

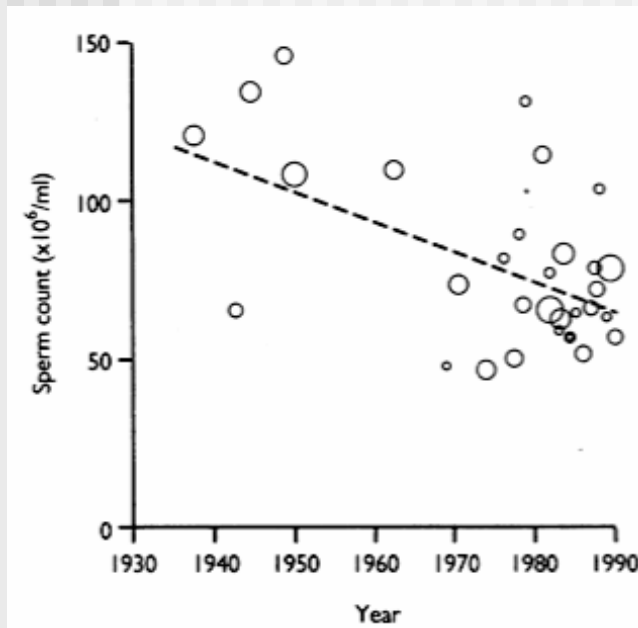
# Sperm mRNA as a Biomarker of Testicular Toxicity

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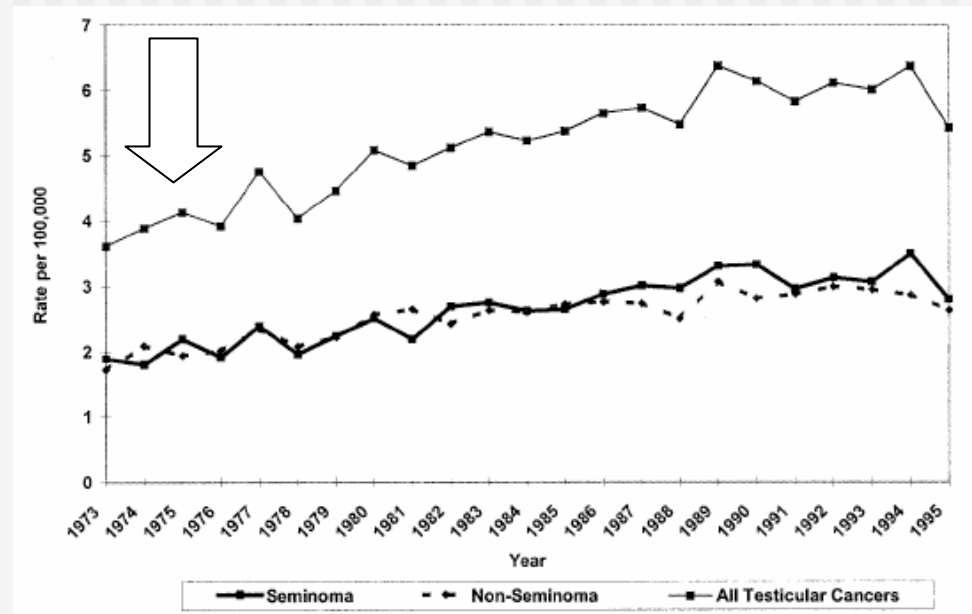
Andrea Barnes

# Male Reproductive Disorders

## Trends in Sperm Counts



## Testicular Cancer Incidence



In industrialized countries:

- Among fertile men, decreasing sperm counts
- Increase in testicular cancer incidence

# Fertility Concerns

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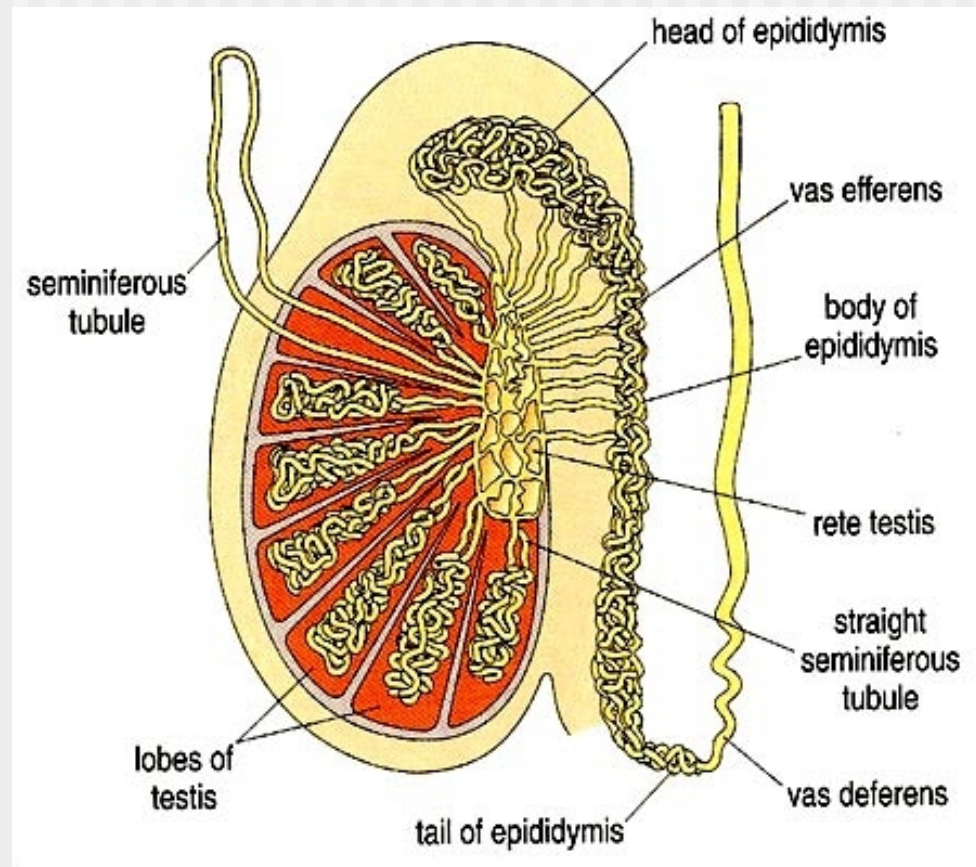
- Infertility affects up to 17% of American couples
- Male factor infertility accounts for 50% of these cases
- >90% of male infertility cases have no identifiable cause

# Environment and Public Health

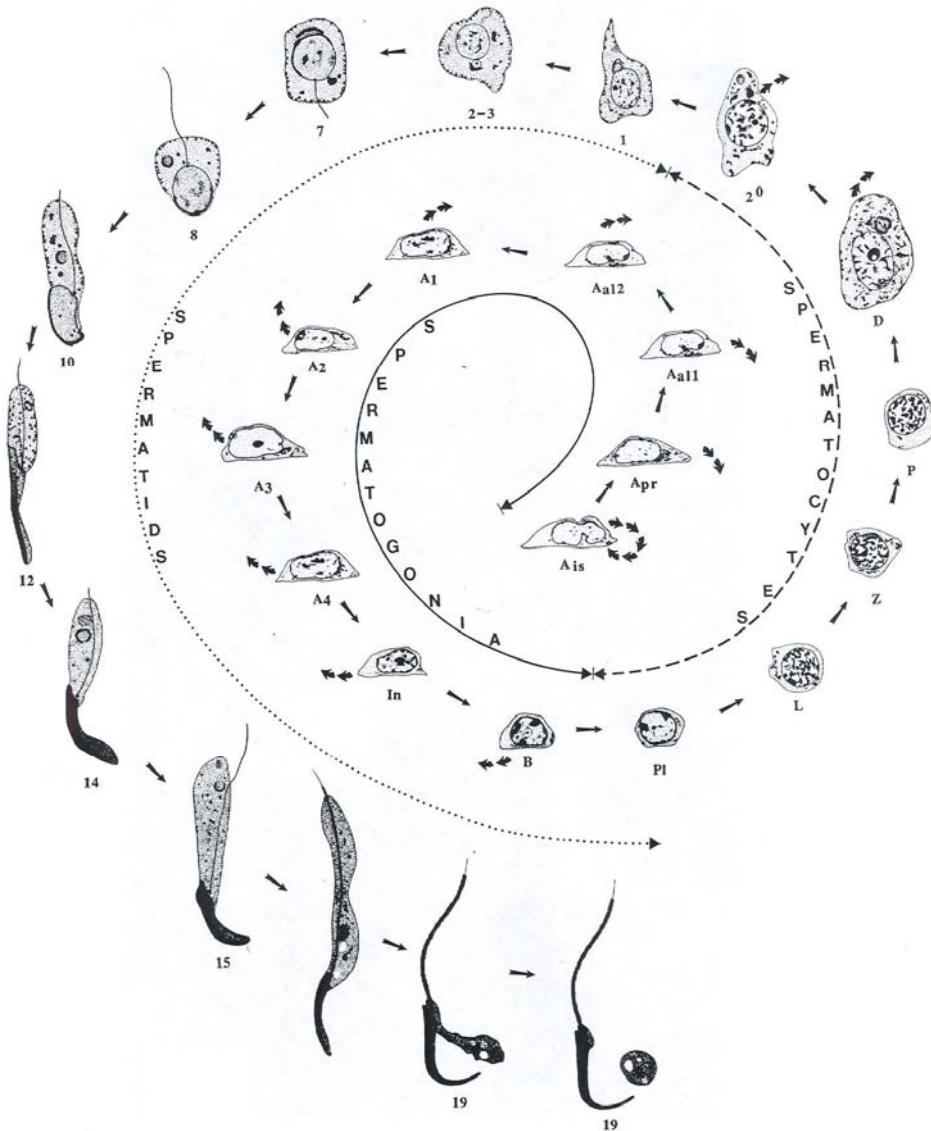
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- Common etiology among the various male reproductive disorders
- Known male reproductive toxicants: phthalates, pesticides, fungicides, dioxins, solvents, lead, cadmium

# The Testis



# Spermatogenesis



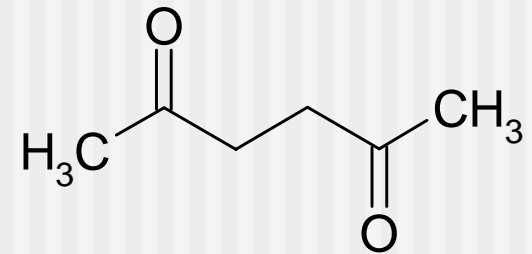
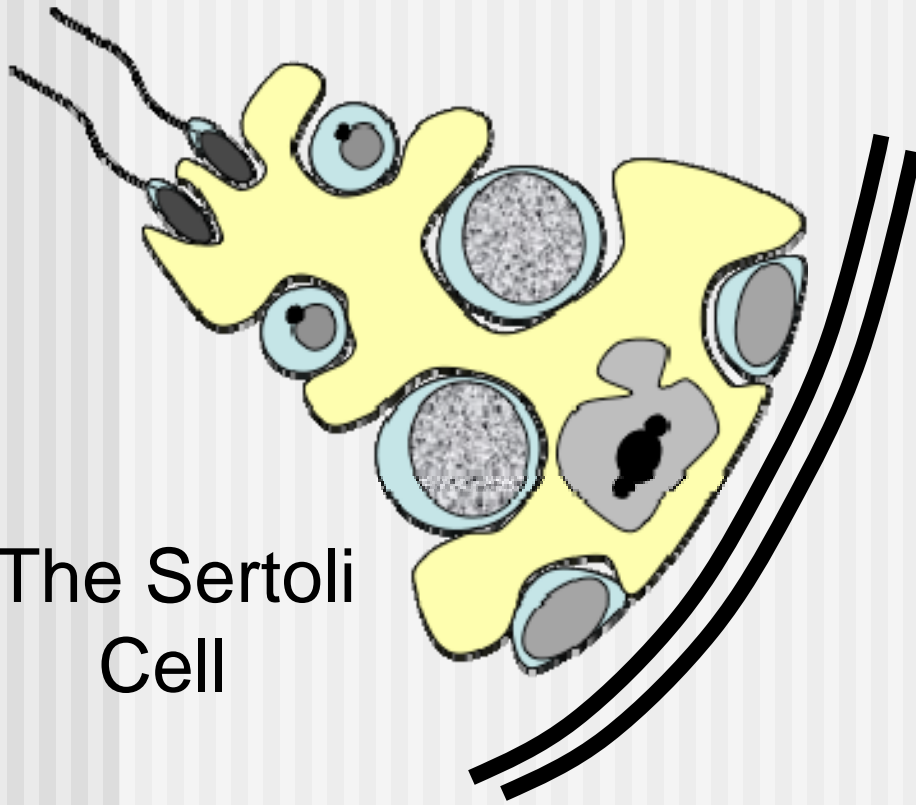
- Germ cells divide and differentiate
- DNA -->mRNA-->protein
- Tight regulation
- Transcription ends, not all mRNA translated
- mRNA remnants in sperm

# Significance of mRNA in sperm

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- A “fingerprint” of spermatogenesis
- Encode genes involved in transcription and regulation in spermatogenesis
- mRNA would be a more sensitive biomarker of testicular toxicity than looking at sperm motility and concentration

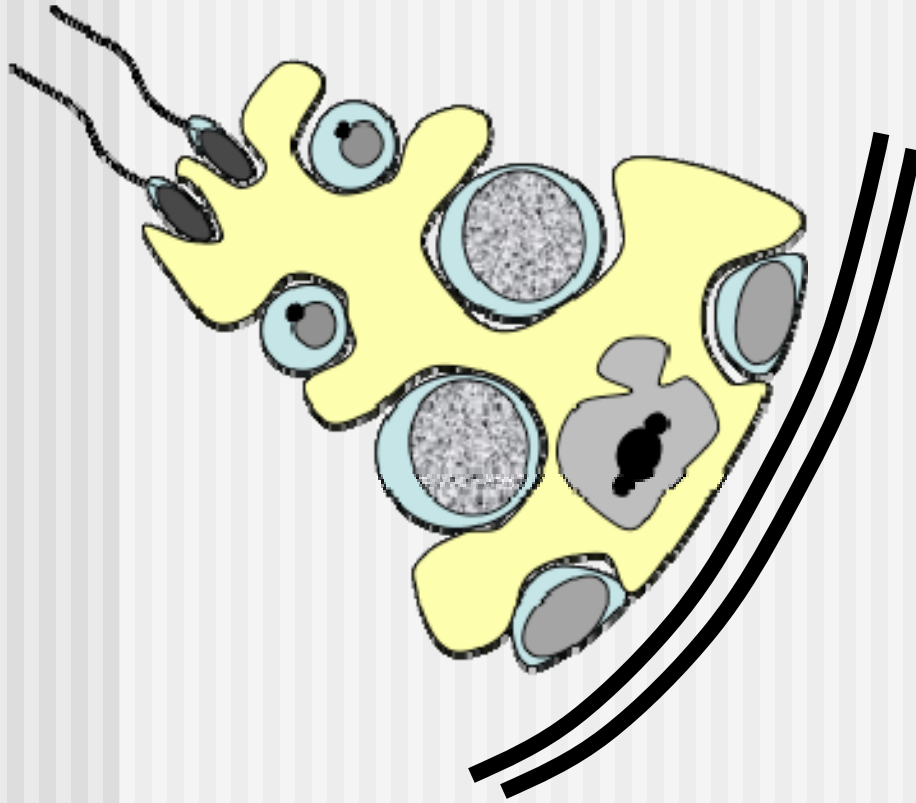
# 2,5-hexanedione (2,5-HD)



- Metabolite of *n*-hexane
- Targets Sertoli cells
- Germ cell death and germ cell loss

# Sertoli Cell: The “Nurse” Cell

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- Supports the developing sperm
- Blood-testis barrier
- Seminiferous tubule fluid

# What mRNA to look for?

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- Choose genes found to have altered expression in infertile men
- In particular, genes that have a protective function
- Link mRNA to functional and histologic evidence in testis samples

# Genes: PRM1 and Bcl2

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- In infertile men:
  - the amount of mRNA transcripts of a specific type of protamine is abnormal
  - The amount of mRNA transcripts of Bcl2, an anti-apoptotic gene
- Protamines replace histones in DNA packaging
- Bcl2 prevents cell apoptosis

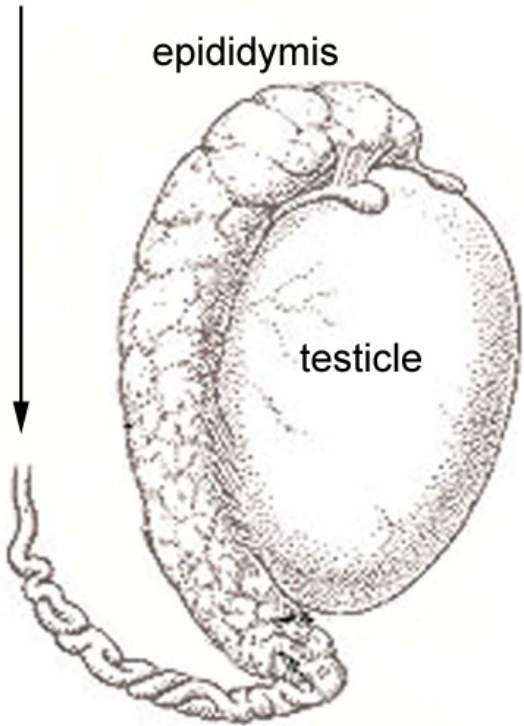
# Methods and Materials

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vas deferens

epididymis

testicle



10 rats: 0.33% 2,5-HD in drinking water, 9 wks

10 rats: control

- Sperm obtained from cauda epididymis
- RNA extraction
- qRT-PCR for PRM1, bcl2

# Results

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- Functional markers
- Histology
- mRNA sperm

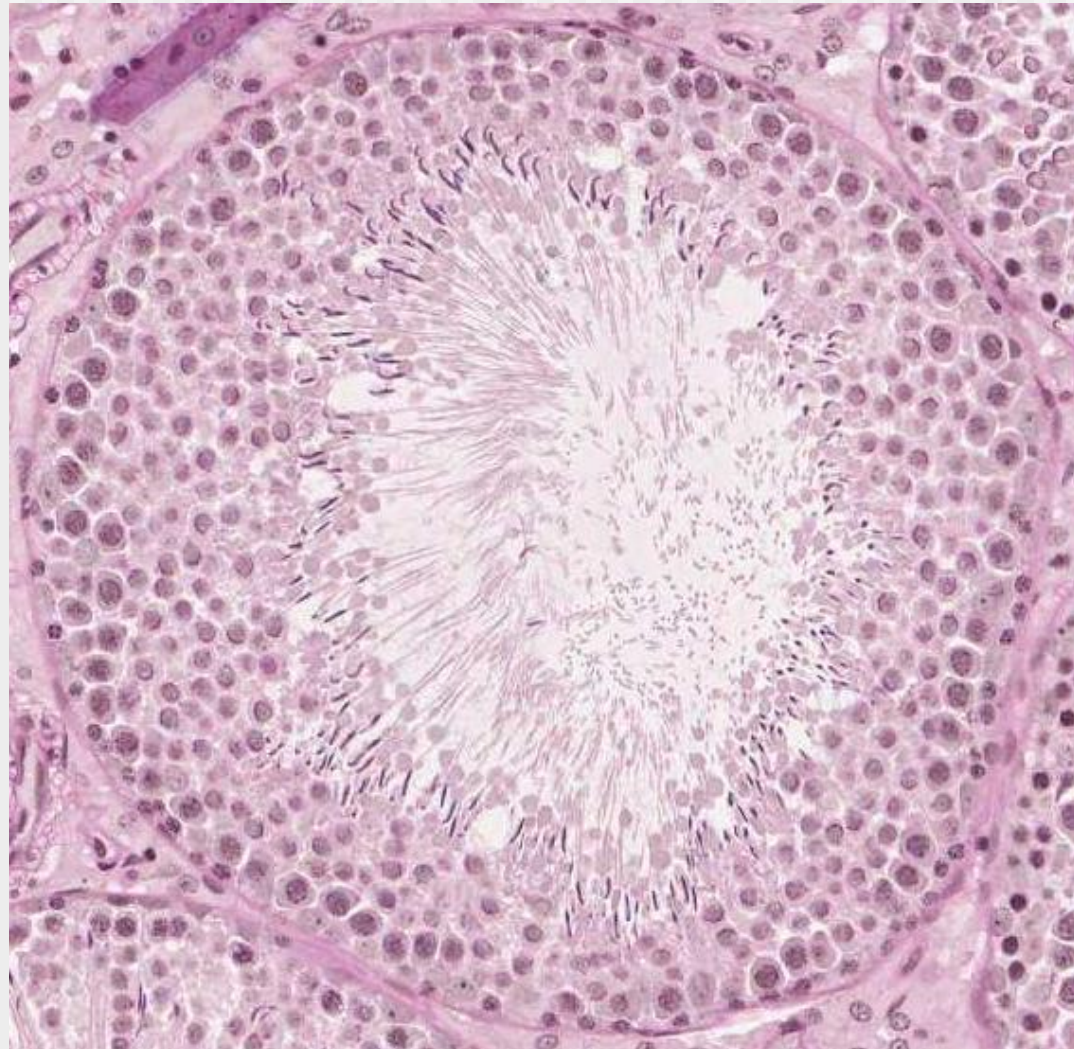
# Functional markers

<b>Weights</b>	Control	2,5-HD
Testis (g)	1.55 ± 0.01	1.51 ± 0.02
Epididymis weight (g)	0.46 ± 0.01	0.43 ± 0.01*
<b>Sperm Counts</b>		
Testis spermatid heads/g (x10 <sup>-8</sup> )	1.75 ± 0.05	0.96 ± 0.02*
Caput Epididymis sperm/g (x10 <sup>-8</sup> )	2.37 ± 0.14	2.26 ± 0.05
HD, 2,5-hexanedione; mean ± SEM; *, p < 0.05 by t-test		

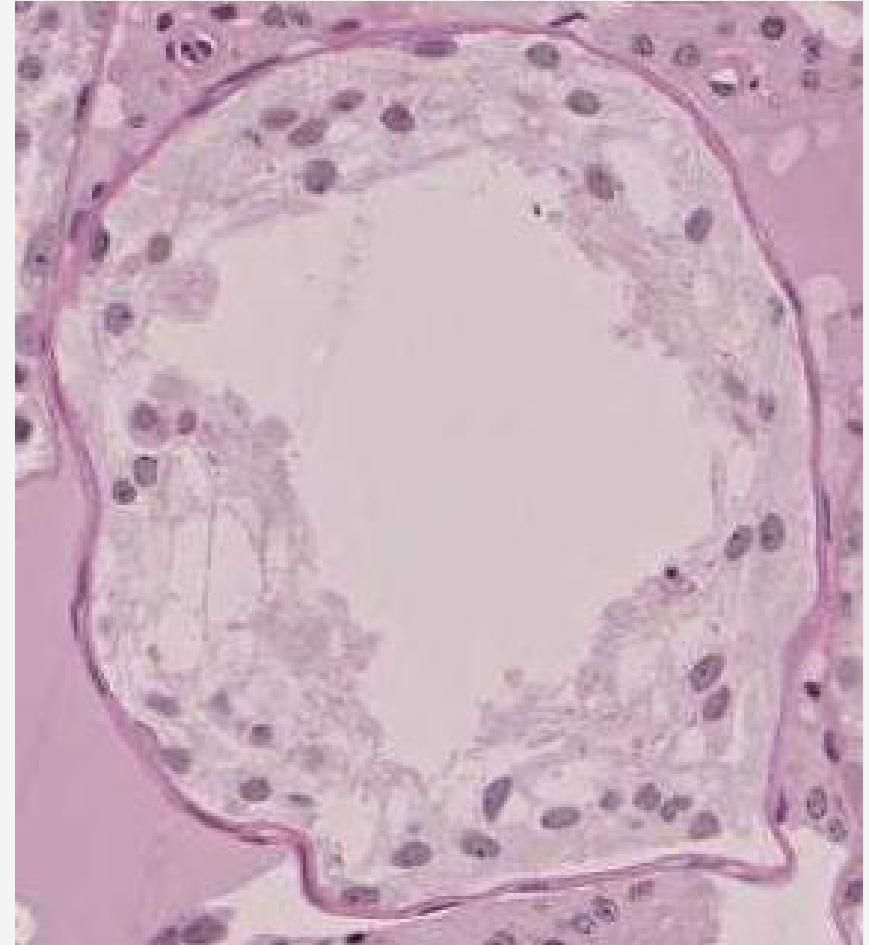
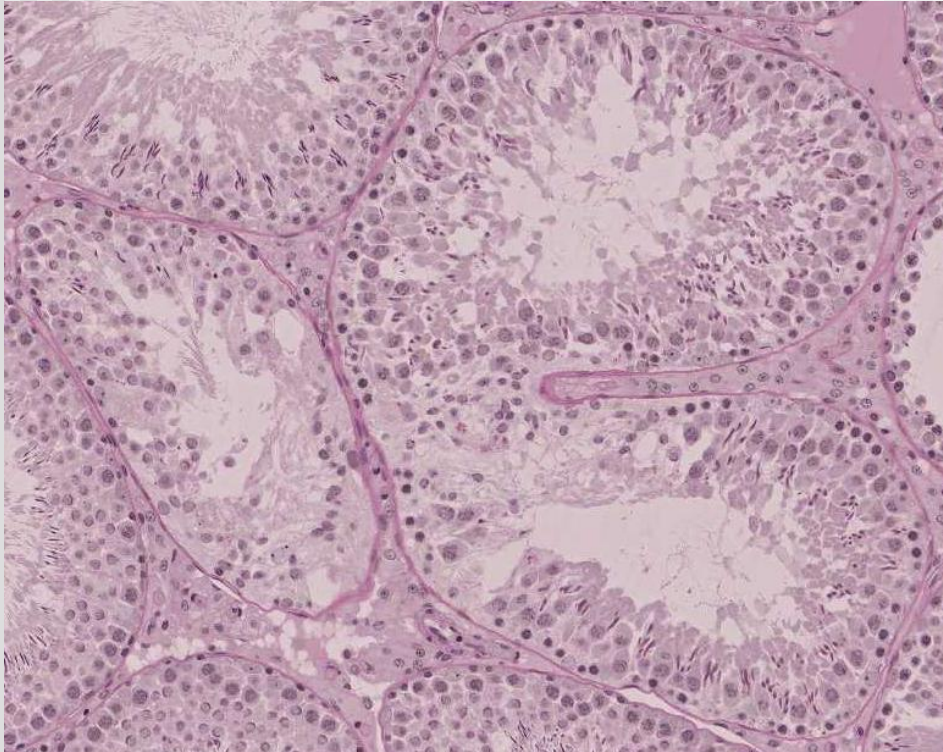
# Histology

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Control  
Rat:



# Histology Endpoints

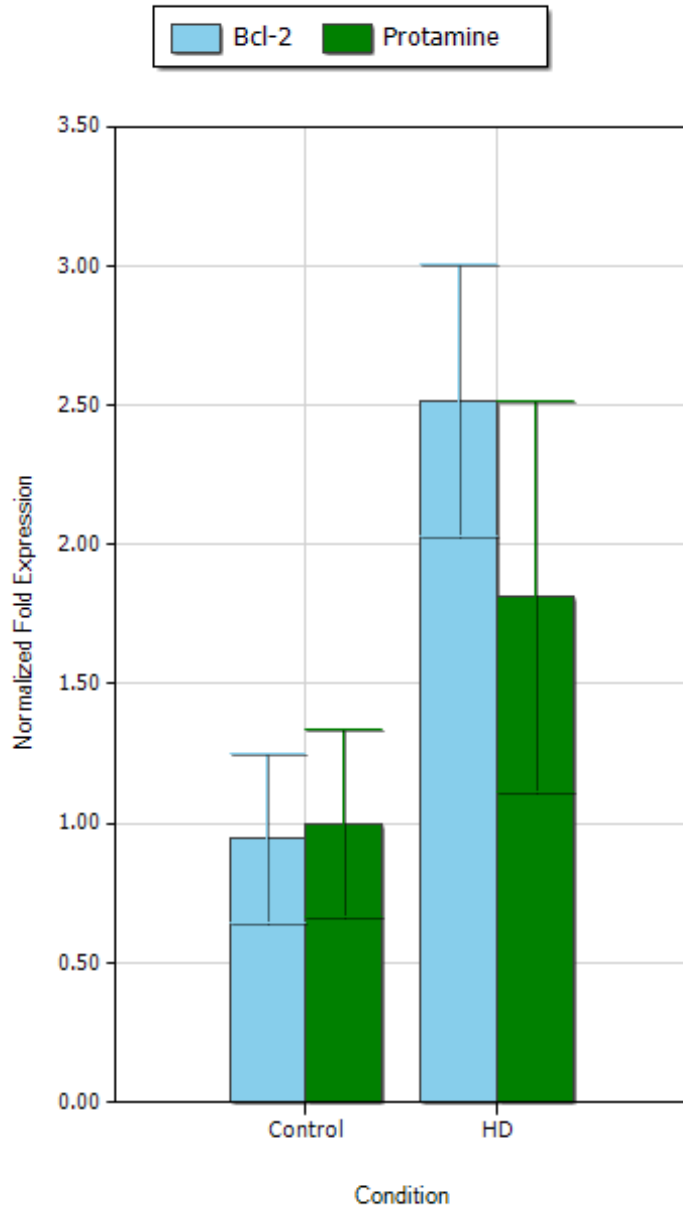


- Mild focal atrophy

# Spermatid Head Retention



# Gene Expression



- bcl2: 2.5-fold increase
- PRM1: 1.7-fold increase

# Results Summary

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- Functional markers
  - Decrease in epididymis weight
  - Decrease in the number of testis spermatid heads
- Histology
  - Mild focal atrophy
  - Spermatid head retention
- Sperm mRNA
  - Increase in protamine-1 & bcl2 transcripts

# Significance

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- Sperm mRNA can give information about exposure history
- Biomonitoring to establish long-term trends

# Future Directions

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- Explore other possible genes
- Use other toxicants with different cellular targets
- Apply these techniques to humans

# Acknowledgements

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