourinary region. It has been already demonstrated altered morphologic features in the pelvic support connective tissue in women with genital prolapse. However, analysis of nonsupport tissue may provide a more accurate reflection of body collagen. The objective of our study was compare the expression of collagen I, III and four PGs (Decorin, Fibromodulin, Lumican, Biglycan), essential for synthesis and regular assembly and diameter of the collagen fibrils, in uterosacral ligaments and in a nonsupport tissue, the uterine cervix, between premenopausal women with uterine prolapse respect to age matched controls.

Methods: We characterized uterosacral ligaments and uterine cervix of 25 premenopausal women with uterine prolapse and 16 controls. This immunohistochemical study was performed on paraffin-embedded sections.

Results. Uterosacral ligaments of the prolapsed uteri are characterized by a higher expression of collagen III, decorin, fibromodulin and byglican and a lower quantity of collagen I. No differences in the immunoreactivity of Lumican between the two patients groups. The abnormalities of support connective tissue composition are not observed in the uterine cervix of patients with prolapse.

Conclusions. Our results suggest an altered remodeling of connective tissue in the ligaments of premenopausal patients with prolapse, with a significant decrease in collagen I content and an increase in collagen III and PGs expression . In the prolapse patients this abnormal collagen metabolism and organization, mainly related to the observed change in PGs expression, might affect significantly the tensile strength of the connective tissue and consequently the support that is provided by the suspensory apparatus to the uterus. The alterd remodeling of support tissues, not detectable in nonsupport tissues, may suggest a predominant role of local biomechanical stresses (childbirth, chronic straining) in the pathogenesis of prolapse respect to systemic connective tissue deficiency.

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Pregnacy Promotes a Loss of Vaginal Smooth Muscle Contractility Facilitating Vaginal Distention during Delivery. James A Daucher, Jennifer R Rohland, Robin E Gandley, Pamela A Moalli. *Urogynecology, Magee Womens Research Institure, Pittsburgh, PA, USA.*

Objective: To achieve vaginal delivery with minimal maternal injury, vaginal smooth muscle (SM) contractility likely decreases. The objective of this study was to characterize changes in rat vaginal SM contractility in pregnancy that affords circumferential vaginal distension.

Methods: A tubular segment of the vagina of 8 virgin, 8 late pregnant , 8 immediate and 8 late post vaginal delivery rats were mounted in an organ bath between a force transducer and an adjustable support block. Dose response curves to norepinephrine (NE) and potassium (K+) were used to assess contractility. Relaxation capacity was determined in NE preconstricted vagina, using cumulative doses of sodium nitroprusside (SNP). Differences in active vs. passive length-tension curves were used to measure SM contractility relative to the vaginal wall forces. SM α-actin levels were measured using quantitative confocal immunofluorescence.

Results: Cumulative doses of NE induced a maximum constriction force of 33.1 $\pm$ 6 mN/g in virgin vagina while no measurable force was generated in response to NE in late pregnant or postpartum vaginas. Virgin vagina relaxed with cumulative doses of SNP; no measurable relaxation response was observed from pregnant or postpartum animals. In the presence of the high dose K+ (124mM), virgin vagina generated the greatest contractile force (43.92 $\pm$ 3 mN/g) vs. late pregnant vagina (22.6 $\pm$ 2.4 mN/g, P<0.001) or immediate post partum vagina (26.81 $\pm$ 4 mN/g, P=0.033). Four week postpartum K+ induced forces were similar to virgin levels (43.89 $\pm$ 3 mN/g). SM force generation (difference in active vs. passive length tension curves at 3mm of displacement) was decreased in late pregnancy (15.3 $\pm$ 4 mN/g) compared with virgin (112 $\pm$ 29 mN/g, P < 0.023) and 4 week postpartum vagina (172 $\pm$ 34 mN/g, P < 0.004). The expression of SM α-actin was lowest in late pregnancy (15 $\pm$ 1.5, P < 0.001) and immediate postpartum vagina (23 $\pm$ 1.5, P < 0.001) relative to virgin (33 $\pm$ 3) with a return to virgin levels 4 week postpartum (56 $\pm$ 2).

Conclusions: Vaginal SM contractility diminishes in pregnancy. The altered contractility is mirrored by a decrease in SM α-actin protein expression. Near complete recovery to pre-pregnancy levels occurs by 4 weeks post partum. These functional and biochemical changes likely represent maternal adaptations designed to minimize trauma during passage of the fetus(es).

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Endometrial Biopsy for the Diagnosis of Endometriosis by Detection of Small Endometrial Nerve Fibers. A Double-Blind Study. Moamar I Al-Jefout,[1] Gabrielle M Dezarnaulds,[2] Michael JW Cooper,[2] Natsuko Tokushige,[1] Robert Markham,[1] Ian S Fraser.[1] *[1]The Department of Obstetrics & Gynecology, University of Sydney, Queen Elizabeth II Research Institute for Mothers and Infants, Sydney, NSW, Australia; [2]Royal Prince Alfred Hospital, Camperdown, Sydney, NSW, Australia.*

BACKGROUND: Diagnosis or exclusion of endometriosis usually requires laparoscopy and peritoneal biopsy. We have described a novel and consistent observation of small nerve fibers in the functional layer of eutopic endometrium in women with endometriosis (Tokushige et al, 2006). These nerve fibers are not present in women without endometriosis. This finding allows the possibility

of making the diagnosis of endometriosis from an endometrial biopsy. OBJECTIVE: To evaluate the diagnostic value of examining endometrial biopsy specimens for small nerve fibers in women with pelvic pain or infertility in a double-blinded prospective comparison with laparoscopy. METHODOLOGY: We undertook to compare the detection of endometrial nerve fibers with laparoscopy and peritoneal biopsy for the diagnosis of endometriosis in 38 women (aged 34.4 years; range 22-45 years) who presented with chronic pelvic pain or infertility. Small nerve fibers were detected in the functional layer of endometrium using immuno-histochemical staining with the highly specific pan-neuronal marker PGP9.5, using a carefully validated technique and blinded assessment of nerve fiber density. Laparoscopic assessment of the presence of endometriosis and peritoneal biopsies was undertaken by three experienced gynecologic endoscopic surgeons. Data from these assessments were recorded independently of endometrial findings and maintained under blinded coding until the codes were broken. RESULTS: Small nerve fibers were detected in all 23 of the women in whom endometriosis was surgically diagnosed and in none of the 15 women in whom endometriosis was excluded at laparoscopy, giving the specificity and sensitivity of 100%. The density of nerve fibers in the endometriosis cases was 3.4 per $mm^2 \pm$ 4.3 (mean ± SD). CONCLUSIONS: Endometrial biopsy provided a reliability of detection or exclusion of endometriosis which was equal to that of diagnostic laparoscopy carried out by experienced gynecologic laparoscopists.

Reference: Tokushige N et al. High density of nerve fibres in the functional layer of endometrium in women with endometriosis. Hum Reprod 2006, 21: 782-787.

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ERK1/2 Is Involved in Endometriotic Angiogenesis. William Murk, Yesim Uz, Hakan Cakmak, Aydin Arici, Umit A Kayisli. *Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Background: ERK1/2 are MAPK intracellular signaling proteins involved in cell survival and differentiation. Endometriosis requires angiogenesis for ectopic implant growth. We hypothesized that the endometriotic peritoneal environment, known to be high in estrogen (E2), VEGF, and cytokines such as IL-8 and MCP-1, stimulates angiogenesis in human endometrial endothelial cells (HEEC) through ERK signaling.

Methods: Serial sections from normal (n=24) and ectopic (n=25) endometrium were immunostained for total- (T-) and phospho- (P-) ERK1/2 and CD34 (an endothelial progenitor cell marker); results were quantified by computer densitometry and grouped by menstrual phase. Cultured normal HEEC were treated with control, E2 (10-8M), IL-8, MCP-1, and VEGF (all 2 ng/mL) for 5 & 15 min, and Western blotted for P-/T-ERK (n=3). HEEC were treated with peritoneal fluid (PF; diluted 1:3 in basal media) from normal (n=4) and endometriotic (n=4) women, with or without PD98059 ERK1/2 inhibitor (20 μM) for 48 h, and cell viability was analyzed by MTT assay. Statistical analysis was done with one-way ANOVA.

Results: Tissue staining revealed that ectopic CD34+ endothelial progenitor cells undergoing angiogenesis (vessel sprouting and/or angiogenic cell cord formation) exhibited the strongest levels of P-ERK. HEEC of ectopic foci showed moderate-high P-ERK staining, while surrounding mesothelial capillaries were weak for P-ERK. In normal endometrium, P-ERK was cycle-dependent, with low levels in the late secretory phase vs. other phases ($p<0.05$). P-ERK of ectopic HEEC showed no cycle dependence, with moderate-high levels in all phases. T-ERK in all tissues was high and invariable. In cultured HEEC, treatment with PF from endometriotic women significantly increased HEEC viability after 48 h compared to normal PF ($p<0.05$). This effect was abrogated by ERK1/2 inhibitor. Among factors known to be high in endometriotic PF, VEGF increased P-ERK in cultured HEEC in 5 and 15 min ($p<0.05$), while E2, IL-8, and MCP-1 had no effect.

Conclusions: High P-ERK found specifically in sprouting endothelial progenitor cells and focal ectopic capillaries suggests that ERK1/2 is involved in endometriotic angiogenesis. The peritoneal microenvironment of endometriosis may be persistently stimulating ERK-mediated endometrial angiogenesis through VEGF. Further investigation into ERK1/2 inhibitors may offer a novel treatment of endometriosis.

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Hypoxia Induces the Adhesion Phenotype by Reducing Superoxide Dismutase Levels. Ghassan M Saed, Zhong L Jiang, Nicole M Fletcher, Husam M Abu-Soud, Michael P Diamond. *Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA.*

Introduction: The events leading to the development of post operative adhesions are unknown, though recent observations emphasize the crucial role played by the superoxide ion generated by hypoxia. Exposure of normal peritoneal fibroblasts to hypoxia caused the development of the adhesion phenotype, which is characterized by increased extracellular matrix molecules and inflammatory cytokines as well as high protein nitration and low nitric oxide. Superoxide dismutases (SOD) are a family of metalloenzymes that eliminates superoxide by converting it to hydrogen peroxide, protecting mammalian organisms against superoxide.

Objective: To determine whether SOD is differentially expressed between normal peritoneal and adhesion fibroblasts and whether its expression is modulated by hypoxia.

Methods: Fibroblasts from normal peritoneal and adhesion tissues were cultured under normal (20% O_2) and hypoxia (2% O_2) conditions for 24 and 48 hours. Real time RT/PCR was utilized to measure the absolute mRNA levels for SOD in both cell lines before and after hypoxia exposure.

Results: Normal peritoneal fibroblasts exhibited significantly higher basal mRNA levels for SOD (49.7 pg/μgRNA, $p<0.05$) as compared to adhesion fibroblasts (38.1 pg/μgRNA, $p<0.05$). Short exposure to hypoxia resulted in a significant increase in SOD mRNA levels to 63.3 and 52 pg/μgRNA in normal and adhesion fibroblasts respectively, $p<0.05$. In contrast, long exposure to hypoxia resulted in a significant decrease in SOD mRNA levels to 41.5 and 25.7 pg/μgRNA in normal peritoneal and adhesion fibroblasts respectively, $p<0.05$.

Conclusion: SOD mRNA levels are lower in adhesion as compared to normal fibroblasts, both basally and following short and longer exposure to hypoxia. Reduced SOD expression creates an milieu with greater free radical levels, which leads to the development of the adhesion phenotype. Enhancement of SOD levels and/or function are likely to be of benefit for reduction of postoperative adhesion development.

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Simvastatin Inhibits Development of Experimental Endometriosis; Role of Inhibition of MMP-3. Kaylon L Bruner-Tran,[1] Kevin G Osteen,[1] Antoni J Duleba.[2] *[1]Women Reproductive Health Research Center, Vanderbilt University, Nashville, TN, USA; [2]Ob/Gyn, University of California, Davis, Sacramento, CA, USA.*

Objectives: Development of endometriosis requires ectopic attachment and proliferation of endometrial tissue. The invasive process required to establish endometriosis may involve matrix metalloproteinases (MMPs), including MMP-3. Recently, we have demonstrated that statins inhibit proliferation of endometrial stroma. This study evaluated the effects of simvastatin on a nude mouse model of endometriosis and on modulation of MMP-3.

Methods: Proliferative phase human endometrial biopsies were established as organ cultures or utilized for stromal cell isolation. To establish endometriosis in the nude mouse, endometrial tissues were maintained in 1 nM estradiol (E) for 24 hrs and subsequently injected intraperitoneally into ovariectomized nude mice. Mice were treated with E (8 μg, silastic capsule implants) and simvastatin (5-25 mg/kg/day) by gavage for 10 days beginning 1 day after tissue injection. Control mice received E+vehicle. Subsequently, animals were sacrificed and endometrial implants were evaluated. Studies of endometrial stroma involved plating the cells in the presence of E (1 nM) or E+medroxyprogesterone acetate (MPA; 50 pM) with and without simvastatin (1-10 μM). Some cultures additionally received interleukin-1α (IL-1α, 200 ng/ml). Conditioned media was subjected to Western analysis for expression of MMP-3.

Results: In vivo studies demonstrated a dose-dependent inhibitory effect of simvastatin on number and volume of endometrial implants in mice. At the highest dose of simvastatin (25 mg/kg/day), the number of endometrial implants was decreased by 83% and the volume by 98% in comparison to the control group. Isolated stromal cells expressed abundant levels of MMP-3 following treatment with E, but minimal levels in cultures additionally receiving MPA or simvastatin. Although IL-1α induced a dramatic increase in MMP-3 secretion from cells pretreated with E alone, treatment with either MPA or simvastatin prevented this induction. Cultures receiving E+MPA+simvastatin were the most resistant to MMP-3 induction by IL-1α.

Conclusions: Simvastastin exerted a potent inhibitory effect on development of

endometriosis in the nude-mouse and inhibited MMP-3 expression in human endometrial stroma. The present findings may lead to the development of novel treatments of endometriosis involving statins.
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K-ras Actived Immunocompetent Retrograde Menstruation Model of Endometriosis. Ching-wen Cheng,[1,2] Di Licence,[1,2] Cristin G Print,[1,3] D Stephen Charnock-Jones.[1,2] *[1]Pathology, University of Cambridge, Cambridge, United Kingdom; [2]Obstetric & Gynaecology, University of Cambridge, Cambridge; [3]Department of Molecular Medicine & Pathology, University of Auckland, Auckland, New Zealand.*
Objective: To establish a new murine model of endometriosis that mimics the retrograde menstruation theory using immuno-competent mice.
Method: Ovariectomised donor female *K-ras$^{V12+/-}$/Cre$^{+/+}$/ROSA26R-LacZ$^{+/+}$* transgenic mice were treated sequentially with steroid hormones. To induce decidualization and activation and K-ras transgene, 16 mg/kg b-NF dissolved in maize oil was injected into the uterine lumen. Tissue degeneration mimicking menstruation was induced by hormone withdrawal. Menstruating endometrial tissue was collected from donor mice 60 hours after last hormone treatment, re-suspended in matrigel, and implanted subcutaneously in female C57BL/6 recipients. Immunohistochemical and morphometric methods were used to characterize the endometriosis-like lesions. Microarray analysis (illumina, n=6 in each group), was used to study the molecular changes in "menstruating" uteri following K-ras activation.
Result: Simple transplantation of decidualised endometrial tissue into immunocompetent animals does not lead to endometriotic lesion development but using tissue with the genetic modification described here overcomes this. Viable endometriosis-like lesions are visible 28 days after implantation. These lesions are histologically similar to those seen in man with intact glands, functional blood vessels, leukocyte infiltration and collagen deposition. Statistical analysis revealed that 220 transcripts were differentially regulated in the Ras activated and control tissue. Gene ontology (GO) analysis indicated that transcripts associated with epithelial cell function and differentiation were over represented within this gene set (chloride transport and epidermis development) as were those associated with the acute inflammatory response and neutrophil chemotaxis.
Conclusion: We developed a new model of endometriosis using immuno-competent mice to mimic retrograde menstruation induced endometriosis. This permits for the first time, the ready use of transgenic and knock-out tools to investigate the cellular and molecular mechanisms underlying endometriosis. Since the animals have an intact immune system it also allows the testing of therapeutic agents that modify the inflammatory response.

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***STRA6* Is Expressed and Induced by Progesterone in the Endometrium and Is Absent in Endometriosis.** Mary Ellen Pavone, Serdar E Bulun, Scott S Reierstad, Joy Innes, Magdy P Milad, You-Hong Cheng. *Obstetrics and Gynecology, Northwestern Univeristy, Chicago, IL, USA.*
OBJECTIVE:
Retinoic acid (RA) plays important roles in development, growth and differentiation by regulating the expression of target genes. In the mouse endometrium, RA deficiency leads to hyperkeratinization, while high concentrations of retinoids promote secretory characteristics. This leads many to believe that RA mediates important actions of progesterone in the endometrium and may account for progesterone resistance in endometriosis. The mechanism for regulation of RA production by progesterone is unknown. Moreover, the conversion from retinol to retinoic acid is not different between normal endometrium and endometriotic tissue. We hypothesize that retinol intake into cells rather than conversion of retinol to RA is the critical step that determines RA activity in the endometrium and endometriosis.
STRA6, a widely expressed multitransmembrane domain protein and member of a group of "stimulated by retinoic acid" genes, has been identified as the retinol binding protein receptor. It is strongly expressed in adult mice in several tissues including the female genital tract. We hypothesize that RA activity in the endometrium may be regulated by *STRA6*. Here we investigate the differential expression of *STRA6* in the endometrium and endometriosis.
DESIGN:
We studied primary stromal cells isolated from the eutopic endometrium of disease free women and walls of ovarian endometriomas from women with endometriosis with respect to *STRA6* expression and regulation by progesterone.
MATERIALS AND METHODS
Primary culture of eutopic endometrial and endometriotic stromal cells were treated with 10^{-7}M estradiol (E_2), 10^{-7}M R5020, a progesterone agonist, or vehicle for 24 hours. Real-time PCR was employed to quantify *STRA6* mRNA levels. We treated cells for 48 hours and quantified protein levels by immunoblotting.
RESULTS
Basal mRNA of *STRA6* levels in endometrial cells (n=8), were >1,000 fold higher than those in vehicle-treated endometriotic cells (n=8, P<.001). In endometrial stromal cells (n=8), R5020 stimulated *STRA6* mRNA levels by 1.7-3 fold. R5020 induced *STRA6* protein levels in endometrial stromal cells (n=3).
CONCLUSIONS
STRA6 is highly expressed and regulated by progesterone in endometrial stromal cells, whereas it is practically undetectable in endometriotic cells. *STRA6* may mediate important actions of progesterone in endometrium.

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Aromatizable C_{19}-Steroids Upregulate Aromatase Expression in Human Endometrial Cells Via Recruitment of SF-1 to *CYP19 IIa* Promoter. Orhan Bukulmez,[1] Daniel B Hardy,[2] Bruce R Carr,[2] Tannaz Toloubeydokhti,[1] Ruth A Word,[2] Carole R Mendelson.[1,3] *[1]Obstetrics and Gynecology, University of Florida College of Medicine, Gainesville, FL, USA; [2]Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, TX, USA; [3]Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX, USA.*

Upregulation of aromatase expression in endometrial cells disseminated into the peritoneal cavity (PC) may influence their survival and persistence via local estrogen synthesis. The inducing factors for the upregulation of aromatase in endometriosis are not well defined, but increased expression of SF-1 has been suggested to play an important role. Given that the aromatase substrate androstenedione (A4) is the predominant steroid in peritoneal fluid, we aimed to determine whether A4 has a role in the regulation of aromatase expression in human endometrium. We found that culture of primary endometrial stromal cells and explants with A4 (5-10 nM) for 24 h significantly upregulated expression of aromatase mRNA transcripts containing exon *IIa* at their 5'-ends. Moreover, in human endometrial surface epithelial cells (HES), dose-response studies with A4 (5-100 nM) revealed that 10 nM A4 maximally upregulated expression of both aromatase and SF-1. When tissue samples were evaluated from women with endometriosis and control endometrium, expression of aromatase mRNA mirrored SF-1. Treatment of HES cells (24 h) with tritiated A4 demonstrated its metabolism to estradiol (E_2), testosterone (T), dihydrotestosterone (DHT) and androstanediol (A-diol). Although equimolar concentrations of A4, T and E_2 upregulated aromatase and SF-1 mRNA expression in HES cells, the non-aromatizable androgens, DHT and A-diol, had no effect. A positive feedback role of estrogen in aromatase upregulation was suggested by the finding that the estrogen receptor antagonist, ICI 182,780, markedly diminished aromatase and SF-1 mRNA expression induced by A4. Finally, chromatin immunoprecipitation revealed that A4 significantly enhanced recruitment of SF-1 to its response element (-136 bp) upstream of *CYP19* exon *IIa*. Collectively, our findings strongly suggest that exposure of endometrial cells within the PC to C_{19}-steroids may cause an acute upregulation of *CYP19* gene expression through their aromatization to estrogens. Thus, estrogen may play a critical positive feedback role in the pathogenesis of endometriosis. Supported by NIH R01-DK031206.

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The HPA Axis and Depression/Anxiety Scores in Chronic Pelvic Pain Patients. B Stegmann,[1] B Frank,[2] J Gemmill,[1] G Chrousos,[1] M Ballweg,[3] P Stratton.[1] *[1]RBMB, NICHD/NIH, Bethesda, MD; [2]SOM, Wake Forest University, Winston-Salem, NC; [3]Endometriosis Association, Milwaukee, WI.*
Stress, pain, anxiety and depression adversely affect the hypothalamic-pituitary-adrenal (HPA) axis. We examined the influence of depression and anxiety on the HPA axis response to Corticotropin-Releasing Hormone (CRH) testing in women with and without chronic pelvic pain (CPP). **Methods:** Healthy women, aged 18-50, with & without CPP, with regular menses and off hormonal contraception were studied. None had pelvic infections, untreated depression, manic depression, fibromyalgia, or chronic fatigue syndrome. After ovine CRH injection (1 mcg/kg), serial blood samples (-5, 0, +15, +30, +45 minutes) were obtained for ACTH and cortisol measurements. Depression and anxiety

were scored using the Duke Quality of Life Questionnaire. HPA response was abnormal if peak ACTH levels were > 55 pg/mL without a rise in cortisol levels. 4 subject groups were: no pain and normal ACTH response (NPNR), pain and normal ACTH response (PNR), no pain with an abnormal ACTH response (NPAR) and pain with an abnormal ACTH response (PAR). Student t-test was used for comparisons. **Results:** 31 women (21 CPP, 10 controls) had a mean age of 33.5 ± 9.1 years (range: 21-47). 18 women responded normally (10 PNR, 8 NPNR) and 13 women responded abnormally (11 PAR, 2 NPAR). Peak and absolute rise in ACTH were significantly higher in abnormal (A) vs normal (N) responders; (peak: 85.2 A vs 33.5 pg/mL N, p<0.001; absolute: 51.2 A vs 27.5 pg/mL N, p=0.024). There was a significant difference within groups: peak ACTH: 88.5 PAR vs 37.1 pg/mL PNR, p=0.001; 67.5 NPAR vs 20.3 pg/mL NPNR, p<0.001; absolute ACTH: 72.2 PAR vs 28.2 pg/mL PNR, p=0.001, 56.1 NPAR vs 20.4 pg/mL NPNR, p<0.002). However, total cortisol level was not different between or within the 2 groups (23.2 A vs 20.0 mcg/dL N, p=0.08). Mean depression score did not differ among CPP patients (34.7 PAR vs 35.2 PNR, p=0.95), but differed among controls (25 NPAR vs 4.3 NPNR, p=0.01). Anxiety scores did not differ between or within groups (29.5 NPAR vs 8.4 NPNR p = 0.12; 40 PAR vs 39.7 PNR, p=0.66). **Conclusions:** Chronic pelvic pain is associated with an abnormal HPA response, regardless of anxiety or depression symptoms. Abnormal HPA responses in control women, however, appear to be influenced by depression. The mechanism and clinical significance of these findings should be explored.

Support: RBMB/NICHD/NIH and Endometriosis Association.

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Experimental Murine Endometriosis Induces Altered Endometrial Receptivity in Eutopic Endopmetrium. Banghyun Lee, Hongling Du, Hugh S Taylor. *Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.*

Objective: The eutopic endometrium in women with endometriosis demonstrates altered endometrial receptivity and altered gene expression. It is unknow if the endometrium is defective giving rise to a predisposition toward endometriosis or alternativly if the endometriosis causes the altered endometrial receptivity. Here we created experimental endometriosis in a mouse model through allotransplantation of the uterus to the peritoneal cavity in immunocompetent mice and examined the expression of several markers of endometrial receptivity in the eutopic endometrium.

Materials and Methods: The uterus of 8-week-old CD1 female mice was transected at cervicovaginal junction and each horn divided and transplantated into the abdomidnal cavity of eight CD1 mice. Seven controls received sham surgery only. After 14 weeks the uterus was removed, snap-frozen in Trizol. Total RNA was extracted and cDNA generated. Quantitative real time RT-PCR using SybrGreen was performed and normalized to β-actin. Fold changes in normalized Hoxa10, Hoxa11, BTEB1, EMX2, IGFBP1, integrin β-3, total progesterone receptor (PR-AB) and progesterone receptor-B (PR-B) were assessed. All experiments were conducted in duplicate, repeated at least three times and compared using Mann-Whitney Rank Sum Test.

Results: Hoxa10, Hoxa11, BTEB1, EMX2, and IGFBP1 mRNA expression showed 0.57-fold (P=0.046), 0.25-fold (P<0.001), 0.50-fold (P=0.043), 0.23-fold (P=0.038), and 0.09-fold (P<0.001) decrease in the uterus of mice with experimental endometriosis compared with controls. PR-AB and PR-B mRNA showed 2.7-fold (P=0.016) and 8.39-fold (P=0.006) increase in endometriosis group compared to controls, respectively, however the ratio of PR-B to PR-AB (B/AB) showed no significant change in both groups (20.98 vs. 7.53 , endometriosis vs. control, P>0.05). There was no significant change in Integrin β-3 mRNA expression (1.09-fold, P>0.05).

Conclusion: We demonstrate significant changes in multiple markers of endometrial receptivity in eutopic endometrium after induction of endometriosis. These findings suggest that origionally normal endometrium can develop defects with the creation of endometriosis; an abnormal endometrium is not a prerequisite for the development of endometriosis or associated abnormalities. This data also suggest the existance of a signal conduction pathway from endometriosis that alteres endometrial gene expression.

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Endometrial Expression Patterns of Relaxin and Relaxin Receptor mRNA Suggest Involvement of Relaxin in Endometriosis. Sara S Morelli,[1] Felice Petraglia,[2] Gerson Weiss,[1] Stefano Luisi,[2] Pasquale Florio,[2] Jeff Gardner,[1] Andrea Wojtczuk,[1] Laura T Goldsmith.[1] [1]*Obstetrics, Gynecology and Women's Health, New Jersey Medical School, Newark, NJ, USA;* [2]*Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Siena, Italy.*

Objective:

In normal endometrium, relaxin is a potent inhibitor of matrix metalloproteinases, which have been implicated in the invasive process of endometriosis. We tested the hypothesis that relaxin plays a role in endometriosis by comparing expression of relaxin and its LGR7 receptor in normal human endometrium to levels in samples from patients with endometriosis.

Materials and Methods:

Total RNAs, extracted from ectopic (n=8) and eutopic (n=11) endometrium of patients with endometriosis and from endometrium of normal controls (n=12), were reverse transcribed into cDNAs. Real-time PCR was performed using primers previously shown to identify human LGR7 relaxin receptor mRNA, H2 relaxin mRNA, and beta-actin mRNA, with SYBR-Green detection of double stranded DNA products. The comparative C_t method ($2^{-\Delta\Delta Ct}$) determined relative LGR7 and relaxin mRNA expression (normalized to beta-actin mRNA expression).

Results:

Relaxin mRNA was detectable in normal endometrium from 9 of 12 (75%) control patients. In contrast, relaxin mRNA was detectable in a lower proportion of samples [9 of 19 (47.4%)] from patients with endometriosis, among whom relaxin mRNA was detectable in a lower proportion of ectopic samples [2 of 8 (25%)] than in eutopic samples [7 of 11 (63.6%)]. LGR7 relaxin receptor mRNA was detectable in all samples, with lower expression in endometriosis samples than in endometrium from normal controls. Relaxin receptor LGR7 mRNA levels vary with cycle phase, with greater expression in the secretory phase (SP) than in proliferative phase (PP): in normal controls 5.9-fold higher levels in the SP than PP; and in endometriosis patients 3.6-fold higher levels in the SP than PP. In both phases, LGR7 mRNA levels were lower in ectopic samples than in either eutopic samples (6.2-fold lower in PP and 19.0-fold lower in SP) or endometrium from normal controls (6.5-fold lower in PP and 15.6-fold lower in SP). Eutopic endometrium had similar LGR7 mRNA expression to controls throughout the cycle.

Conclusions:

Decreased local expression of relaxin and relaxin receptor mRNA in ectopic endometrium from patients with endometriosis throughout the menstrual cycle suggests that relaxin may be protective against endometriosis.

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Ovarian Reserve after Laparoscopic Cystectomy of Endometriotic Ovarian Cysts. Shinichi Hayasaka, Takashi Murakami. *Obsterics and Gynecology, Tohoku University, Sendai, Japan.*

Objective

Laparoscopic ovarian cystectomy is generally recommended for endometriotic ovarian cysts because it has been associated with a higher pregnancy rate and a lower recurrent rate. However, residual ovarian function after laparoscopic cystectomy of endometriotic ovarian cysts has been questioned. In this study, we retrospectively evaluated ovarian response to hyperstimulation in women selected for IVF and ICSI cycles who previously underwent laparoscopic cystectomy of a monolateral endometriotic ovarian cyst.

Methods

A retrospective review of the patients between January 2003 and September 2007 was performed. The operated ovary and contralateral intact ovary were compared in terms of number of oocytes retrieved, number of follicles with a mean diameter > 15mm at the time of hCG administration. The patients who had recurrent endometriotic ovarian cysts were excluded.

Results

In total, ten patients were identified. The mean(±SD)number of oocytes retrieved was 4.1±2.4 in the control ovary and 1.1±1.4 in the previously operated ovary(P=0.031). The mean(±SD)number of follicles with a mean diameter > 15mm was 5.6±2.5 in the control ovary and 1.8±1.1 in the previously operated ovary(P=0.0004). The age of patients and diameter of the operated ovary had little influence on the difference between the response of the control ovary and one of the previously operated ovary.

Conclusion

Laparoscopic cystectomy of endometriotic ovarian cysts is associated with a significant reduction in ovarian reserve.

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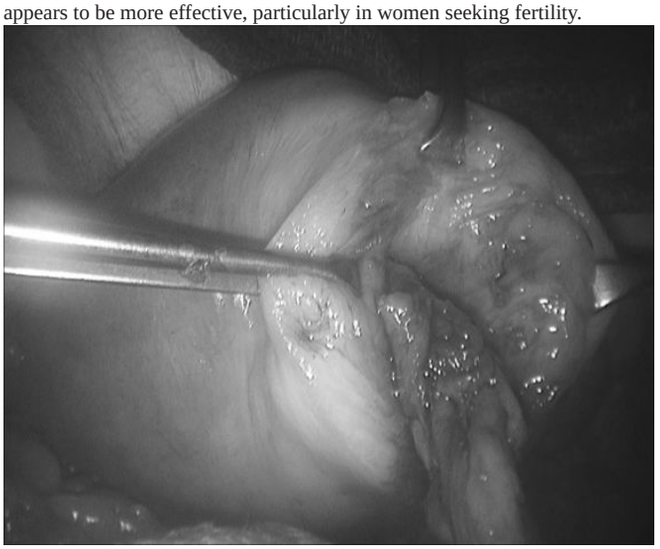
A Uterine Cystic Mass: An Unusual Presentation of Adenomyosis. Ziad R Hubayter, Jennifer Chao, Lisa A Kolp. *Gynecology and Obstetrics, Johns Hopkins School of Medicine, Lutherville, MD, USA.*

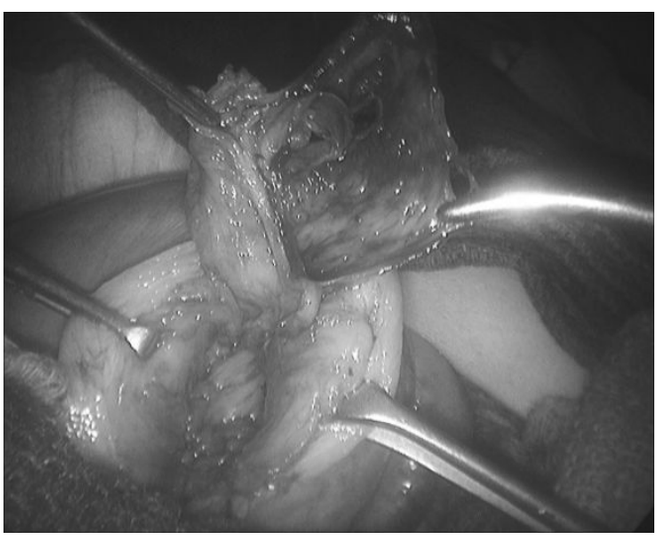
Objective: To increase awareness of potential presentations of adenomyosis and the differential of a uterine mass.

Material and Method: In a tertiary medical center, a patient was being evaluated for a uterine cystic mass and cyclic dysmenorrhea. The patient is a 26 year old nulliparous woman who has been complaining of cyclic dysmenorrhea for several years. The pain starts with the onset of menses and lasts around 2 weeks. The patient did not improve on contraceptives. The patient had prior laparoscopy and imaging studies which misdiagnosed the mass as either a leiomyoma or an adnexal hemorrhagic cyst. The patient underwent exploratory laparotomy and resection of the mass.

Results: On ultrasound, the mass appeared as an echodense cyst within the uterus. Intraoperatively, a 3 cm thick-walled well circumscribed uterine chocolate cyst was identified. The resection of the cyst was performed in similarly to an ovarian cystectomy. Tissue examination confirmed the diagnosis of cystic adenomyosis. Following surgical intervention, the patient reported significant improvement of symptoms.

Conclusion: This case highlights an unusual presentation and treatment of adenomyosis and cyclic dysmenorrhea. Cystic adenomyosis should be on the differential of uterine cystic mass, particularly in young women with cyclic dysmenorrhea and menorrhagia. The earlier misdiagnosis probably resulted from the unfamiliarity of physicians with this condition. Similar clinical presentations may occur with congenital uterine anomalies (with an obstructed uterine segment) and cystic degeneration of a leiomyoma. The incidence and pathogenesis of cystic adenomyosis are unknown. Oral contraceptives may be helpful as a first line therapy. However, resection of the adenomyotic cyst appears to be more effective, particularly in women seeking fertility.





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Inflammatory Cytokine Imbalance Persists in Cultured Eutopic Endometrial Cells of Patients with Endometriosis. Melis Altan,[1] Dana Shanis,[2] Eric Grund,[1] Kristen Crandall,[3] Larry Barmat,[4] Stephen Palmer.[1] *[1]Reproductive Health, EMD Serono Research Institute, Rockland, MA, USA; [2]Ob/Gyn, University of Connecticut Health Center, Farmington, CT, USA; [3]Biology, Cornell University, Ithaca, NY, USA; [4]Ob/Gyn, Abington Memorial Hospital, Abington, PA, USA.*

Objective: It is hypothesized that abnormalities within the eutopic endometrium of females with endometriosis can cause the ectopic growth of the endometrial tissue at extrauterine sites. Previous studies have shown that gene expression in eutopic endometrium of women with endometriosis is markedly different from disease-free females. Inflammatory cytokines and receptor-dependent kinase pathways are widely recognized as therapeutic targets for immune disorders, which is believed to be the underlying pathogenesis of endometriosis. In this study we asked whether responses of primary eutopic endometrial cell cultures are dysregulated between females with or without endometriosis.

Methods: A total of 9 biopsies of endometriotic eutopic endometrium (EEE) and disease-free endometrium (DFE) were obtained from proliferative phase females. The primary cell cultures established from these biopsies were treated with TNFa to induce expression of inflammatory mediators. Parallel cultures were also treated with kinase inhibitors of p38, MEK, PI3K and IKK. After a period of 24 or 48 hours, concentrations of IL-6, MCP-1, and GM-CSF in cell culture supernatants were analyzed by ELISA.

Results: In EEE, basal concentrations of MCP-1 and GM-CSF were 2-3 times higher, while IL-6 was 10 times higher compared to DFE. As expected, TNFa treatment stimulated higher levels of cytokine secretion in DFE mimicking disease state, however, the same treatment had almost no effect on EEE. Kinase inhibitors were very effective in lowering the cytokine levels of DFE, however, their effect on EEE were less dramatic.

Conclusion: EEE expresses higher levels of inflammatory cytokines under basal conditions, which is in concert with the current literature. Our results validate that high IL-6 levels in endometrium are diagnostic for females with disease. The increase of GM-CSF, IL-6 and MCP-1 following TNFa treatment was expected in DFE however; TNFa's effect was blunted in EEE, which implies that EEE are already highly activated. The effect of kinase inhibitors on cytokine production from EEE was unaltered relative to DFE, which implies that TNFa stimulated kinase pathways are modified even in EEE.

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Characterization of *Mig-6* Expression in Normal and PCOS Endometrium Suggests a Mechanism for Endometrial Hyperplasia and Cancer. Bruce A Lessey,[1] Steven L Young,[2] Sandra F Larson,[3] Francesco J DeMayo,[4] Jawook Jeong,[4] Ricardo F Savaris,[5] Linda C Giudice.[5] *[1]Department of Obstetrics and Gynecology, Greenville Hospital System/University of South Carolina, Greenville, SC, USA; [2]Department of Obstetrics and Gynecology, University of North Carolina, Chapel Hill, NC, USA; [3]Department of Biology, Furman University, Greenville, SC, USA; [4]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA; [5]Obstetrics and Gynecology, University of California, San Franscisco, San Franscisco, CA, USA.*

Endometrial cancer is the fourth leading cause of cancer in women, and hyperplasia and adenocarcinoma are more commonly seen in women with polycystic ovary syndrome (PCOS). *Mig-6*, a negative inhibitor of EGF signaling, is expressed in normal secretory phase endometrium and associated with hyperplasia in *Mig-6* knockout mice. **Objective**: We examined and compared endometrium from normal and PCOS women for *Mig-6* expression and characterized its regulation using *in vivo* and *in vitro* models. **Methods**: Immunohistochemistry (IHC) and real-time PCR were performed in endometrium from normal (n=20) women and PCOS (n=15) women. Regulation of *Mig-6* was studied in Ishikawa and ECC-1 endometrial cell lines, treated with sex steroids or EGF. Endometrial xenografts from normal and PCOS women were implanted in ovxd Rag2-γ(c) immunocompromised mice, treated with E_2 or E_2 + P pellets to mimic a natural cycle. **Results**: *Mig-6* protein expression was low in the functionalis of the proliferative phase and high in the secretory phase; this pattern was reversed in the basalis layer. PCOS secretory phase endometrium had significantly less *Mig-6* protein and mRNA than normal endometrium ($p < 0.01$). Xenografts using PCOS samples had paradoxically elevated expression of *Mig-6* compared to normal, and appeared unresponsive to steroid treatments. *Mig-6* expression in endometrial cell lines was regulated by EGF but not by ovarian steroids, E_2 or E_2 + P. **Conclusions**: *Mig-6* expression is low in proliferating endometrium and regulated by EGF. Risk of hyperplasia or cancer in PCOS women can be explained by altered expression of *Mig-6*.

Reduced expression *Mig-6* provides insight into endometrial function and may lead to better treatment options for disorders of endometrial proliferation including endometriosis, adenomyosis, endometrial hyperplasia and cancer. Supported in part by NIH-U54-HD35041(SLY), U54HD055764 (LCG) and U54HD77495 (FJD).

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Could Androgens Influence Human Luteal Cells Function? Anna Tropea,[1] Mariateresa Orlando,[1] Maria Francesca Gangale,[1] Federica Romani,[1] Isamaria Loiudice,[1] Federica Tiberi,[2] Stefania Catino,[3] Antonio Lanzone,[3] Rosanna Apa.[1] *[1]Cattedrà di Fisiopatologia della Riproduzione, Università Cattolica del S. Cuore (UCSC), Roma, Italy; [2]Istituto Scientifico Internazionale (ISI) "Paolo VI", UCSC, Roma, Italy; [3]Istituto di Ricerca "Associazione Oasi Maria SS ONLUS", Troina (EN), Italy.*

Objective.

In PCOS patients reproductive dysfunctions are frequently observed even if ovulation occurs. An impaired luteal function could partially explain this subfertility, since luteal steroidogenesis deficiency and premature luteal degeneration have been described in PCOS patients. Based on the frequent observation of high plasmatic levels of androgens in PCOS, we investigated whether hyperandrogenism could negatively affect luteal function.

To this aim, we tested the in vitro effects of androgens on Progesterone (P) and on Vascular endothelial growth factor (VEGF) production by human luteal cells. Indeed, VEGF is essential for normal luteal development and function being an important regulator of angiogenesis and vascular permeability. Since Prostaglandins (PGs) play a central role in modulating luteal function, the influence of androgens on PGE2 and PGF2a secretion was also investigated.

In order to investigate whether testosterone and androstenedione act directly or after local aromatisation, we tested the *in vitro* effects of estriol, estrone and 17-β-estradiol on P, VEGF, PGE_2 and $PGF_{2\alpha}$ secretion by human luteal cells. Moreover, we tested the effects of testosterone and androstenedione in presence of exemestane – an aromatase inactivator.

Methods.

Highly purified human luteal cells were cultured for 24 h with medium alone (control) or in presence of increasing concentrations of testosterone or dihydrotestosterone or androstenedione (from 10^{-10} to 10^{-6}M) or in presence of increasing concentrations of estriol, estrone and 17-β-estradiol (from 0.1 to 10 ng/ml). Moreover, testosterone and androstenedione 10^{-7} M were tested in presence of exemestane (from 4 to 400 nM). In the culture medium VEGF, P, PGE_2 and $PGF_{2\alpha}$ secretion was assayed by commercially available ELISA kits. In order to evaluate the influence of androgens and estrogens on VEGF mRNA expression on luteal cells real-time RT-PCR has been performed.

Results.

Our results demonstrated that testosterone, androstenedione and dihydrotestosterone were all able to significantly reduce VEGF secretion in human luteal cells, while no effect was seen on VEGF mRNA expression. Androgens were also able to significantly decrease P and $PGF_{2\alpha}$ secretion, while an increase was observed on PGE_2 production.

Moreover our preliminary results demonstrated that in human isolated luteal cells estriol, estrone and β-estradiol at all tested doses are able to mimic androgens effects on P and PGE_2 production. On the contrary estrogens were able to increased VEGF release. Estrogens seemed to have no effect on $PGF_{2\alpha}$ released. Data regarding exemestane inhibition of testosterone and androstenedione are still in progress.

Conclusion.

Increased levels of androgens were able to decrease luteal VEGF, P and $PGF_{2\alpha}$ release and might be involved in PCOS-luteal phase defect. Nevertheless, the observed effects could probably be attributed – at least in part – to estrogens locally produced via the aromatase enzyme, rather than be directly mediated by testosterone and androstenedione.

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The *In Vitro* Effect of Proghrelin-Derived Peptides on Purified Human Luteal Cells. Anna Tropea,[1] Mariateresa Orlando,[1] Maria Francesca Gangale,[1] Federica Romani,[1] Isamaria Loiudice,[1] Federica Tiberi,[2] Stefania Catino,[3] Antonio Lanzone,[3] Rosanna Apa.[1] *[1]Cattedrà di Fisiopatologia della Riproduzione, Università Cattolica del S. Cuore (UCSC), Roma, Italy; [2]Istituto Scientifico Internazionale (ISI) "Paolo VI", UCSC, Roma, Italy; [3]Istituto di Ricerca "Associazione Oasi Maria SS ONLUS", Troina (EN), Italy.*

Introduction.

Ghrelin, well known mediator of neuroendocrine effects, has been demonstrated to have a role as signal for energy insufficiency. Several evidences suggested that ghrelin might also operate as regulator of reproductive function. Indeed, we recently demonstrated that both P and VEGF released were significantly decreased by ghrelin in isolated human luteal cells. Moreover ghrelin was also able to reduce prostaglandin (PG) E_2 and increase $PGF_{2\alpha}$ luteal cells release.

In the present work we investigated whether two ghrelin-related peptides derived by the same ghrelin precursor (unacylated ghrelin and obestatin) might affect human isolated luteal cells function. Conventionally regarded as an inert form of ghrelin, unacylated ghrelin has been recently proven as biologically active in specific cellular contexts. Obestatin has been suggested to directly control porcine ovarian cell functions.

In these assumptions, we investigated whether unacylated ghrelin and obestatin could directly affect luteal progesterone (P) release. Moreover, since Vascular Endothelial Growth Factor (VEGF) is known to play a critical role in luteal development and function, the influence of unacylated ghrelin and obestatin on luteal VEGF, $PGF_{2\alpha}$ and PGE_2 production was also analysed.

Methods.

Highly purified human luteal cells were incubated for 24h with medium alone (control) or with hCG (100 ng/ml) or with $CoCl_2$ (10 μM) or in presence of increasing concentrations of unacylated ghrelin (1-100 nM) and obestatin (1-500 nM). In the culture medium VEGF, $PGF_{2\alpha}$, PGE_2 and P secretion was assayed by commercially available ELISA kits. Moreover real-time RT-PCR has been performed in order to evaluate whether unacylated ghrelin and obestatin could affect VEGF mRNA in human luteal cells.

Results.

Our preliminary results demonstrated that either unacylated ghrelin or obestatin are able to negatively affect luteal steroidogenesis. Moreover, both peptides seemed to increase the release of two luteotropic factors -VEGF and PGE_2- and to reduce $PGF_{2\alpha}$ release – a luteolytic prostanoid – from isolated human luteal cells. Finally data regarding the expression of VEGF mRNA are still in progress.

Conclusions.

Our results suggest that unacylated ghrelin and obestatin – two ghrelin-related peptides – could play a role in regulating luteal function affecting both luteal steroidogenesis and luteotropic/luteolytic imbalance.

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SK3 Channel Down-Regulation Is Essential for Normal Delivery; the Potential Role of Transcription Factors Sp1 and Sp3. Stephanie L Pierce,[1] Jessica DK Kresowik,[2] Kathryn G Lamping,[3] Sarah K England.[1] *[1]Department of Molecular Physiology and Biophysics, University of Iowa Carver College of Medicine; [2]Department of Obstetrics and Gynecology; [3]Department of Internal Medicine and Pharmacology and Veterans Affairs Medical Center, Iowa City, IA.*

The mechanisms responsible for labor progression have yet to be fully elucidated. In a previous study, over-expression of small conductance calcium-activated K^+ channel isoform 3 (SK3) in transgenic mice compromised parturition, suggesting a role for SK3 channels in this process. Based on these findings, we hypothesized that SK3 channel expression is reduced late in pregnancy to enable the uterus to produce the forceful contractions required for parturition. We investigated the effects of SK3 channel expression on gestation and parturition by comparing transgenic mice over-expressing mice SK3 ($SK3^{T/T}$) mice to wild-type (WT). In WT mice, SK3 transcript and protein are significantly reduced during pregnancy. The force produced by uterine strips from $SK3^{T/T}$ mice on the day of delivery was significantly less than WT or SK3 knockout control ($SK3^{DOX}$), and the contractile force reached WT levels by application of the SK3 channel inhibitor, apamin. Moreover, lipopolysaccharide and RU486, which induce pre-term labor in WT mice, failed to result in completion of delivery in $SK3^{T/T}$ mice. Thus, stimuli that initiate parturition under normal circumstances are insufficient to coordinate the uterine contractions needed for the completion of delivery when SK3 channel activity is in excess. The mechanism(s) down-regulating this channel in the uterus during pregnancy is unknown. The SK3 gene promoter region contains two Specificity Protein (Sp) binding sites; 1) Sp1, is a transcription factor known to enhance the transcription of genes in response to estrogen, and 2) Sp3, which competes for the same binding motif as Sp1, reduces gene expression. Sp1 protein expression in mice uteri dramatically decreases in late pregnancy, while Sp3 protein level remains consistent. Our data indicate that SK3 channels must be downregulated for the gravid uterus to generate labor contractions sufficient for delivery in both term and preterm mice. The changes in SK3 channel expression may be transcriptionally regulated by Sp1 and Sp3.

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Changes in Metabolic Profile of Placental Villous Explants Cultured in Different Oxygen Tensions. Alexander E Heazell,[1] Marie C Brown,[2] Warwick B Dunn,[2] Stephanie A Worton,[1] Nicola Robinson,[1] Philip N Baker,[1] Douglas B Kell.[2] *[1]Maternal and Fetal Health Research Group, University of Manchester, Manchester, United Kingdom; [2]Manchester Interdisciplinary Biocentre, University of Manchester, Manchester, United Kingdom.*

Background Pre-eclampsia (PE) is associated with systemic maternal endothelial dysfunction, which is thought to result from the presence of circulating factors released following placental damage. It is hypothesised that this occurs as a result of reduced oxygen (O_2) delivery. Some features of placental pathology seen in PE, such as increased apoptosis, can be reproduced by culture of placental explants in 1% O_2.

Metabolomics operates to study 'all' metabolites within a biological system. This strategy has previously identified differences in maternal plasma between normal pregnancies and those complicated by PE. In these experiments we used metabolomics to investigate differences in the metabolic profile of explants cultured in different O_2 tensions.

Hypothesis The metabolic profile of placental villous explants is altered by culture in different O_2 tensions.

Methods Placental villous explants were cultured with either 10% serum (n=10) or in serum-free conditions (n=6) for 96h in 20%, 6% and 1% O_2. After 96h, conditioned culture medium and tissue were collected and snap frozen. Tissue was homogenised in 50% ice cold methanol prior to analysis. Samples were analysed using Gas Chromatography-Mass Spectroscopy (GC-MS). Samples cultured at 20%, 6% and 1% O_2 were compared to identify concentration differences in metabolites in conditioned cultured medium and tissue lysate. Kruskal-Wallis test was used for statistical analysis. Due to the large number of metabolites identified, a p value of 0.001 was considered significant.

Results The mean intra-assay variability was 9.1%. There were no differences in the metabolic profile of conditioned culture medium from 6% and 20% O_2. Several metabolites differed in culture medium from 1% compared to 6% and 20% O_2 including: deoxyribose, glycerol and threionic acid. These changes were present in both serum and serum-free conditions. Using these metabolites alone, culture in 1% O_2 could be completely discriminated from 6% and 20% O_2. Deoxyribose was also elevated in tissue lysate from 1% O_2 compared to 6% O_2.

Conclusion This metabolomic strategy can identify differences in metabolic profile in placental tissue cultured in 1% O_2. These novel compounds may provide further insight into pathophysiology of PE.

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Metabolic Footprinting of Placental Explants Using UPLC/LTQ-Orbitrap Mass Spectrometry. Marie Brown,[1] Alexander Heazell,[2] Warwick B Dunn,[1] Louise C Kenny,[3] Stephanie Worton,[2] Douglas B Kell,[1] Philip N Baker.[2] *[1]School of Chemistry, University of Manchester, Manchester, United Kingdom; [2]Division of Human Development, University of Manchester, Manchester, United Kingdom; [3]Department of Obstetrics and Gynaecology, University College Cork, Cork, Ireland.*

Objective

A strategy of metabolic footprinting (the study of extracellular metabolites, which are related to intracellular metabolism) was used to detect a wide array of low molecular weight metabolites. Metabolic profiles were employed to differentiate between placental explants cultured at different oxygen tensions. Two separate studies were combined to validate initial observations.

Methods

Metabolic footprints of placental villous explants, obtained from uncomplicated pregnancies, (n=5) were cultured for 96h in 1%, 6% and 20% oxygen. Conditioned culture medium was then collected and frozen, prior to analysis by UPLC/LTQ-Orbitrap mass spectrometry in both negative and positive ion mode. Samples cultured at 1%, 6% and 20% were compared to identify differences in the metabolic profiles. This procedure was repeated for 6 different explants and the results compared across the 2 studies in order to validate initial findings.

Results

Approximately 350 unique peaks were detected in both sample sets in negative ion mode and 300 peaks in positive ion mode. A number of metabolites were identified as being significantly different using Kruskal-Wallis (pval<0.01) under different oxygen tensions. Some differences were seen between the results obtained from both runs due to separate batch preparation of the medium but good reproducibility was obtained for many metabolites between batches. Metabolites of biological interest included amongst others 2-deoxyribose, valine, tyrosine and aspartic acid. The largest differences were those seen between O_2 1% and O_2 6%.

Conclusion

Comprehensive metabolic profiles were detected and employed to identify differences in the metabolic footprints of explants cultured under differing oxygen conditions. A number of biologically interesting metabolites were characterized.

Acknowledgements

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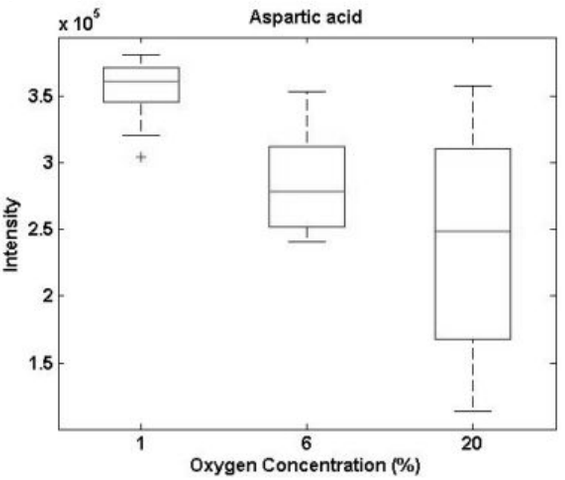

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Leptin Affects the Development of Neural Lineage Cells during the Prenatal Period. Jun Udagawa,[1] Ryuju Hashimoto,[1] Atsuki Ono,[1] Hiroaki Itoh,[2] Hiroki Otani.[1] *[1]Department of Developmental Biology, Faculty of Medicine, Shimane University, Izumo, Japan; [2]Obstetrics and Gynecology, National Hospital Organization Osaka National Hospital, Osaka, Japan.*

Objective: Brain weight and DNA content were reduced in leptin deficient (*Lep^{ob}/Lep^{ob}*) mice postnatally. Leptin is detected in the sera and its receptors are expressed in the brain of the mouse fetus. We examined the role of leptin in the proliferation and differentiation of neural lineage cells in the mouse fetus. **Methods:** The number of total cells in the cerebral cortex was compared between (*Lep^{ob}/Lep^{ob}*) and wild type fetuses from embryonic day (E) 14 to 18. The number of BrdU positive cells was also compared on E14 and E16. BrdU uptake, the ratio of viable colony number to plated cell number, the proportion of multipotent, neuronal or glial progenitor colonies, and the expression levels of *Hes* mRNA were compared between leptin- and non-treated neurosphere cells. Moreover, we examined STAT3 phosphorylation by leptin stimulation with ELISA to investigate whether or not JAK/STAT3 pathway transduces the leptin signal in the prenatal period as in the adult. **Results:** *Lep^{ob}/Lep^{ob}* fetuses had reduced total cell numbers in the ventricular zone (VZ) on E16 and E18, and in the cortical plate on E18. The number of BrdU-positive cells was reduced in VZ of E14 and E16 *Lep^{ob}/Lep^{ob}* fetuses. BrdU uptake and the number of viable colonies were increased by 2 days-leptin treatment in the neurosphere culture. The proportions of glial-restricted and multipotent precursor colonies were increased by leptin, whereas that of oligodendrocyte precursor colonies were decreased. *Hes1* mRNA expression was enhanced in neurosphere cells by leptin. Neither the amount of phosphorylated STAT3 nor that of STAT3 was increased in neurosphere cells by leptin stimulation. **Conclusion:** Our study suggests that leptin maintains the neural stem and glial-restricted precursor cells through upregulation of *Hes1* expression and increases the number of cerebrocortical cells in the mouse fetus, however, JAK/STAT3 pathway does not mediate the leptin signal in undifferentiated neural lineage cells.

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Diabetes Mellitus: A Threat to Male Fertility? IM Agbaje,[1] D O'Neil,[1] C McVicar,[1] C Mallidis,[1] B Schock,[2] AB Atkinson,[3] SEM Lewis,[1] N McClure.[1]
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Introduction. Diabetes (DM) the commonest endocrine disorder, is rising rapidly affecting more men of reproductive age. Studies of its relation to male fertility are limited. Oxidative stress is key in sperm DNA damage and complications of DM. Advanced Glycation End-products (AGEs) are possible mediators. This study compared semen profiles, sperm DNA and AGEs in DM with controls.

Methods. Semen from 26 diabetic and 23 non-diabetic subjects underwent conventional analysis. Sperm DNA was assessed by COMET and Long-PCR. Oxidative DNA damage was determined using HPLC. Immunocytochemistry and ELISA were used to localise and quantify AGE and its receptor (RAGE).and ELISA were used to localise and quantify AGE and its receptor (RAGE).

Results. Sperm from diabetics showed greater nDNA fragmentation (53%vs32%; $p<0.0001$), mtDNA deletions (4.7vs3.1; $p<0.05$) and oxidative DNA damage (2.36vs3.39 8-OHdG 10^{-5}dG; $p<0.05$). There was no difference in semen parameters. AGE and RAGE were present in both groups with significantly higher amounts in diabetics.

Conclusions. This finding has implications for diabetic male reproductive health, as sperm DNA quality is associated with fertility outcomes.

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Allogenic Fetal Cells Persist in the Maternal Circulation. Zev Williams, Dimity Zepf, Janina Longtine, Raymond Anchan, Betsy Broadman, Stacey A Missmer, Mark D Hornstein. *Dept. of Obstetrics and Gynecology, Dept. of Pathology, Brigham and Women's Hospital, Boston, MA, USA.*