CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
REPRODUCTIVE SYSTEM

Third Conference

ORGANIZED BY SOCIETY FOR TOXICOLOGIC PATHOLOGY IN INDIA (STPI)

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The Atria Hotel, # 1, Palace Road, Bangalore - 560 001

Toxicologic Pathology of The Beagle Dog

Evaluation of the male reproductive system in toxicity studies

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Introduction

- The Beagle is the common second species for toxicity testing
- For detecting potential toxicity to the male reproductive tract, reliance is placed on histopathological assessment of the reproductive tissues from the 2/4 week repeated dose studies
- Fertility studies in rodents are generally not conducted prior to FTIH (first time in humans) studies (except for Japan)

Outline

- Sexual maturity
- Background/spontaneous lesions
- Spermatogenesis
  - “Stage aware evaluation” - Staging
- Prostate
- Hormone data in toxicity studies
- Examples of drug-induced testicular toxicity

Points to consider prior to start of study

Age of dogs (sexual maturity)

- Testicular toxicity cannot be adequately detected in the absence of spermatogenesis
- Histologic findings in peripubertal testes can be indistinguishable from treatment related degeneration and depletion of germ cells
- Dogs should be at least 10 months at necropsy to minimize immaturity/peripubertal problems (preferably 12 months or older)

Species	Age at sexual maturity
Rat	8-10 weeks
Mouse	7-8 weeks
Dog	7-12 months
Monkey (Cynomolgus)	4-4.5 years

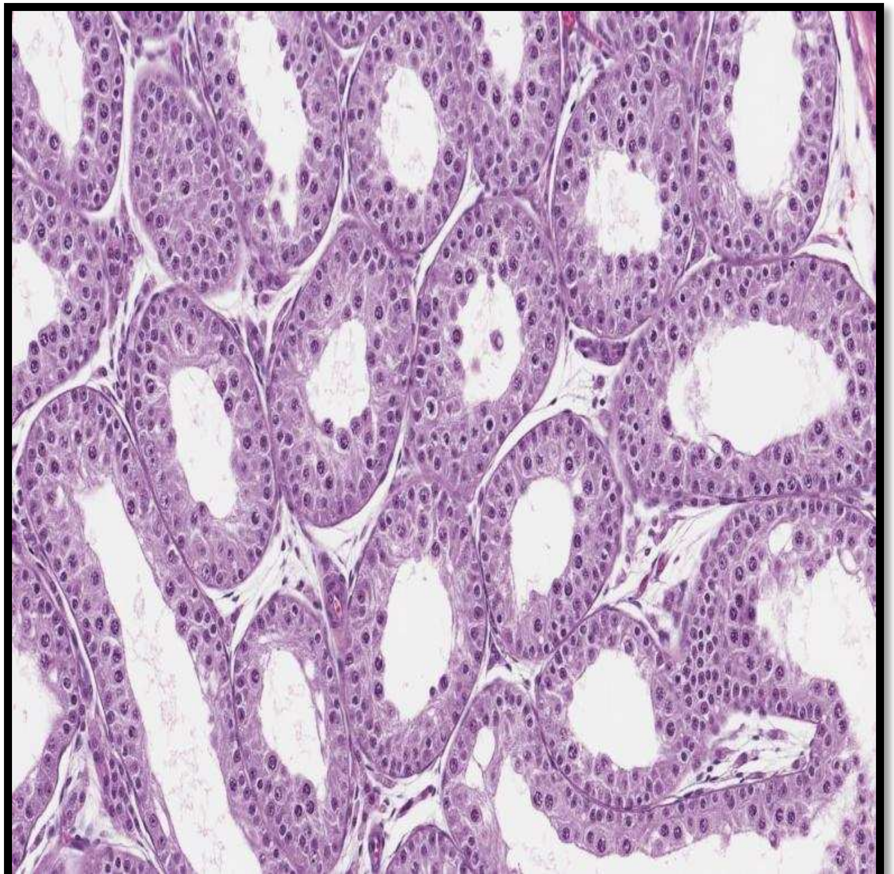
Sexual Maturity

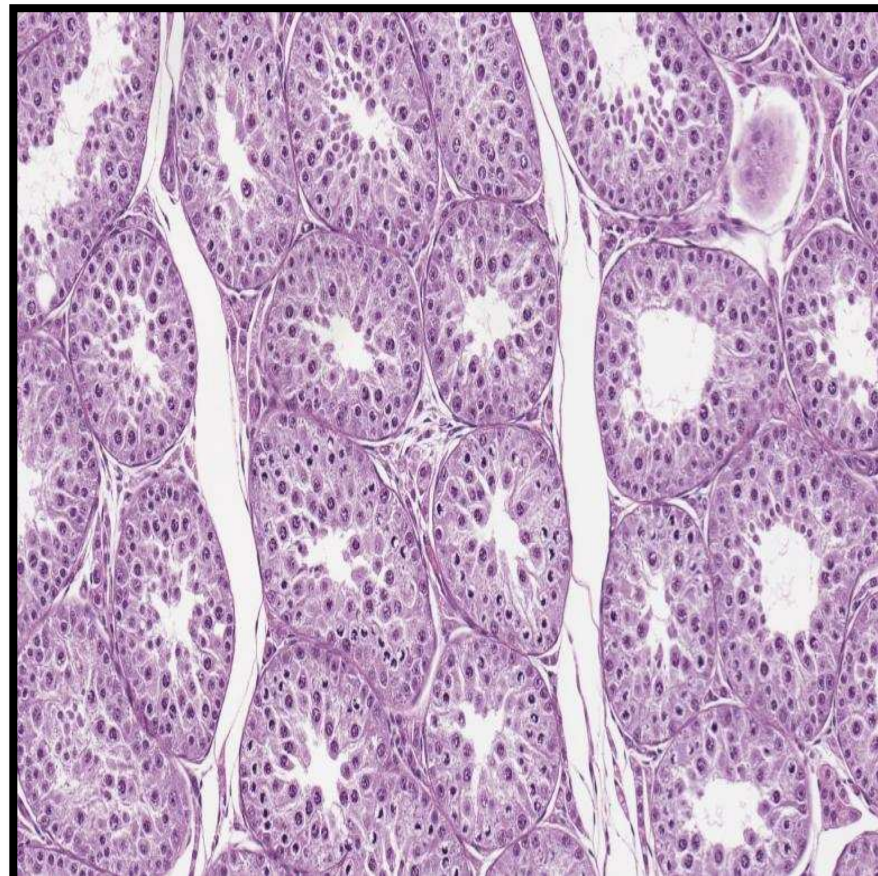
Testes weights from 2 toxicity studies with same compound
Age factor: 6-6.5 month old dogs vs. 10-11 month old dogs

	Absolute	Relative
Control	5.8427	0.073
Low	7.2370	0.087
Mid	6.5490	0.083
High	4.6185	0.065

	Absolute	Relative
Control	12.24	0.133
Low	12.83	0.133
Mid	10.17	0.113
High	11.89	0.130

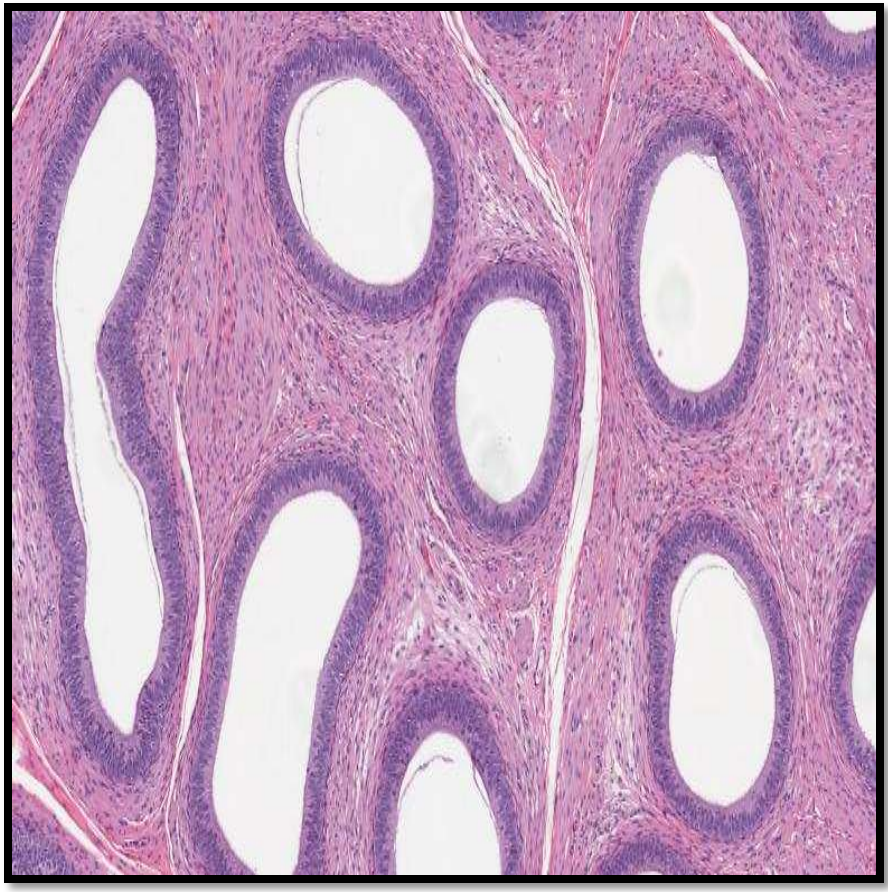
Immature dogs (peripubertal)
Age – 6-6.5 months

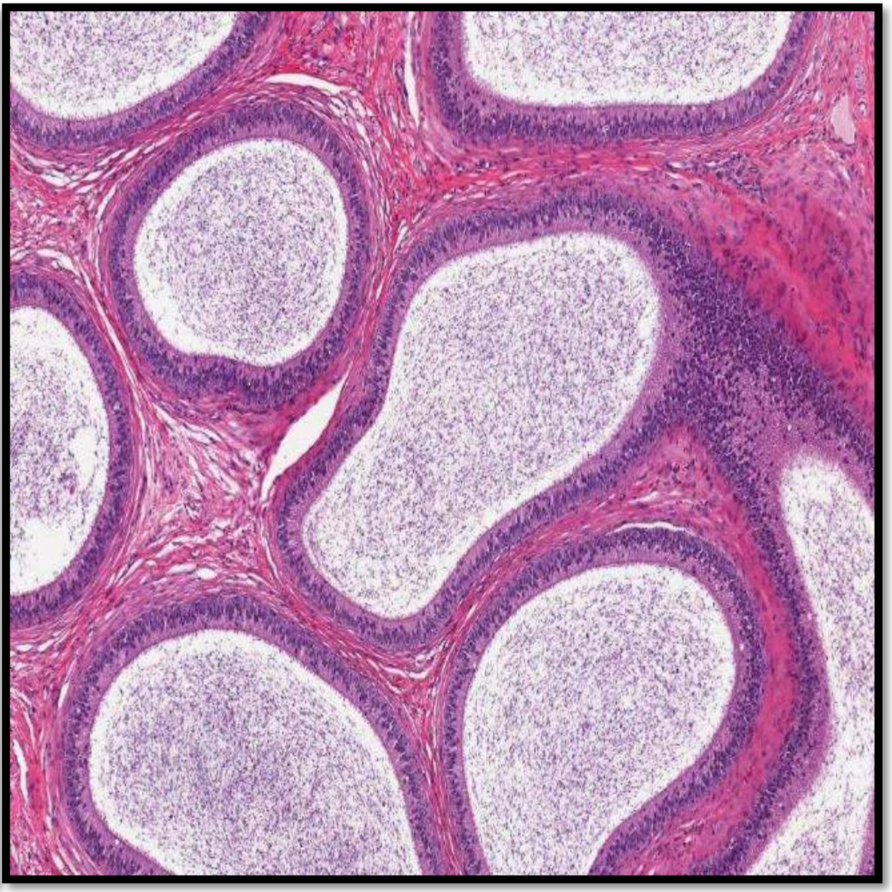




GSK GlaxoSmithKline

Cauda (immature vs. mature)





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Spontaneous/Background Lesions

Rehm S. Spontaneous testicular lesions in purpose-bred beagle dogs. Toxicol Pathol. 2000 Nov-Dec;28(6):782-7.

TABLE 1.—Testicular lesions observed in control beagle dogs from toxicology studies performed in 1988–1999.

Testicular lesions	8–11 months[a] n = 11	12–13 months n = 13	14–17 months n = 16	18–20 months n = 10	Total n = 50
Hypospermatogenesis[b] (n dogs affected)					
Mild	2	2	4	1	9
Moderate	0	3	0	2	5
Severe	1	0	0	0	1
Total incidence n (%)	3 (27%)	5 (38%)	4 (25%)	3 (30%)	15/50 (30%)
Tubular atrophy/hypoplasia[c]					
Incidence n dogs (%)	5 (45%)	4 (31%)	4 (25%)	3 (30%)	15/50 (30%)
Bilateral occurrence n (%)	1/5	3/4	2/4	1/3	6/15 (40%)
No. affected areas/testis in affected dogs	5/8[d] (0.6/testis)	11/8 (1.4/testis)	16/8 (2/testis)	7/6 (1.2/testis)	39/30 (1.6/testis)
Tubules with multinucleated giant cells					
Incidence n dogs (%)	11 (100%)	13 (100%)	16 (100%)	9 (90%)	49/50 (98%)
Bilateral occurrence n dogs (%)	11/11	11/13	15/16	8/9	45/49 (92%)
No. affected tubules/testis in affected dogs	123/20[d] (6/testis)	152/26 (6/testis)	127/32 (4/testis)	98/18[d] (5.5/testis)	500/96 (5/testis)
Tubules with swollen spermatocytes					
Incidence n (%)	11 (100%)	13 (100%)	14 (88%)	10 (100%)	48/50 (96%)
Bilateral occurrence n (%)	11/11	9/13	6/14	6/10	32/46 (70%)
No. affected tubules/testis in affected dogs	64/20[d] (3/testis)	48/26 (2/testis)	36/32 (1/testis)	61/18[d] (3/testis)	209/96 (2/testis)
Retained sperm					
Incidence n (%)	5/11 (45%)	0	1/14 (6%)	0	6/50 (12%)

[a] Age at necropsy.
[b] Bilateral occurrence in all cases.
[c] Tubules lined predominantly by Sertoli cells.
[d] Single dog was excluded from enumeration because of widespread involvement of both testes.

Goedken MJ, Kerlin RL, Morton D. Spontaneous and age-related testicular findings in beagle dogs. Toxicol Pathol. 2008;36(3):465-71.

TABLE 2.—Incidence of hypospermatogenesis and atrophy/hypoplasia by age group.

Age (mo.)	n	Finding	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	No. with both	Total incidence	Percentage incidence
6–7	8	Hypospermatogenesis	2	0	2	1	3	2	6/8	75
		Atrophy/hypoplasia	5	0	1	1	1		3/8	37.5
8	5	Hypospermatogenesis	3	0	1	1	0	2	2/5	40
		Atrophy/hypoplasia	3	0	1	1	0		2/5	40
9	15	Hypospermatogenesis	12	0	2	1	0	3	3/15	20[a]
		Atrophy/hypoplasia	10	1	2	2	0		5/15	33.3
10	8	Hypospermatogenesis	5	1	1	1	0	2	3/8	37.5
		Atrophy/hypoplasia	6	0	1	1	0		2/8	25
11	14	Hypospermatogenesis	14	0	0	0	0	0	0/14	0[a]
		Atrophy/hypoplasia	10	1	1	2	0		4/14	29
12–23	23	Hypospermatogenesis	21	0	0	2	0	2	2/23	8.7[a]
		Atrophy/hypoplasia	19	0	1	3	0		4/23	17.3
24–36	7	Hypospermatogenesis	7	0	0	0	0	0	0/7	0[a]
		Atrophy/hypoplasia	6	1	0	0	0		1/7	14.3

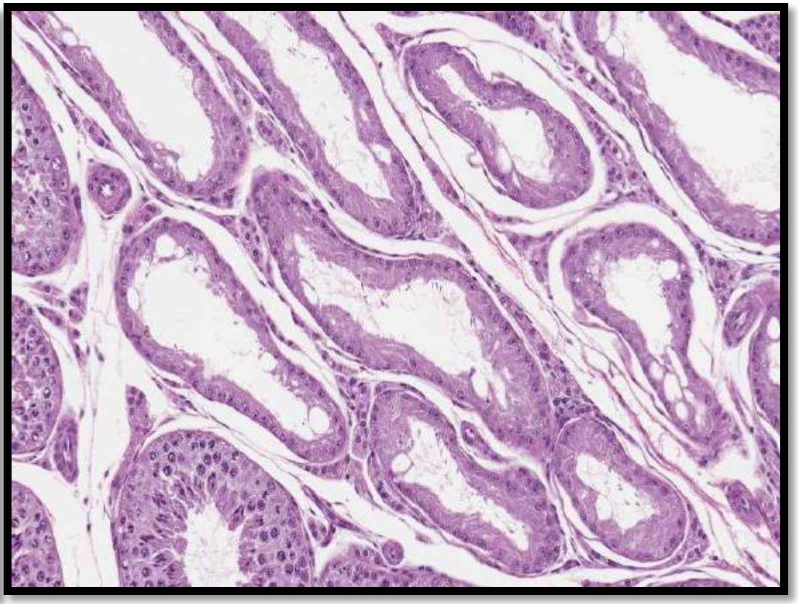
Note: There were no statistical differences among dogs with atrophy/hypoplasia. There were no statistical differences comparing dogs with atrophy/hypoplasia and hypospermatogenesis at any age.

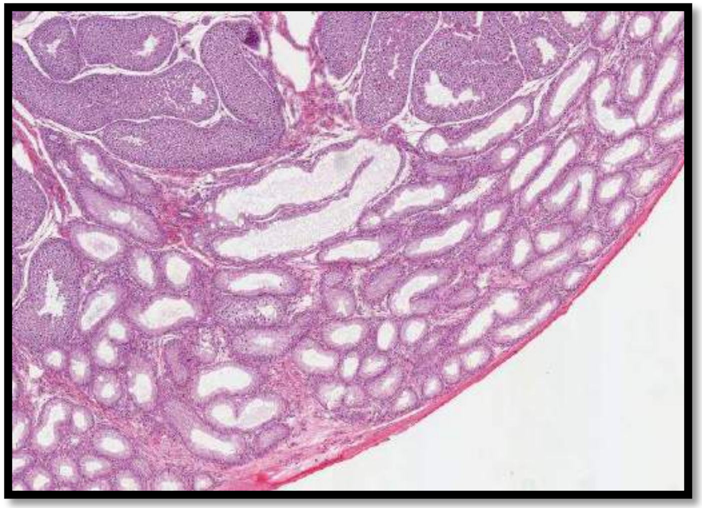
[a] Statistically different from six- to seven-month-old dogs with hypospermatogenesis.

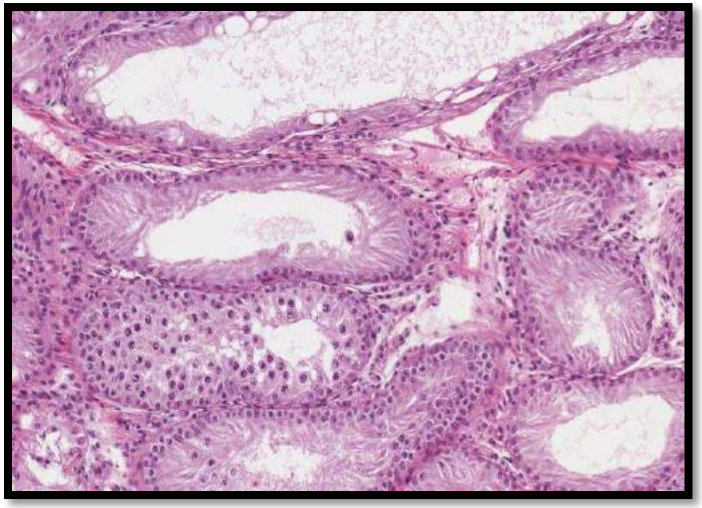
Background/Spontaneous Lesions

- Two main types
  - Hypoplasia (atrophy)
  - Hypospermatogenesis
- Incidence is high
- They are age independent
- Difficult to distinguish and may overlap with drug-induced induced lesions
- Small group size is a major disadvantage
- Use consistent terminology
- Review data after finishing study!

Hypoplasia (atrophy)

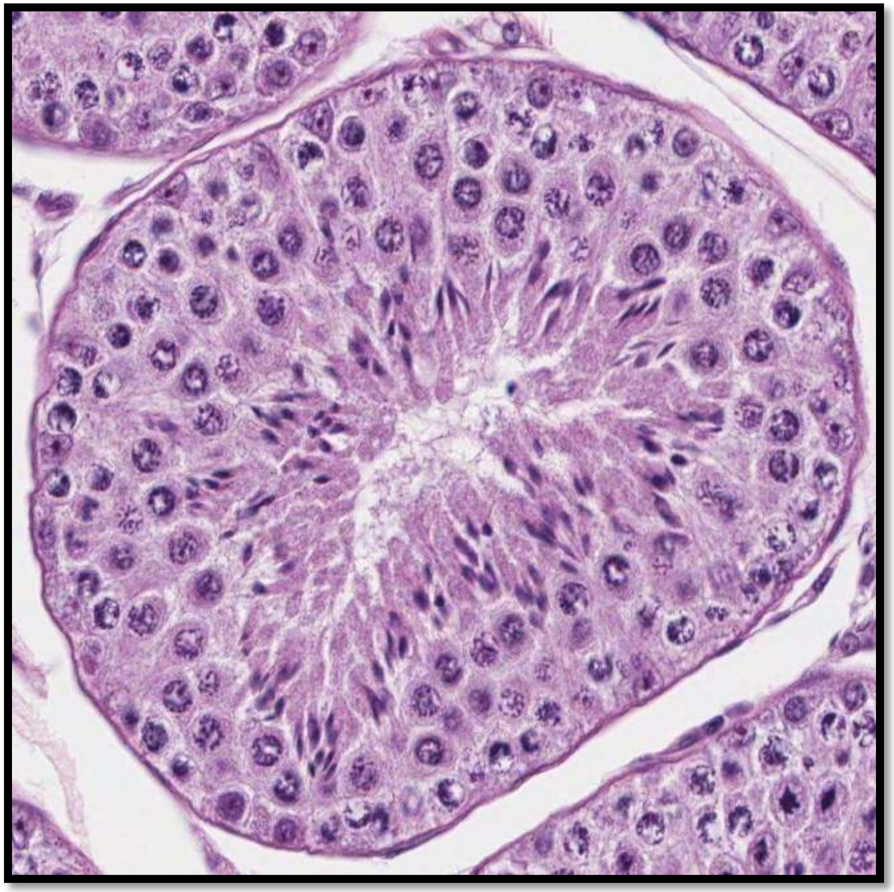


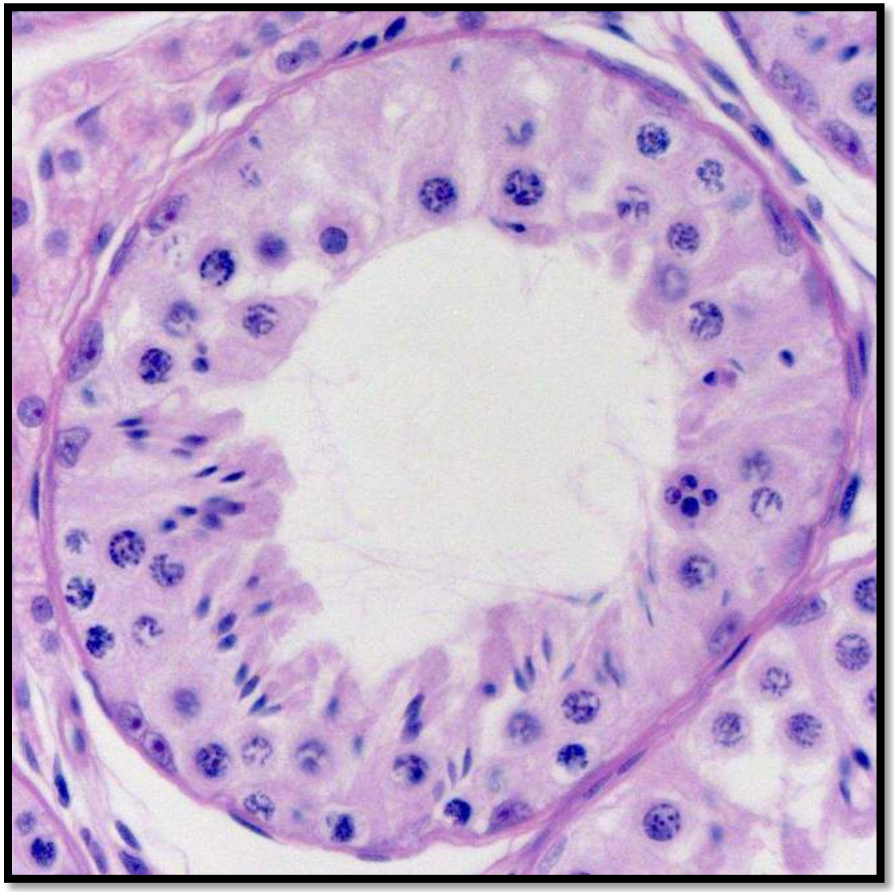




Hypospermatogenesis

Missing elongate spermatids & and spermatocytes
Normal on left and affected tubule on right





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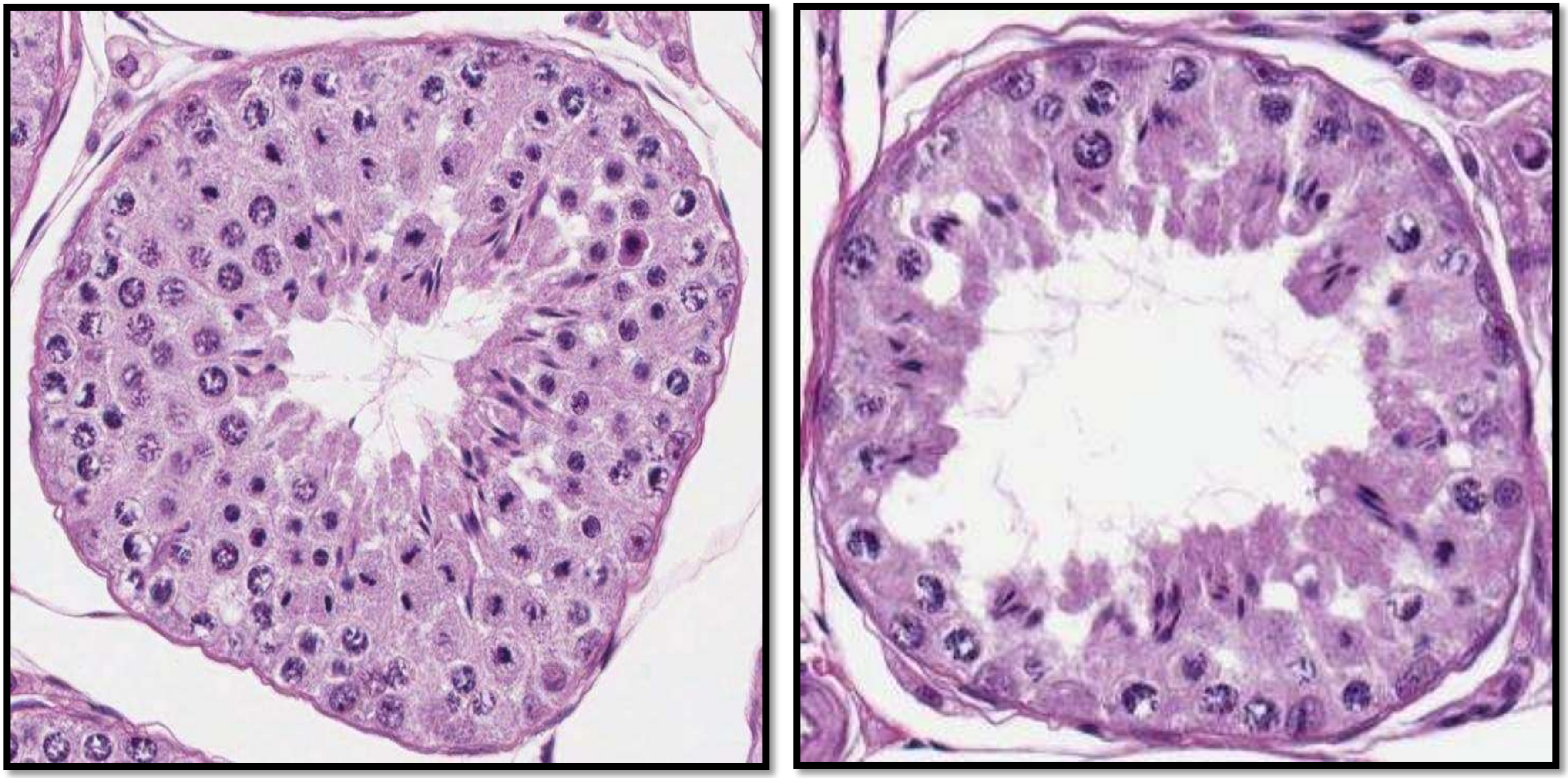
Hypospermatogenesis

Missing round spermatids & and spermatocytes

Hypospermatogenesis

Missing elongate spermatids & and spermatocytes
Normal on left and affected tubule on right



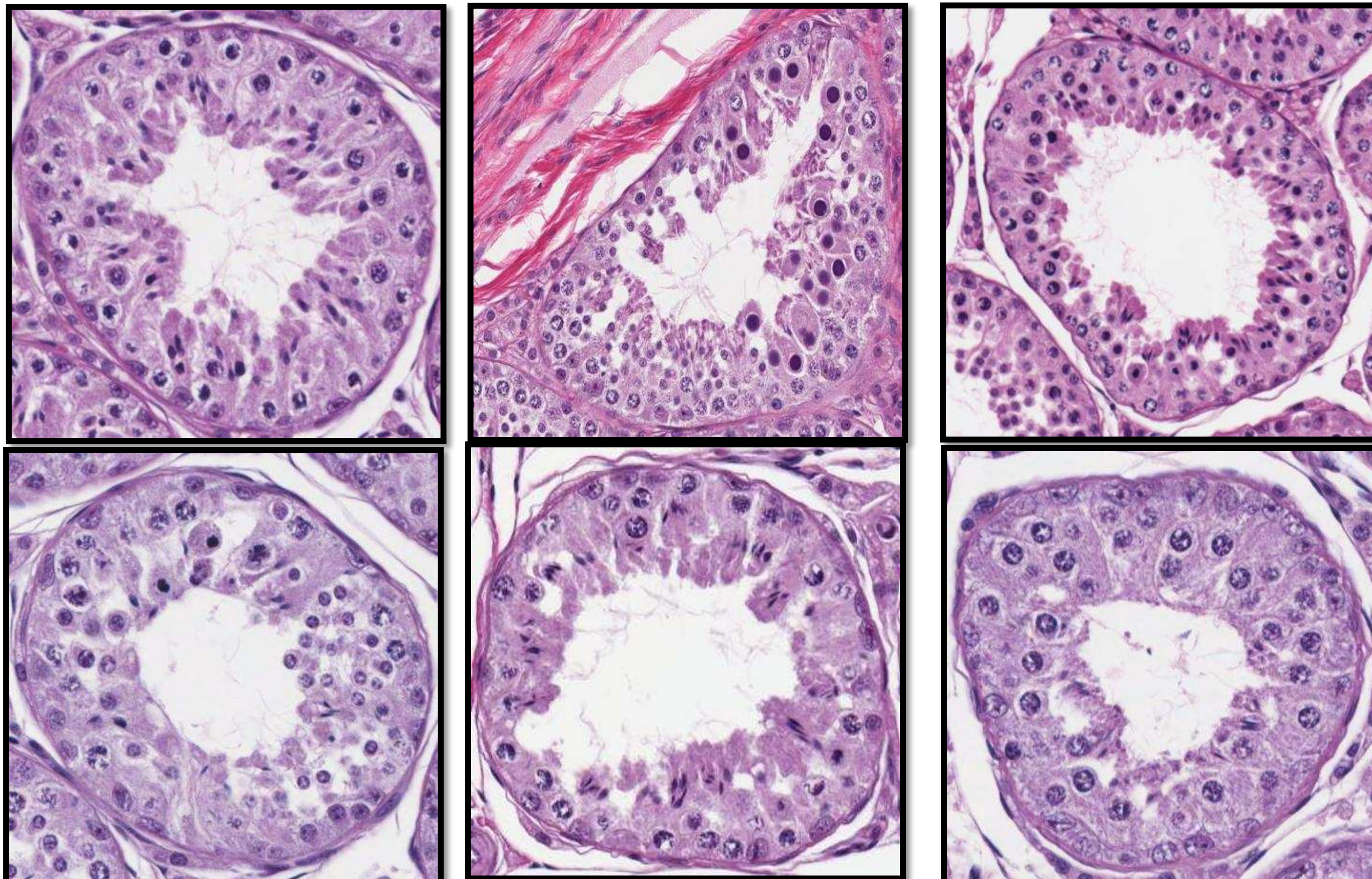
Hypospermatogenesis

Missing elongate spermatids

Normal on left and affected tubules on right

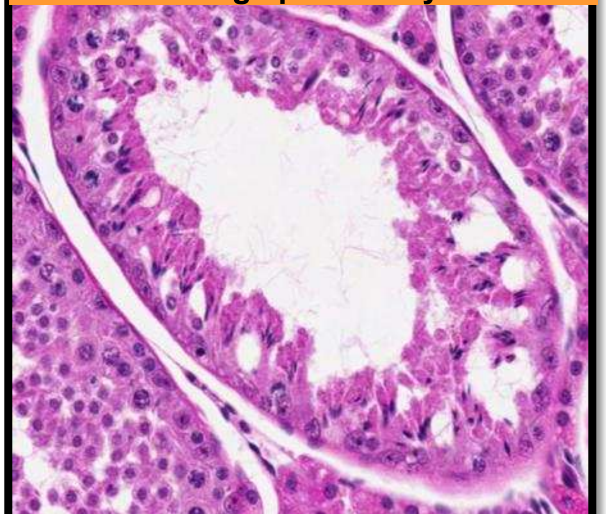
Background – retained spermatids in stage 8

Hypospermatogenesis (Background – Control)



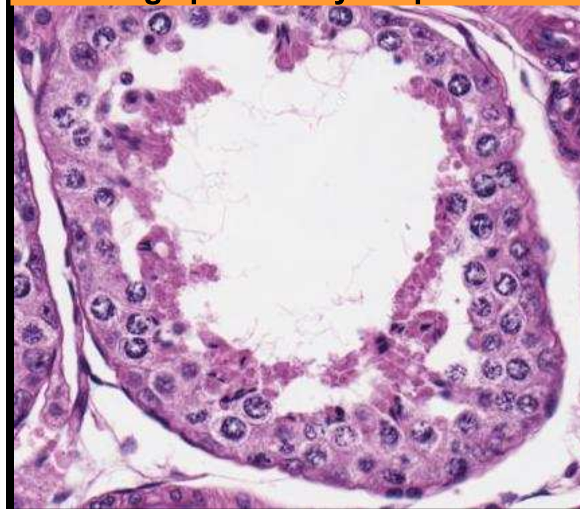
Hypospermatogenesis (Background – Control)

Missing spermatocyes

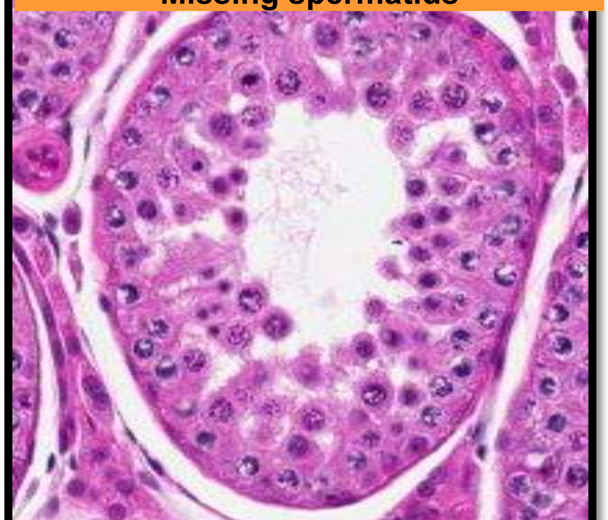


Missing spermatocyes

Missing spermatocyes/spermatids

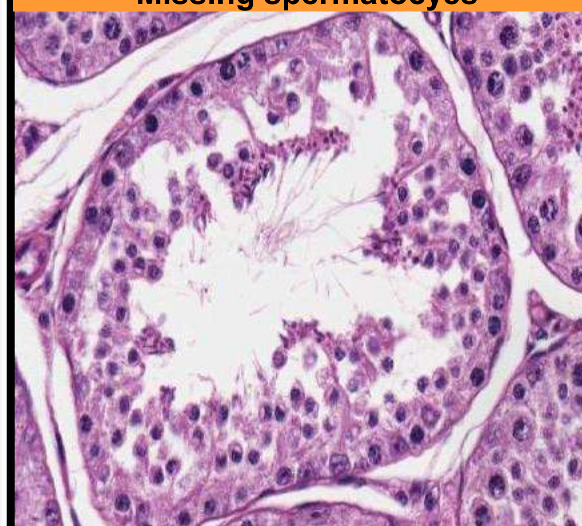


Missing spermatids

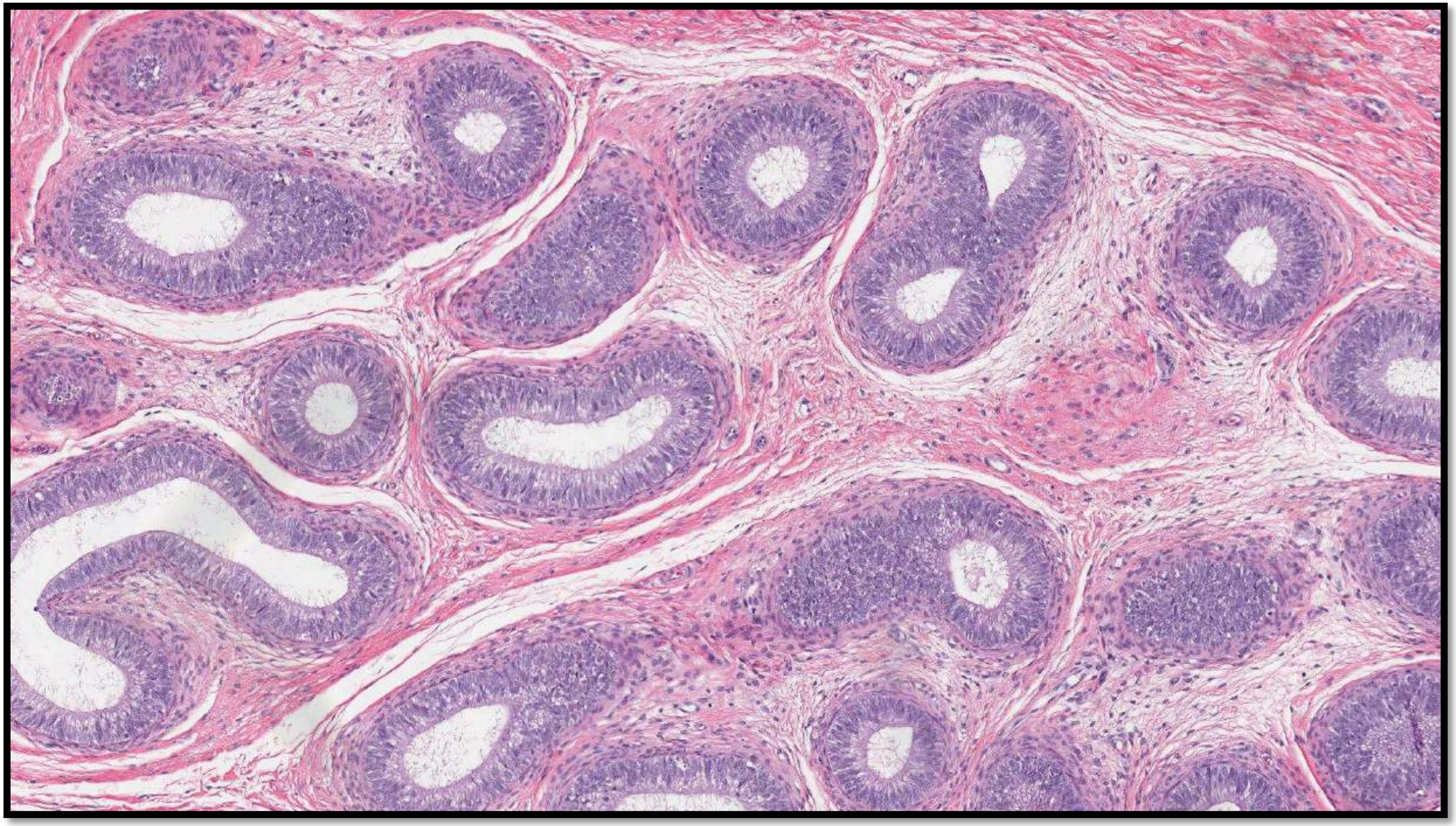


Missing spermatocyes/spermatids

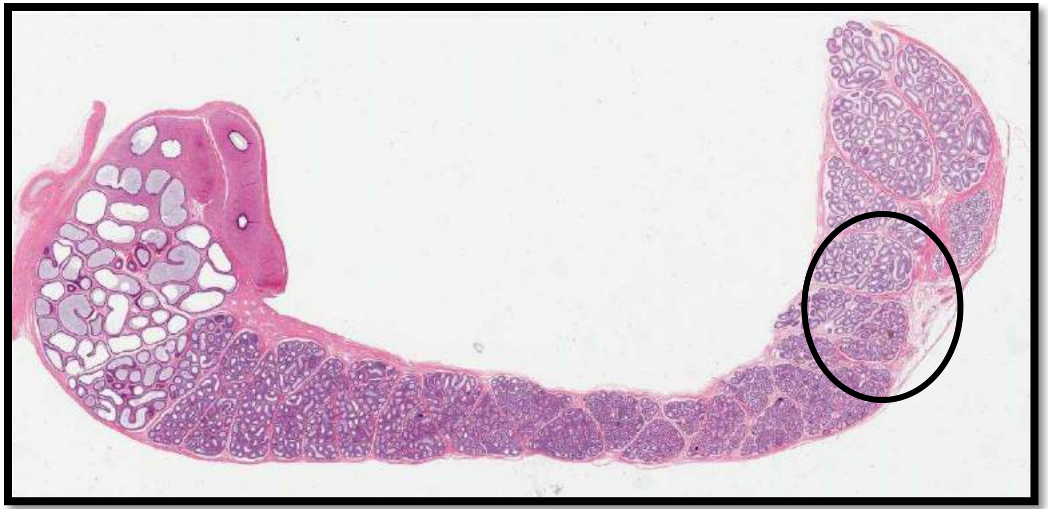
Missing spermatocyes

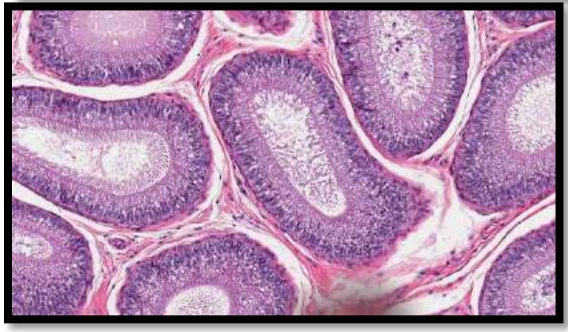


Caput - Inclusions

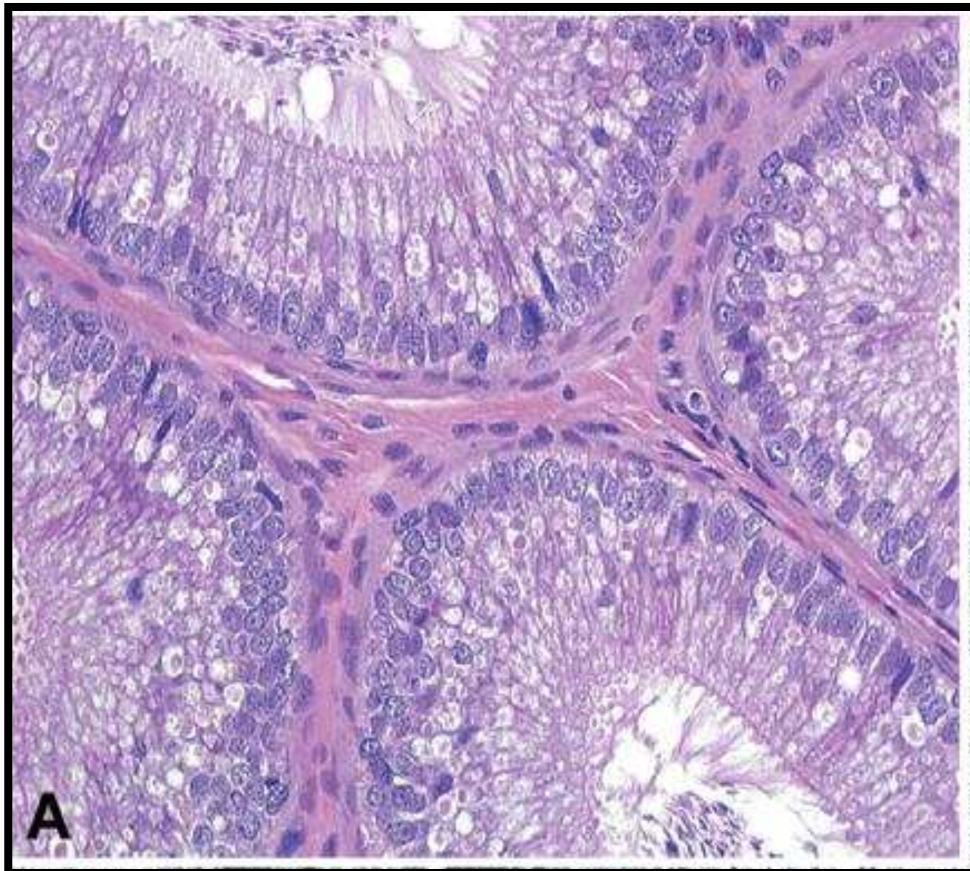


Vacuoles





Epididymal phospholipidosis



(Rudmann et al 2004 – Dopamine D3 antagonist)

Stage aware evaluation – “staging”

Toxicologic Pathology, vol 30, no 4, pp 507–520, 2002

DOI: 10.1080/01926230290105695

Society of Toxicologic Pathology Position Paper

Recommended Approaches for the Evaluation of Testicular and Epididymal Toxicity

Lynda L. Lanning,[1] Dianne M. Creasy,[2] Robert E. Chapin,[3] Peter C. Mann,[4] Norman J. Barlow,[5]
Karen S. Regan,[6] and Dawn G. Goodman[7]

[1]*BioReliance Corporation, Rockville, Maryland, 20850*
[2]*Huntingdon Life Sciences, East Millstone, New Jersey 08875*
[3]*Pfizer Inc, Groton, Connecticut 06340*
[4]*Experimental Pathology Laboratory NorthEast, Galena, Maryland 21635*
[5]*Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina 27709*
[6]*Regan Path/Tox Services, Ashland, Ohio 44805, and*
[7]*Covance Laboratories Inc, Vienna, Virginia 22182*

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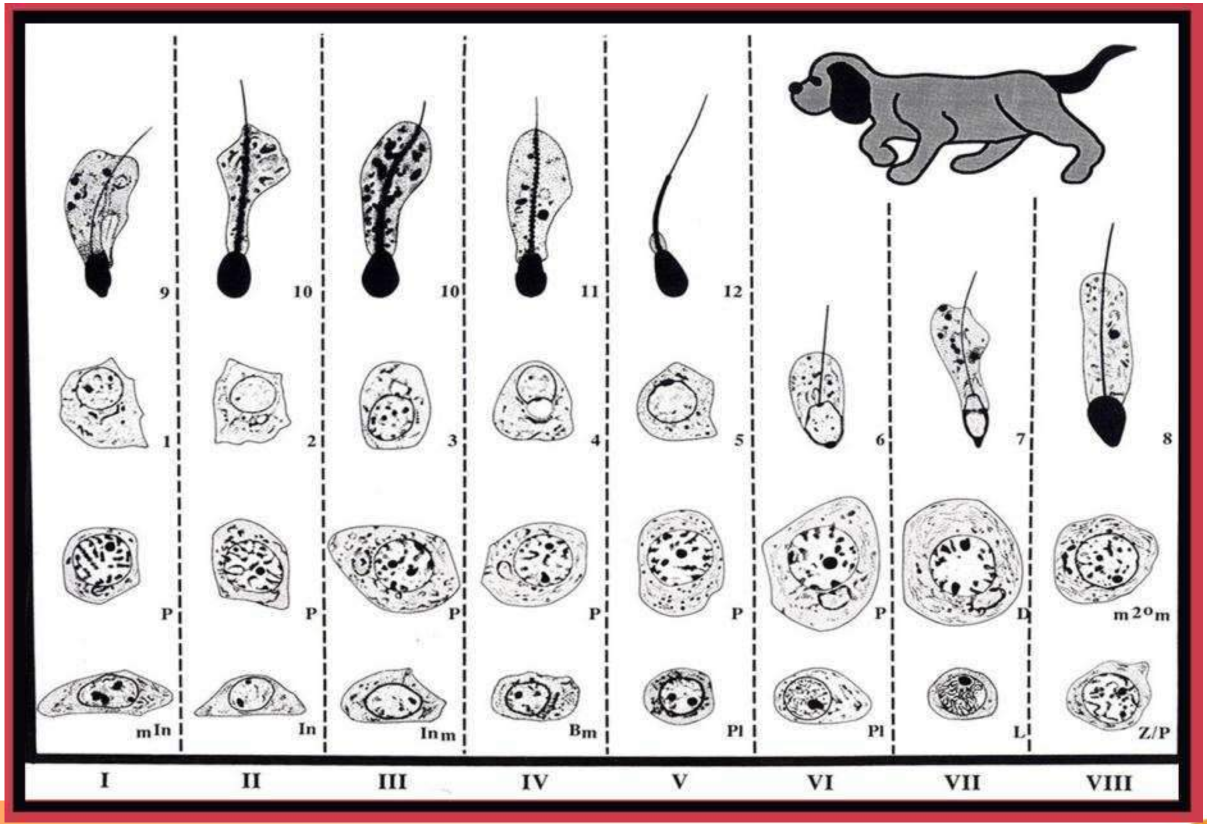
Stage aware evaluation/stage dependent evaluation

- Main objective – designed to maximize the detection of toxic effects
- Early manifestations of damage will be observed.



Stage aware evaluation/stage dependent evaluation

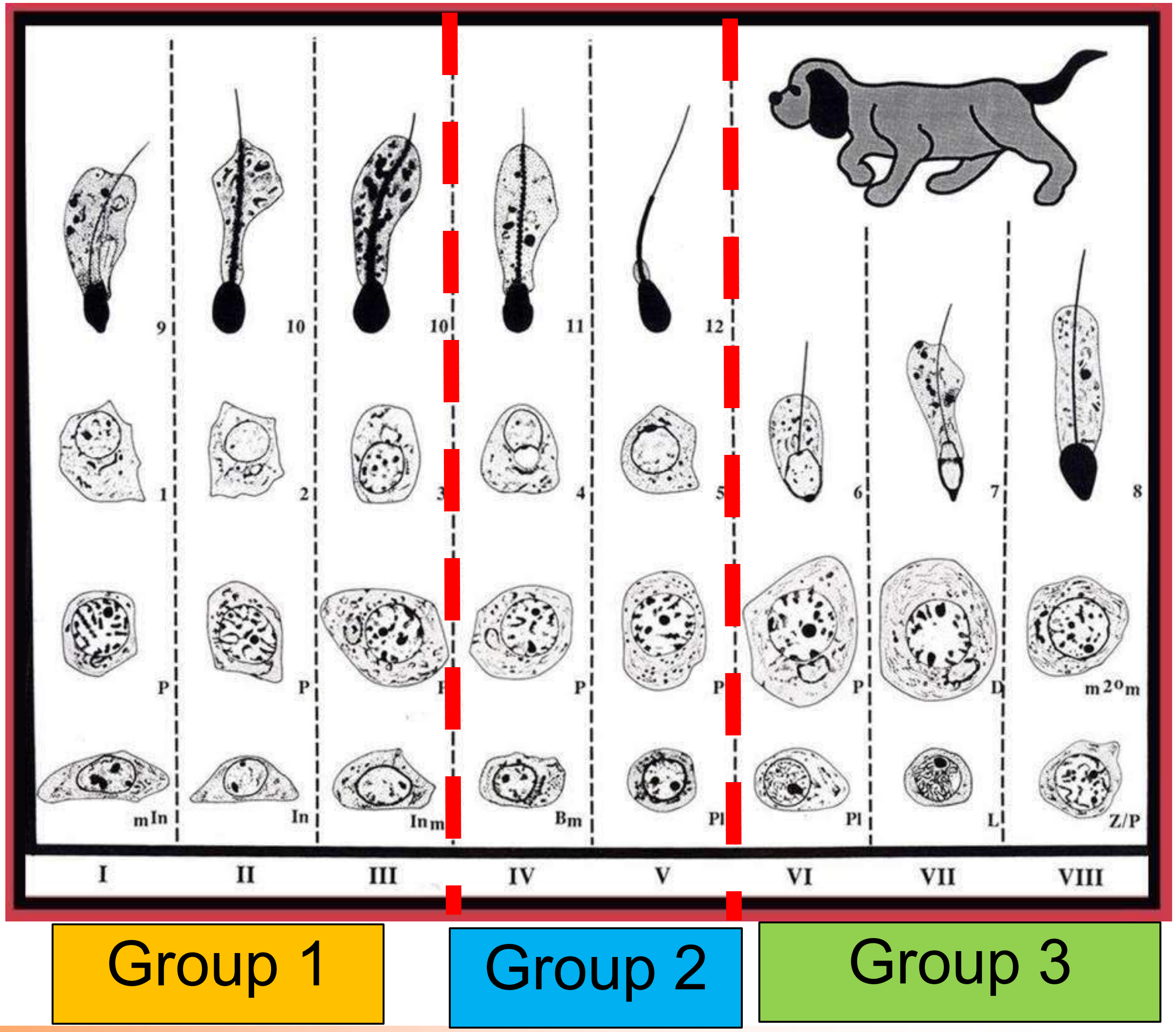
- **Qualitative** histopathologic evaluation of the testis
- Appropriately processed material to identify early treatment-related effects (PAS-H stain not required for dog testes)
- Generally under high magnification (≥20X)
- Requires an understanding of stages
- Early lesions are often subtle (missing germ cell layers) and/or stage/cell specific (retained spermatids)
- Comprehensive approach (weight, histology, and hormonal data), - testis weight, epididymis, accessory sex organs, mammary gland, and pituitary.
- Dependent on the duration of the study and animal age

Grouping of Stages

- Difficult to discern individual stages on HE stained paraffin sections
- **Quote from text book - "dog spermatogenesis was found to be less well organized and less rigidly synchronized" – Russell et al (Histological and histopathological evaluation of the testis).**
- Combine stages for ease of identification
  - **Group 1**. Stage I, II & III - Two generation of spermatids (round & elongate)
  - **Group 2**. Stage IV and V – Thin elongate spermatids line the lumen and are subsequently released
  - **Group 3**. Stage VI, VII, & VIII – One generation of round spermatids that undergo elongation

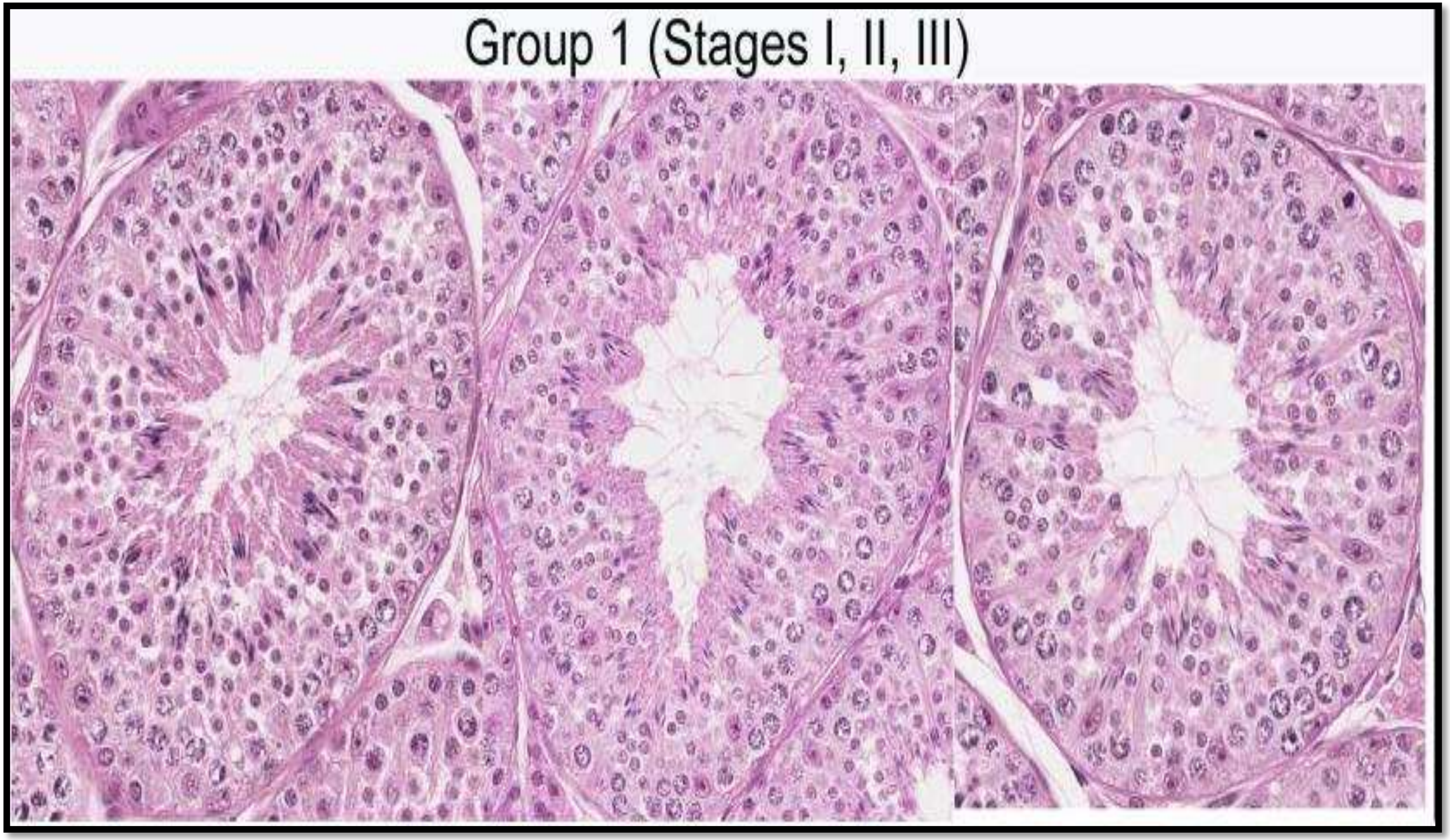
Stages of the dog seminiferous tubules - a simplified algorithm for identification of stages

We are pathologists and not anatomists!



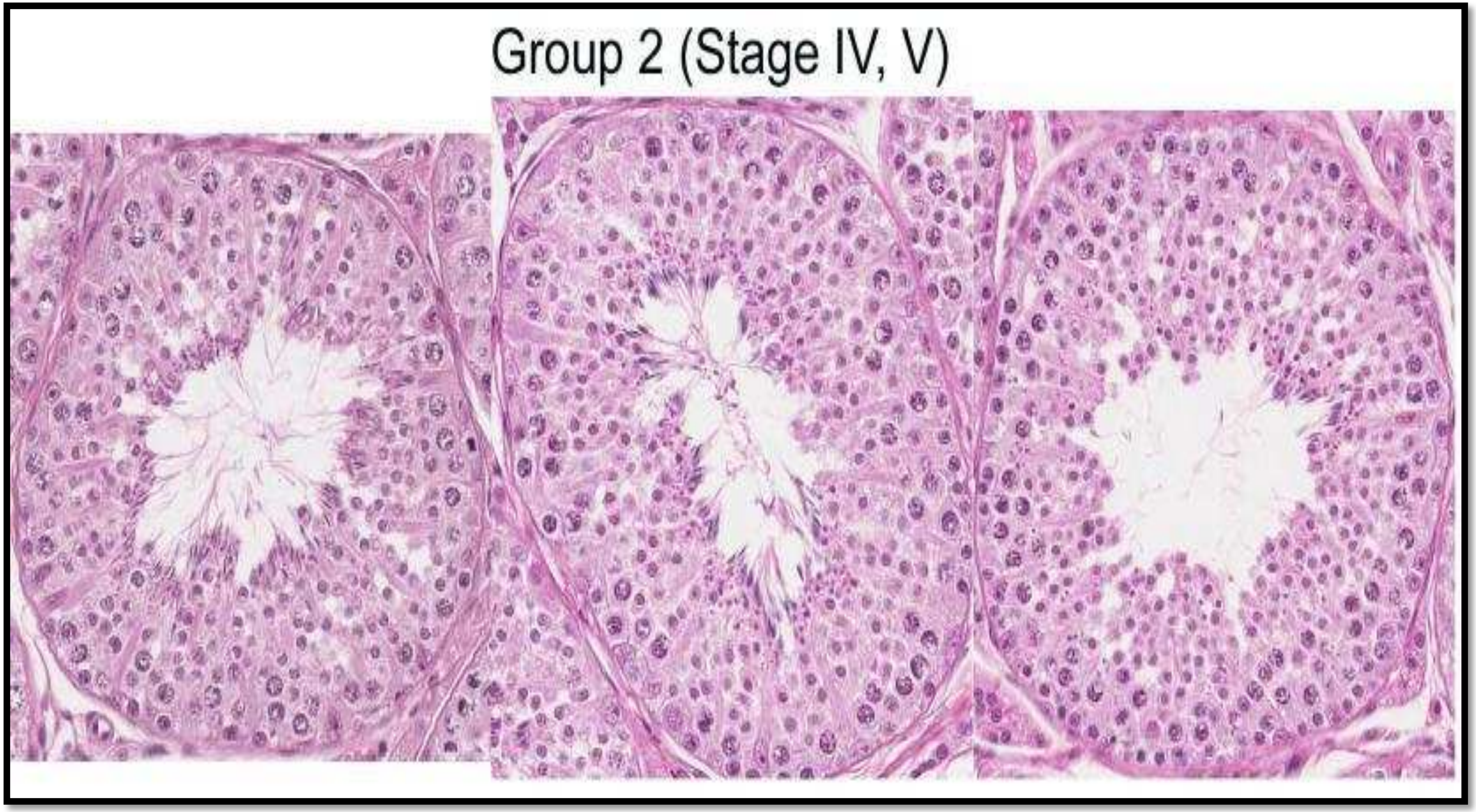
The stages were described using 4 mongrel dogs – Not Beagles!

Group 1



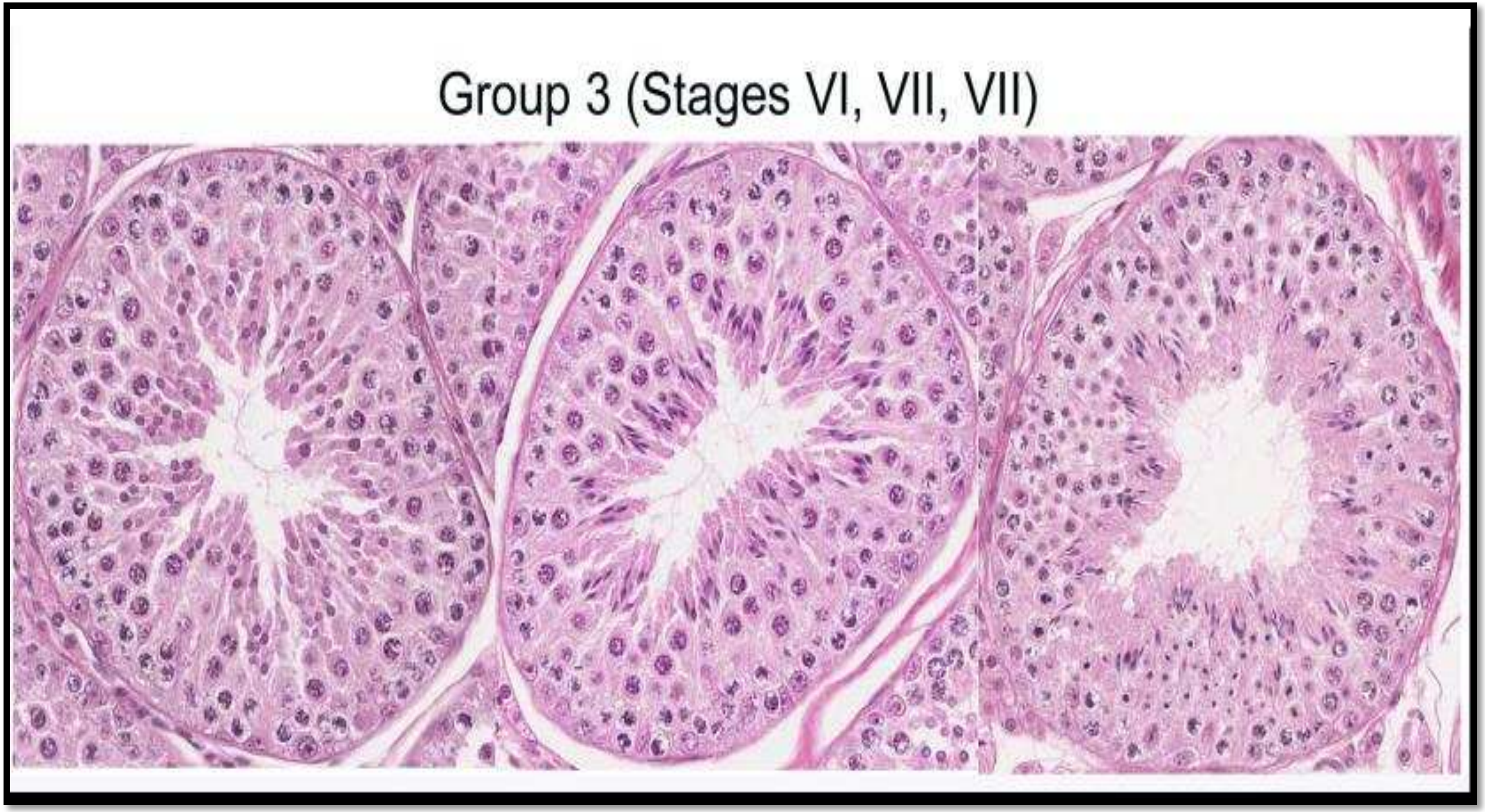
Two generation of spermatids – round & elongate
Stage I can be confused with Stage VIII (meiotic figures are present in Stage VIII and round permatids are not present)

Group 2



Elongate spermatids line lumen, residual bodies are apparent,
Round spermatids are present.
Release of elongate spermatids can occur in Stage IV or V

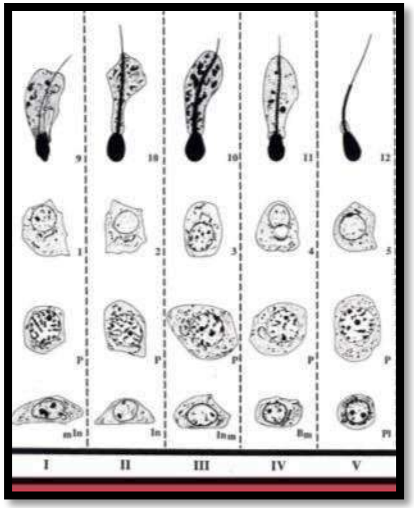
Group 3



Sperm/spermatid release is complete
Only round to elongate spermatids are present
Elongation of “round spermatids “

Stages of the dog seminiferous tubules - a simplified algorithm

Are there 2 generations of Spermatids – round & elongate?



YES

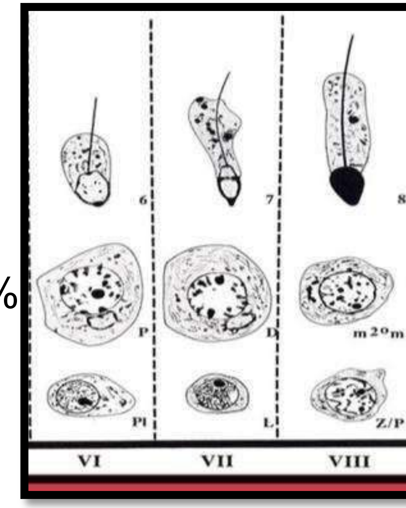
Left side of map – 64%

Stages **I-V**

NO

Right side of map-36%

Stages **VI-VIII**



Do the thin elongated spermatids line the lumen?

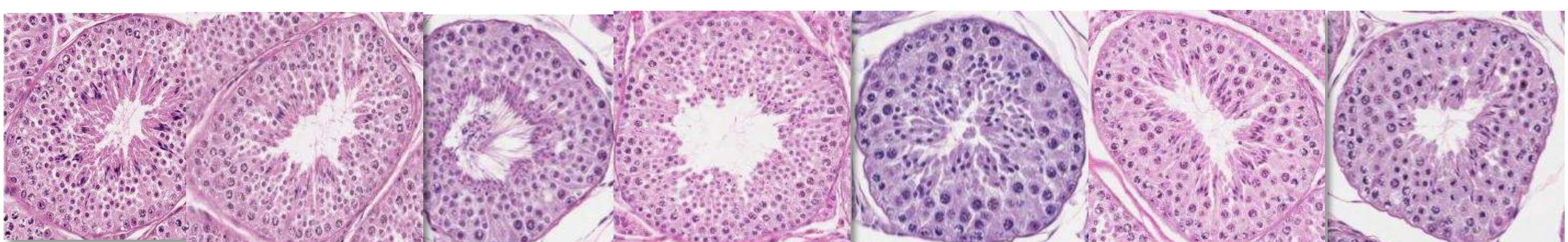
No
I-III

Yes
IV-V

Are meiotic figures present?

No
VI-VII

Yes
VIII



Group 1

Group 2

Group 3

Frequency of stages
(Based on cycle length of 13.6days - 326.4 hrs)

Duration of the Stages of the Cycle in the Dog

Stage[a]	Number of Tubules Scored	% Occurrence	Duration (hours)
I	64	8.48	27.7
II	55	7.28	23.8
III	51	6.75	22.0
IV	211	27.95	91.2
V	102	13.51	44.1
VI	108	14.30	46.7
VII	81	10.73	35.0
VIII	83	10.99	35.9

- Group 1 (22.5%)

Group 2 (41.5%)

Group 3 (36%)

"Don't mind Sundeep. After looking through a microscope all day, anything large startles him."

Test-article vs. background

- Distribution of findings - Focal / Multifocal / Diffuse
- Severity grades - challenging based on finding
- Harmonization of terminology and grading needs to be emphasized on different studies to accurately convey information
- Incidence & Severity based on dose response
- Test article-related changes typically show spectrum
- Stage-associated effect, will typically be bilateral
- Segmental tubular changes
- Typically bilateral if test article-induced
- May not be able to distinguish based on single study
- Seen in 1 month or longer duration
- Look for degenerative changes in lower dose/shorter studies

Terminology Use in GLP Studies

- Review terminology of the male reproductive tract for use in Toxicology Studies
- Pathologists within a group should strive for a common lexicon
- Avoids multiple diagnostic terms for same finding (provides consistency within and across different studies)
- Preferable to use general terms, such as degeneration/necrosis
  - with more specific terms in free text field to characterize the finding or in the report
  - Report text should only use diagnostic terms documented in data capture system
- Keep the audience in mind (will not be a pathologist)
- Terminology used should be sufficiently detailed to communicate specificity of finding to the reader.

Prostate

PROSTATE WEIGHTS

1-Month Dog study (14-16 month old dogs)

	Dose	Prostate Wt. (Abs) **Terminal**	Prostate Wt. (%) **Terminal**	Prostate Wt. (Abs) **Recovery**	Prostate Wt. (%) **Recovery**
Vehicle	0	**3.7223**	**0.037**	**7.8235**	**0.080**
GSK123456	1	**6.5740** ↑	**0.070**		
GSK123456	10	**5.710** ↑	**0.067**		
GSK123456	100	**6.237** ↑	**0.070**	**3.5555** ↓	**0.040**

- Terminal – Weight is higher in treated groups
- Recovery – Weight is lower in treated group (weights increased by 110.18% in controls compared to terminal necropsy)
- No difference in histological appearance between control and treated group

- Conclusion: Drug-related increase in prostate weight?

Prostate weight -
one plausible interpretation (reviewer?)

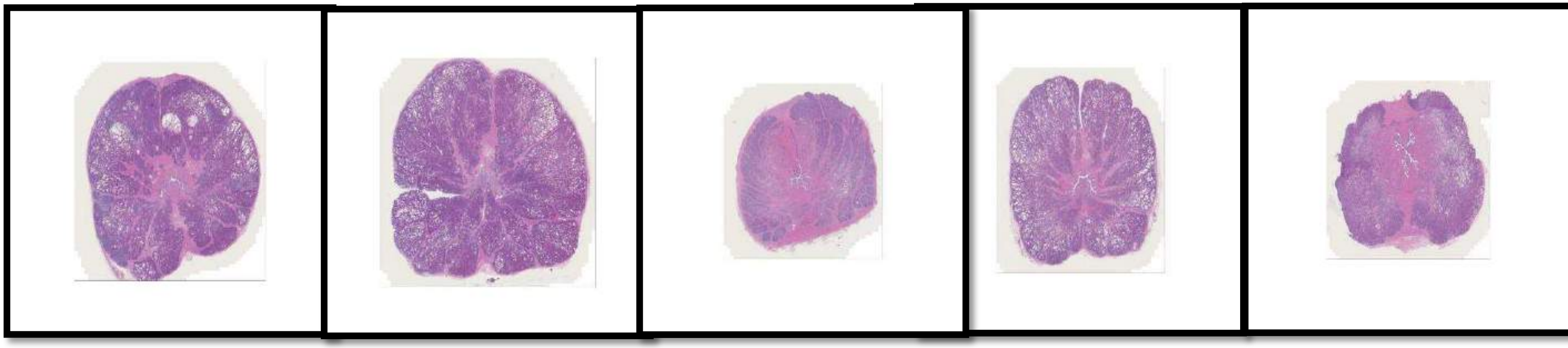
- Interpretation based only on weight alone –
  - Prostate weights are increased at all doses i.e. drug causes hypertrophy/hyperplasia
  - After a 6-week recovery period, decrease in weight (in drug-treated dogs) causes regression of the enlarged prostate
  - Prostate weight at recovery in treated dogs is similar to that noted in control dogs at the end of the study
- Conclusion – The drug has a stimulatory effect on the prostate?

Prostate weight

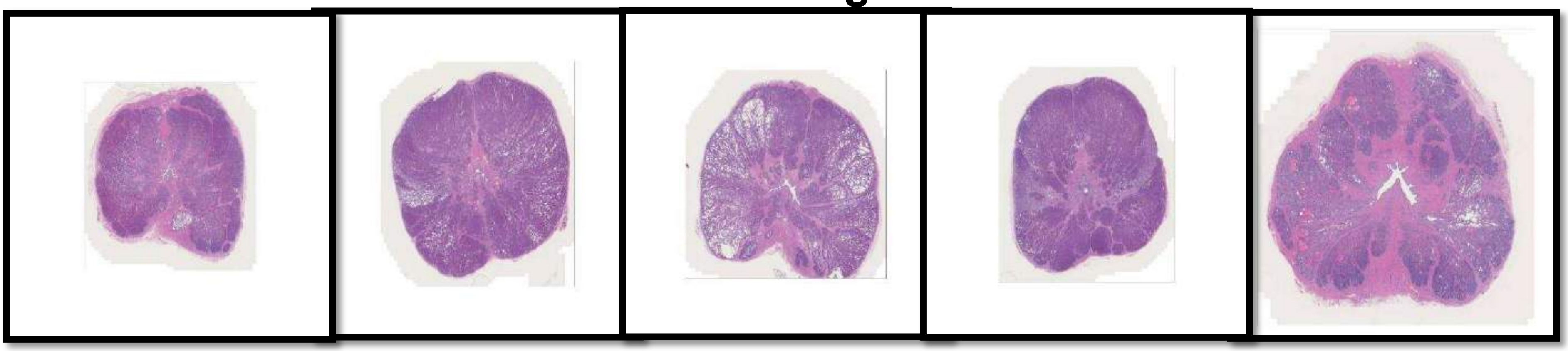
Study Pathologist's interpretation – Literature & Historical Data

- Low weight in concurrent control (3.7223 g) gives the false impression of increased weight in treated dogs
- The low weight in concurrent control dogs is rather unusual
- Literature (Zirkin and Strandberg, 1984)
  - Mature dogs (15-17 month) prostate weight is about 6.4 1.1 g
  - Immature (8.5 months) prostate weight is about 3.2 0.8 g
- Historical control (GSK-RTP)
  - Avg. prostate weight of similar age dogs = 6.2194 g.(Range 2.46 to 12.86
- Prostate of Beagles can still be immature around 1 0.2 yrs

Prostate – Control



Prostate – High Dose



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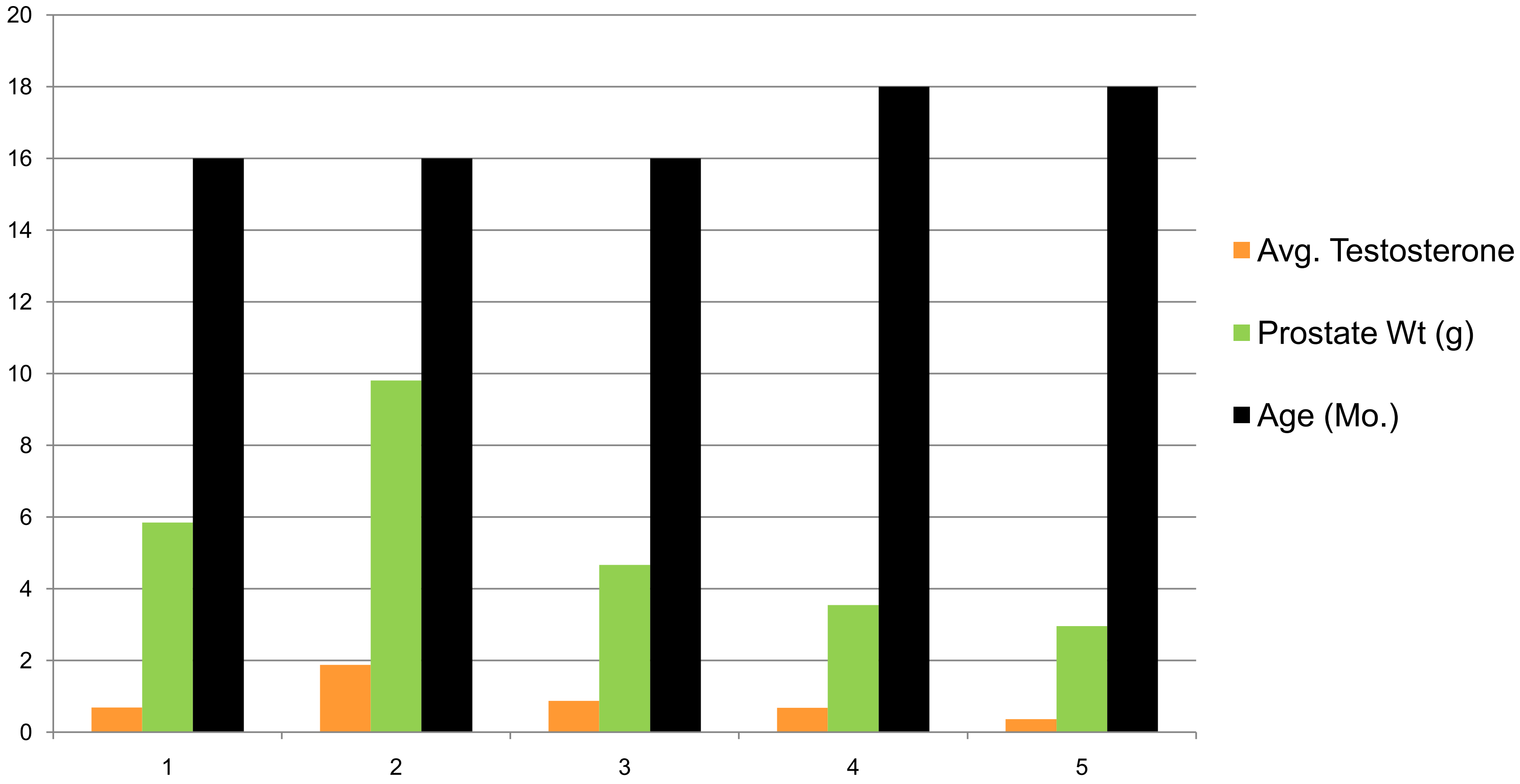
Correlation between serum testosterone, dog's age and prostate weight

Testosterone level is an important factor that influences prostate weight



Dihydrotestosterone concentration of beagle prostatic tissue: effect of age and hyperplasia.
Ewing LL, Berry SJ, Higginbottom EG. Endocrinology. 1983 Dec;113(6):2004-9.

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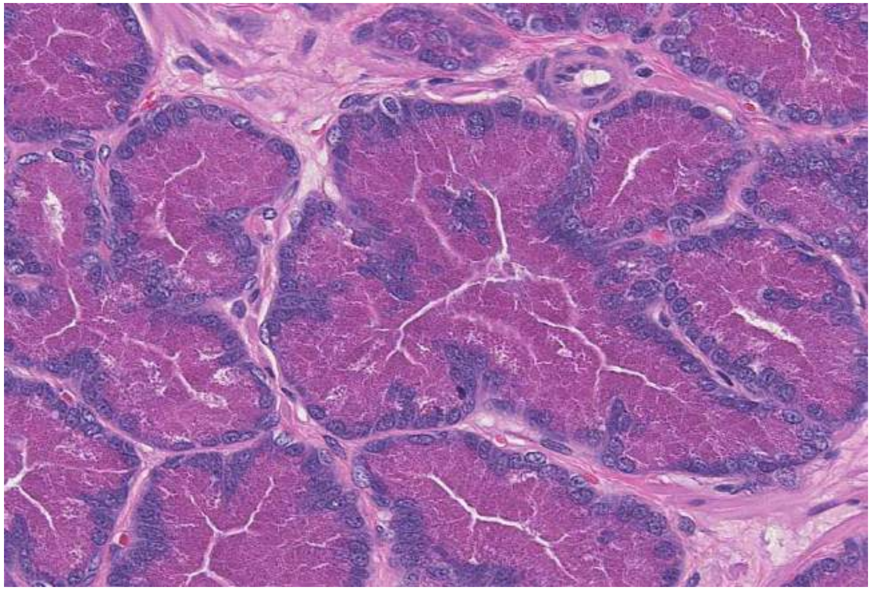
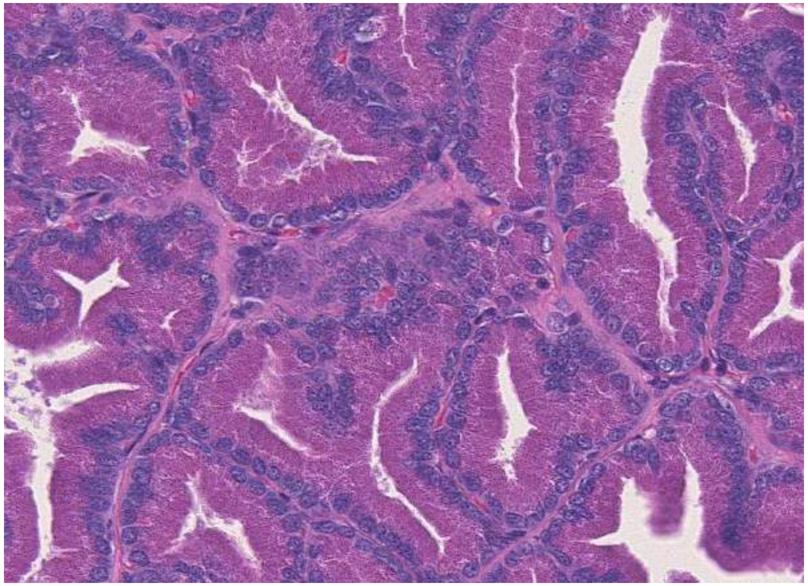
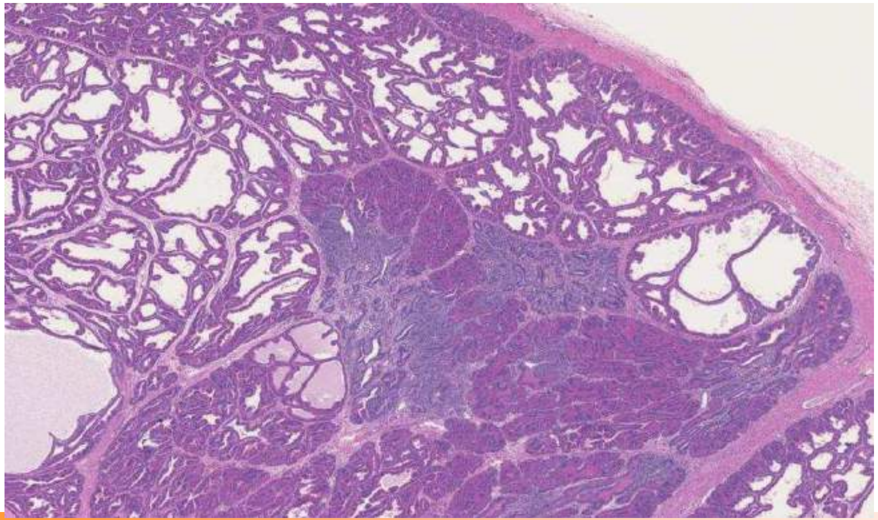
Dog Age vs. Prostate Weight

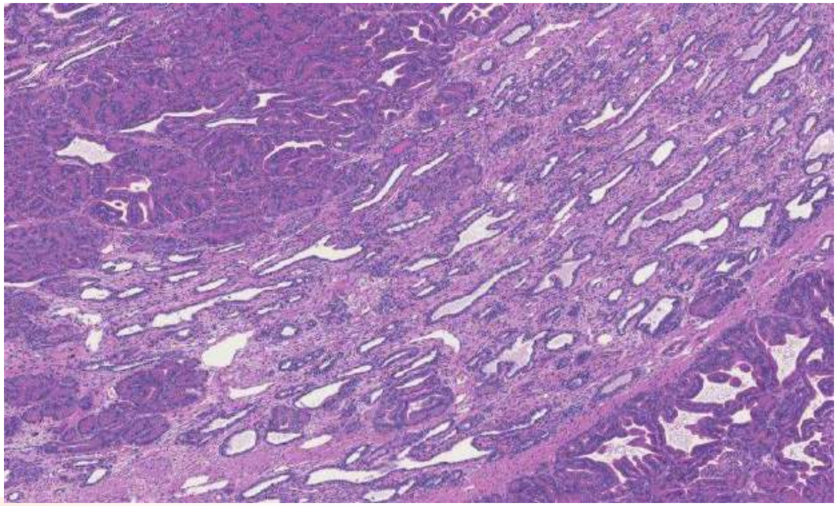
Dog No.	Age - Start of Study	Prostate Weight
1	15 Months	5.842
2	15 Months	9.805
3	15 Months	4.667
4	17 Months	3.541
5	17 Months	2.959
11	17 Months	6.396
12	15 Months	7.174
13	17 Months	6.152
17	15 Months	5.589
18	17 Months	6.861
19	17 Months	4.680
23	17 Months	2.623
24	15 Months	4.488
25	17 Months	5.837
26	15 Months	5.363
27	17 Months	7.511

Control vs Treated

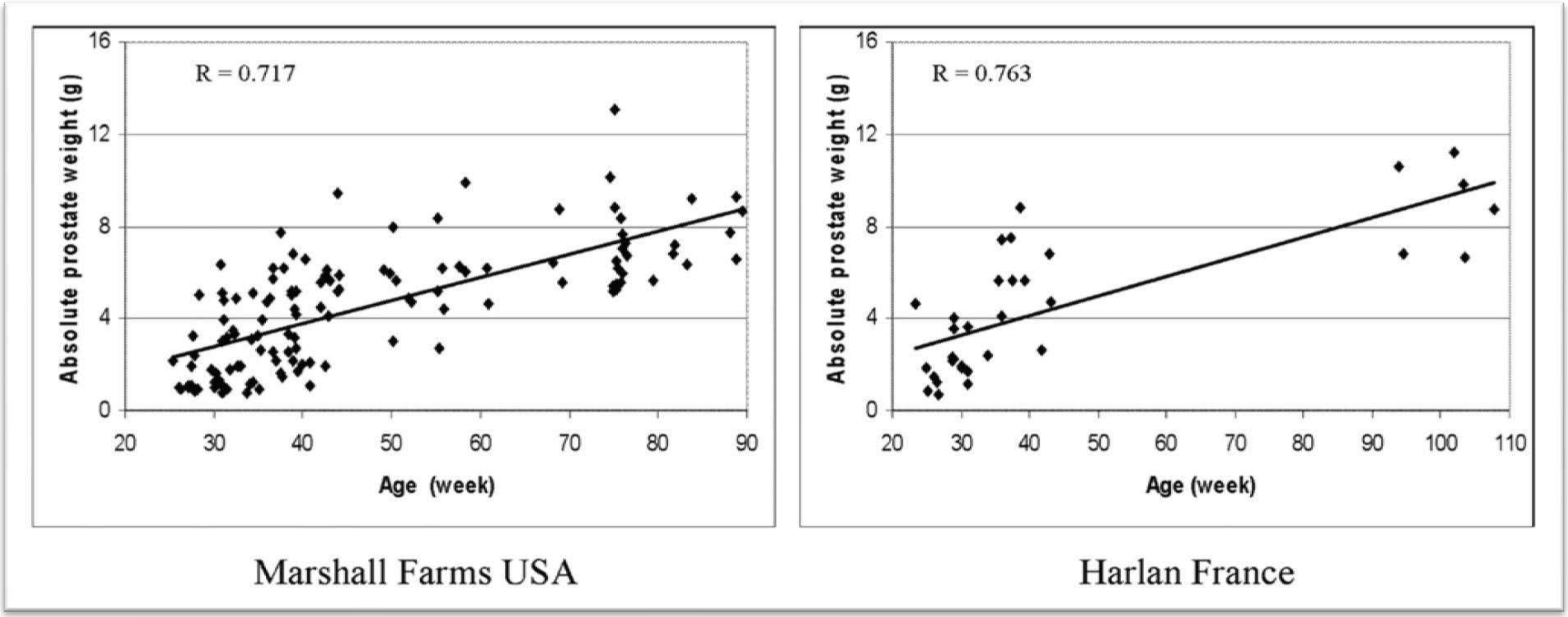








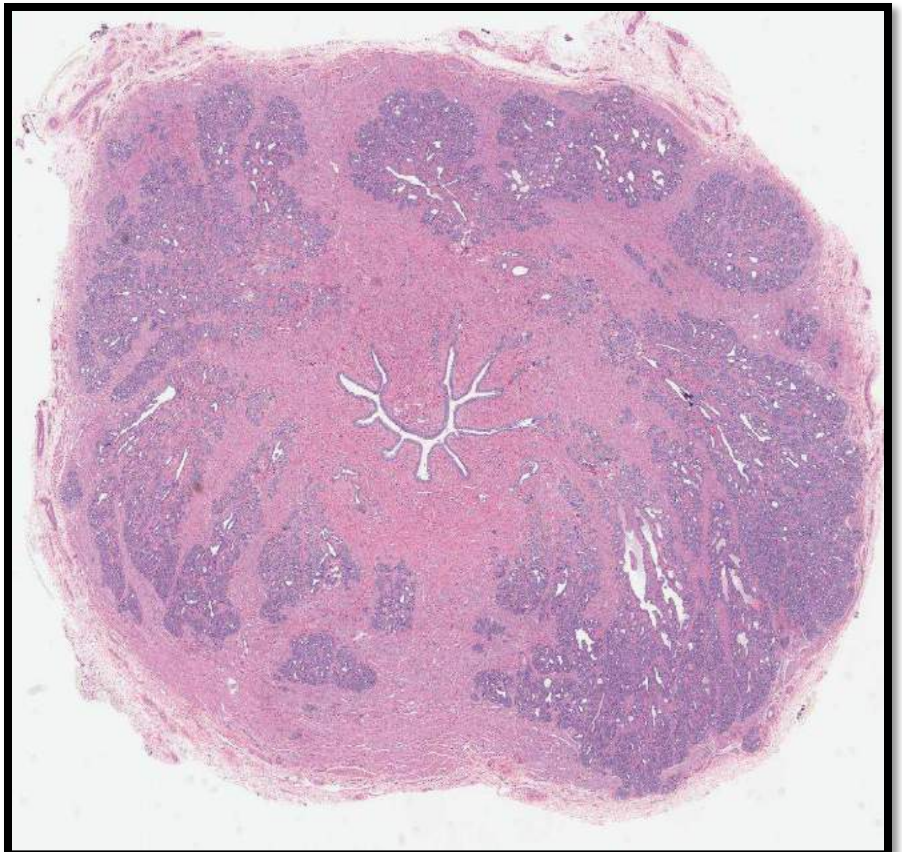
Prostate Weights

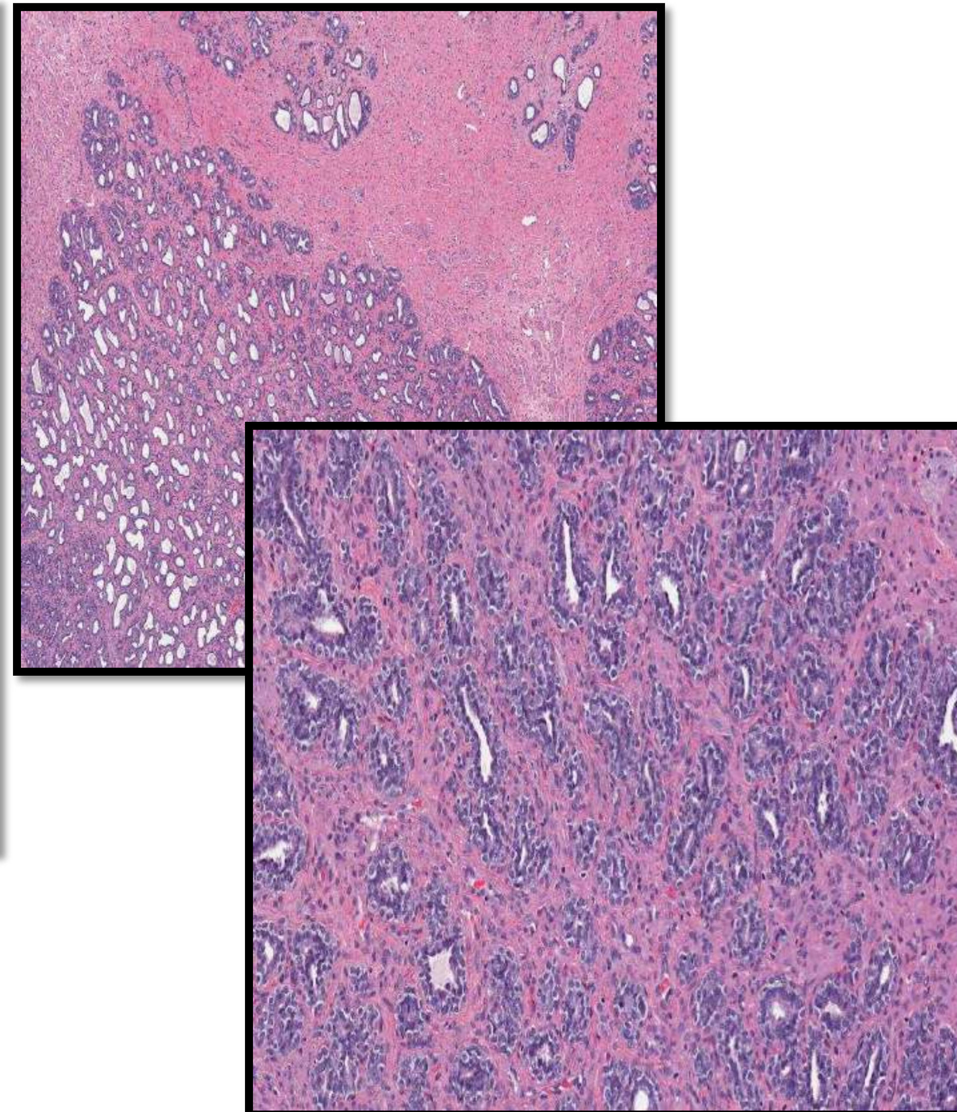


- Positive correlation between absolute prostate weight and age.

Variability in Weight and Histological Appearance of the Prostate of Beagle Dogs Used in Toxicology Studies. Laëtitia Dorso, Franck Chanut, Paul Howroyd, Roger Burnett. Toxicologic Pathology, Vol. 36, No. 7, 917-925 (2008)

Immature Dog





Prostate Weights

- Combine histologic examinations of the prostates and testes for determination of age of sexual maturity.

- Dog vendor
  - Harlan - oldest immature dog was thirty-one weeks old
  - Marshall's – oldest immature dog was forty-one weeks old

- Spontaneous variations in the weight and morphology of the beagle prostate influence the assessment of toxicological data.
- *Immature acini could lead to a misdiagnosis of treatment-related effect of acinar atrophy.*

Variability in Weight and Histological Appearance of the Prostate of Beagle Dogs Used in Toxicology Studies. Laëtitia Dorso, Franck Chanut, Paul Howroyd, Roger Burnett. Toxicologic Pathology, Vol. 36, No. 7, 917-925 (2008)

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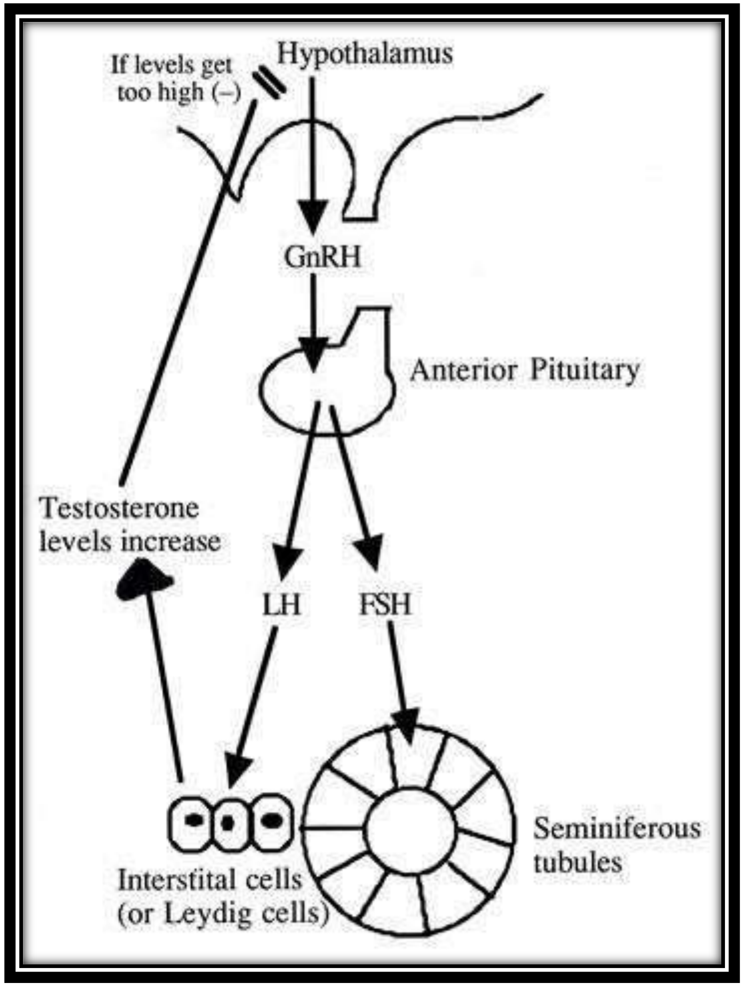
Prostate-Testis Correlation

- Can you identify maturity by looking at the prostate
    - NO

Hormones and Hormone Data

Testosterone Synthesis

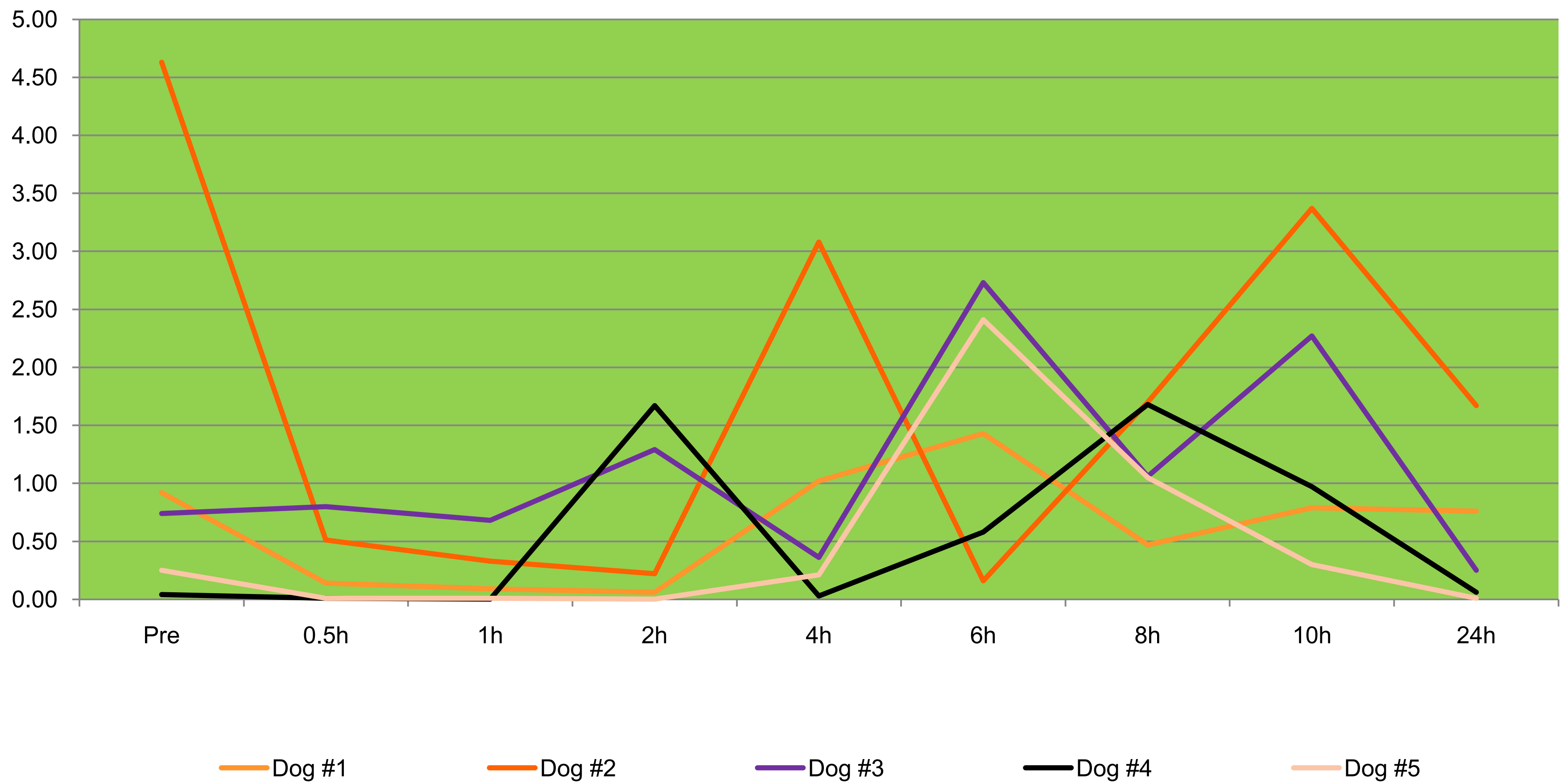


Histopathology vs. hormone data

- Histology is more likely to detect a change in reproductive function than hormone measurement (morphological changes can be easier to identify and/or interpret)
- Single vs. Multiple Time points
- Understand and recognize normal histology.

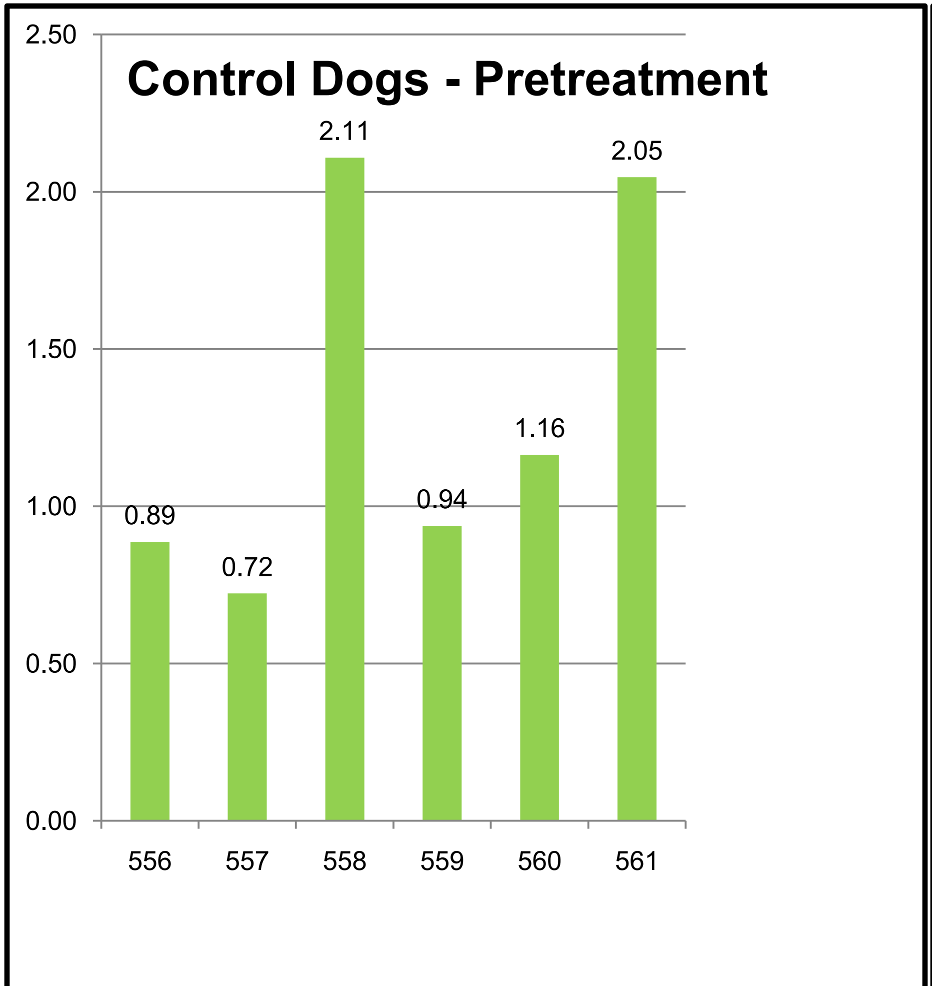
24 hour serum testosterone (ng/ml) profile in control dogs

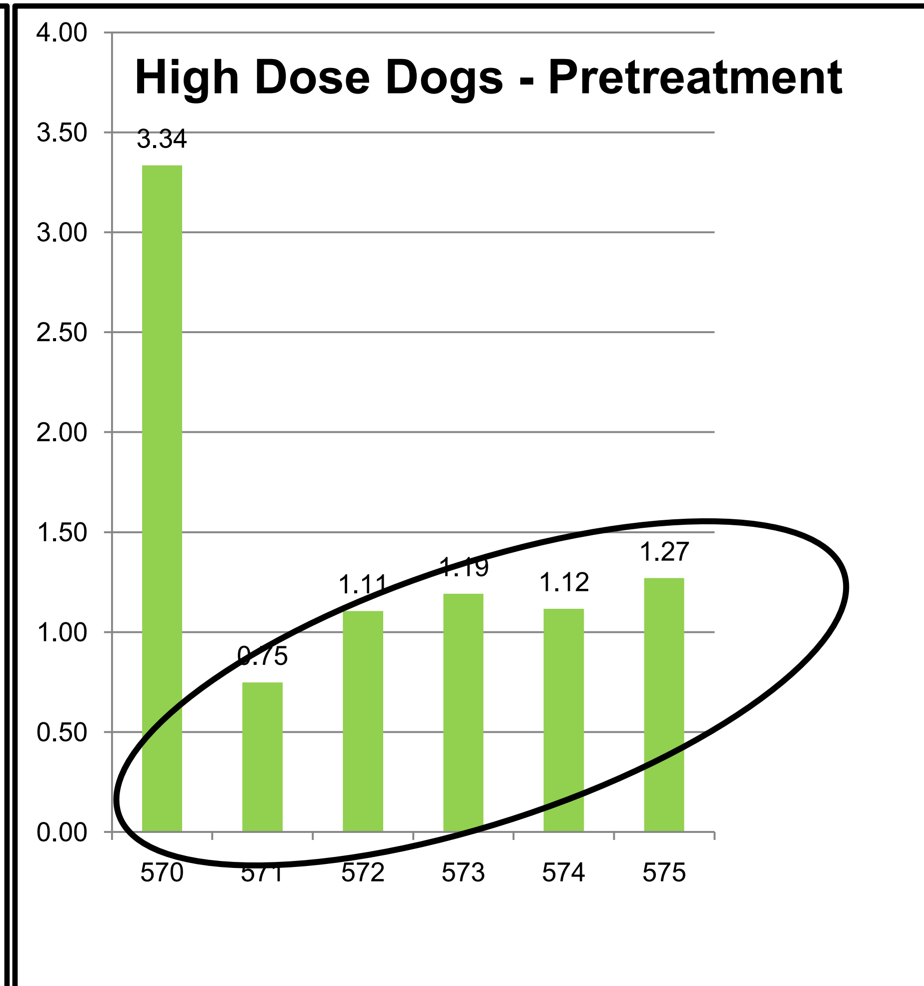
(single vs. multiple time points)



Serum Testosterone (ng/mL)

Avg. of 8 data points collected over a 10 hour period prior to start of 3-month study (Day -7)





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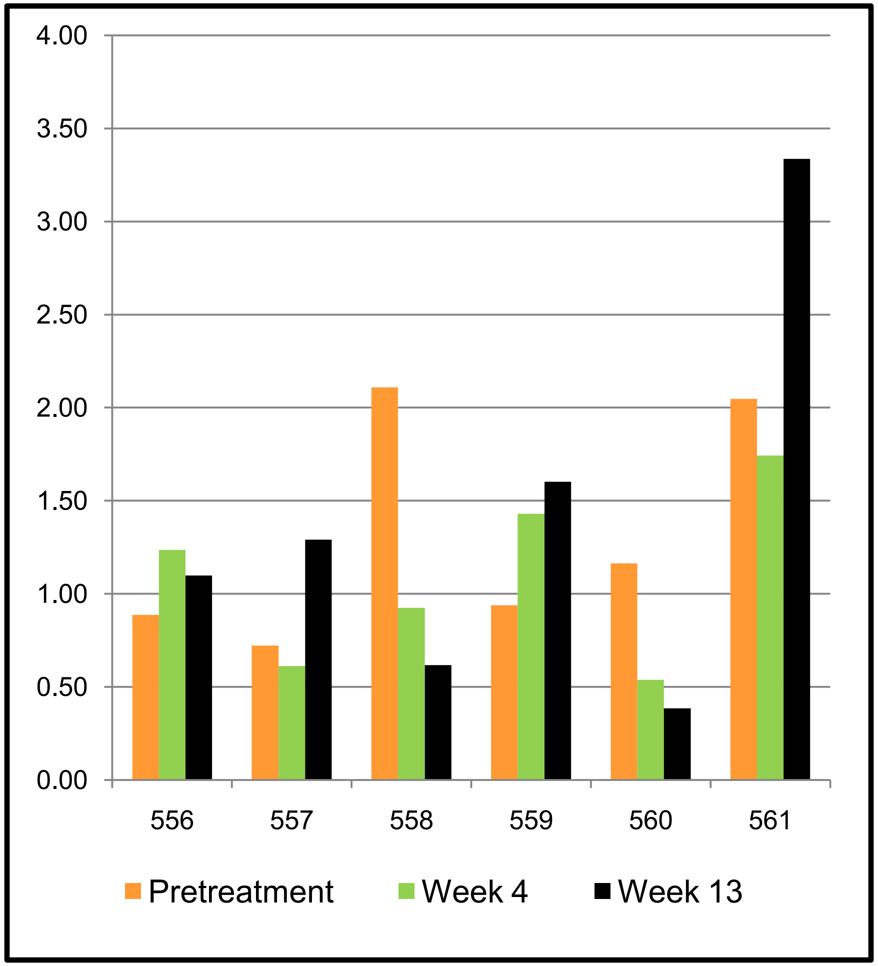
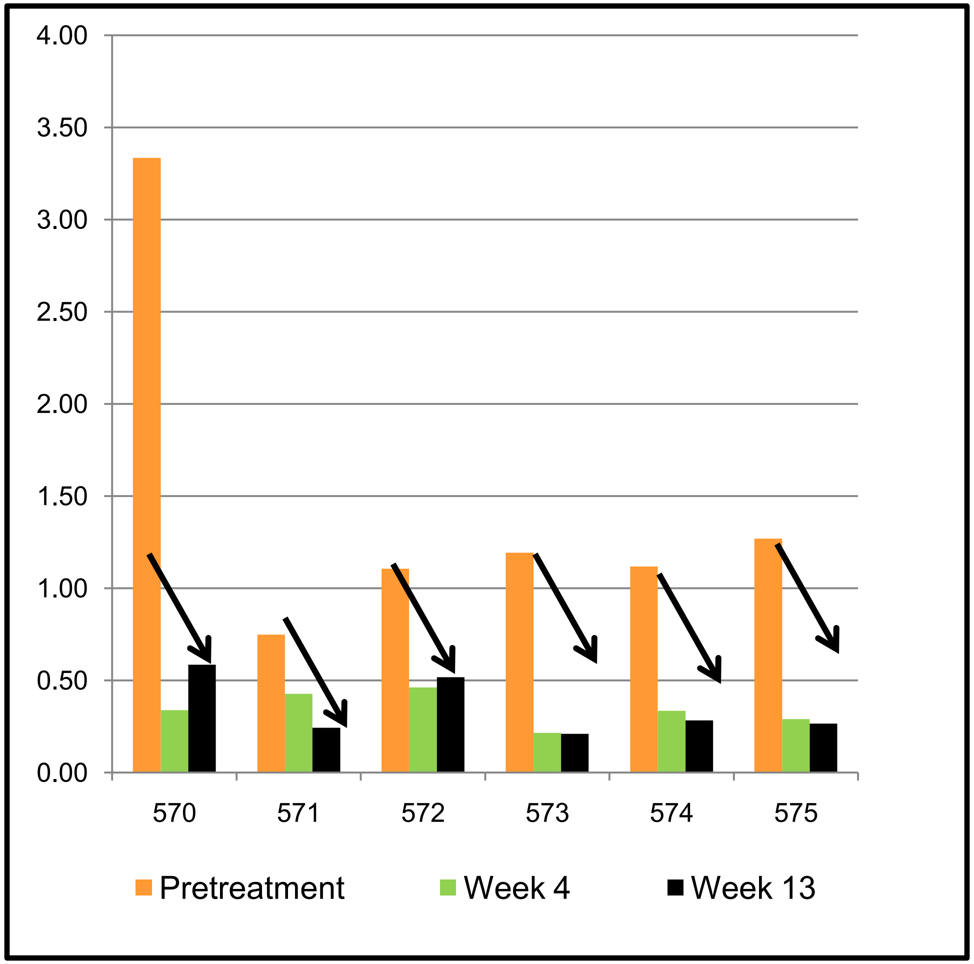
Serum testosterone in control and high dose dogs from a 3-month study

Avg. of 8 data points collected over a 10 hour period





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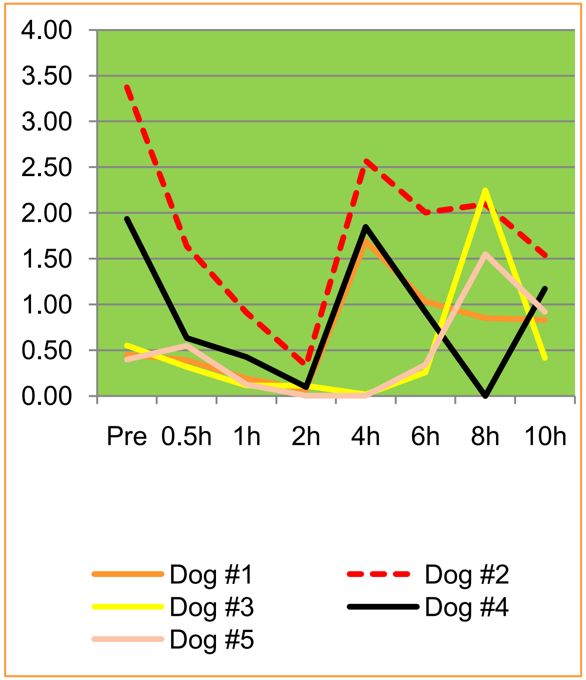
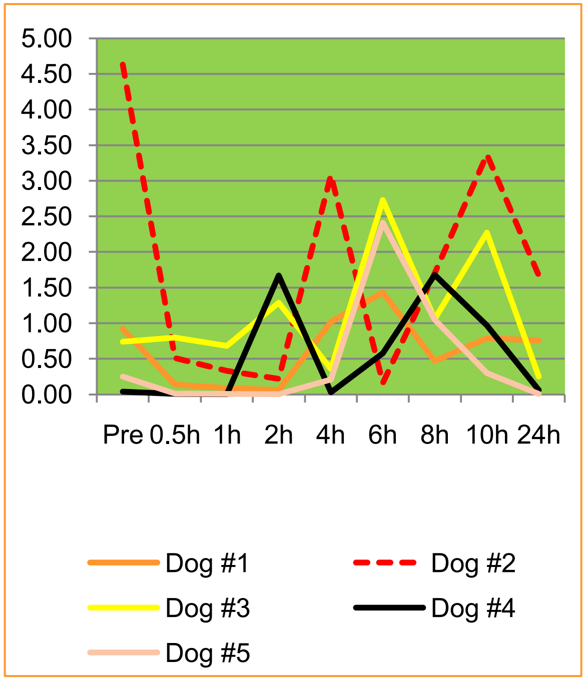
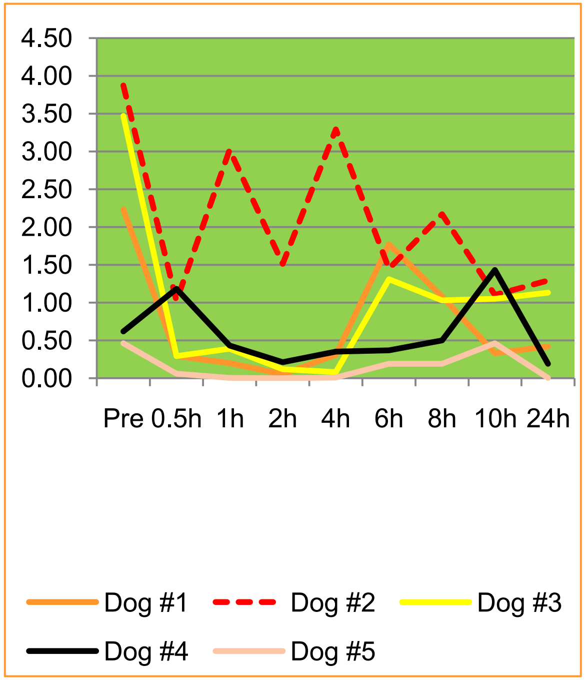
Serum Testosterone (ng/mL)

Study Day -7, 1, 28

Hormone levels fluctuate throughout the whole day.







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Examples of test article-induced testicular toxicity

Antibacterial

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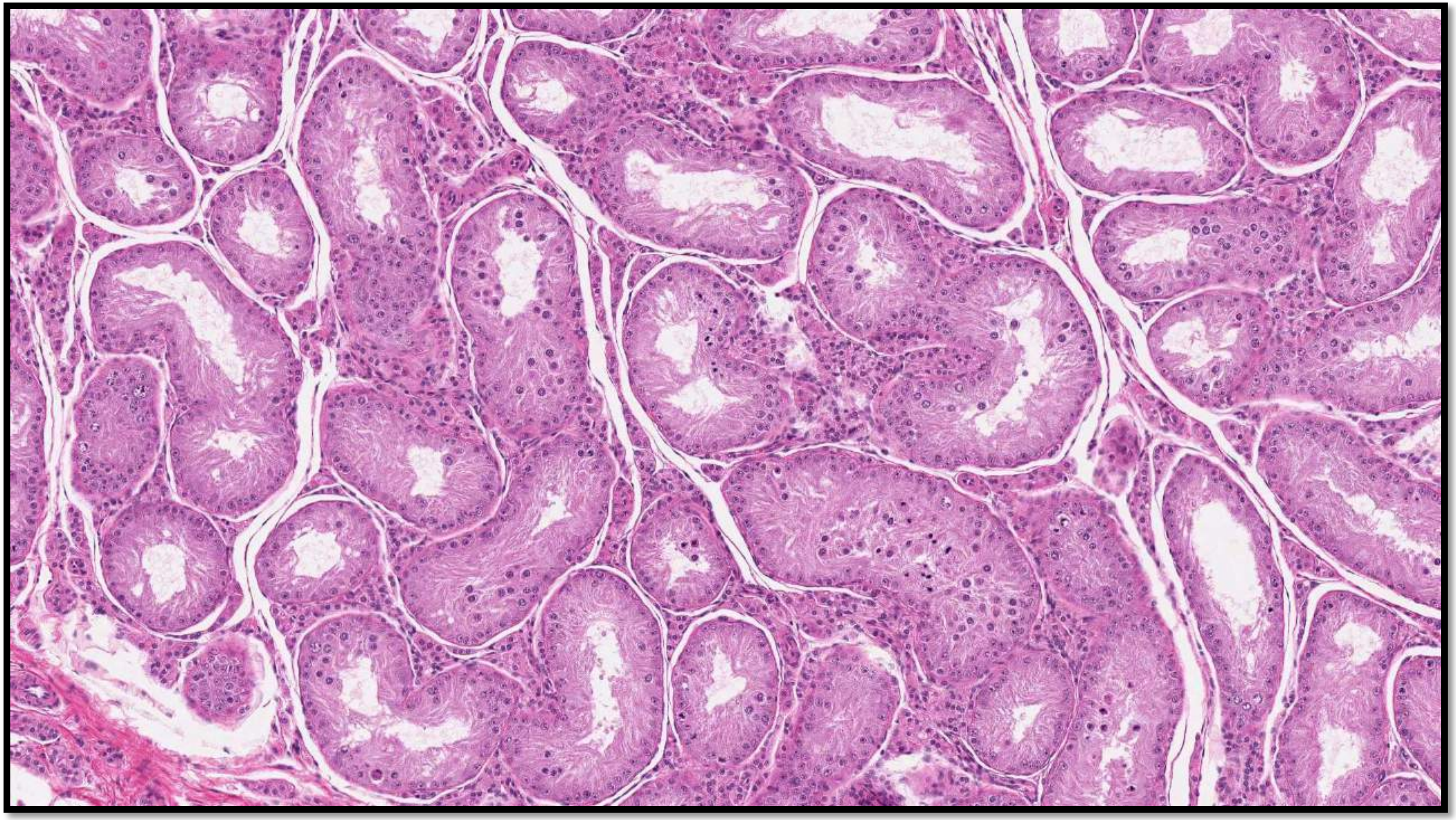
Antibacterial



SERM

Birth Defects Research (Part B) 80:225–232 (2007)

Review Article

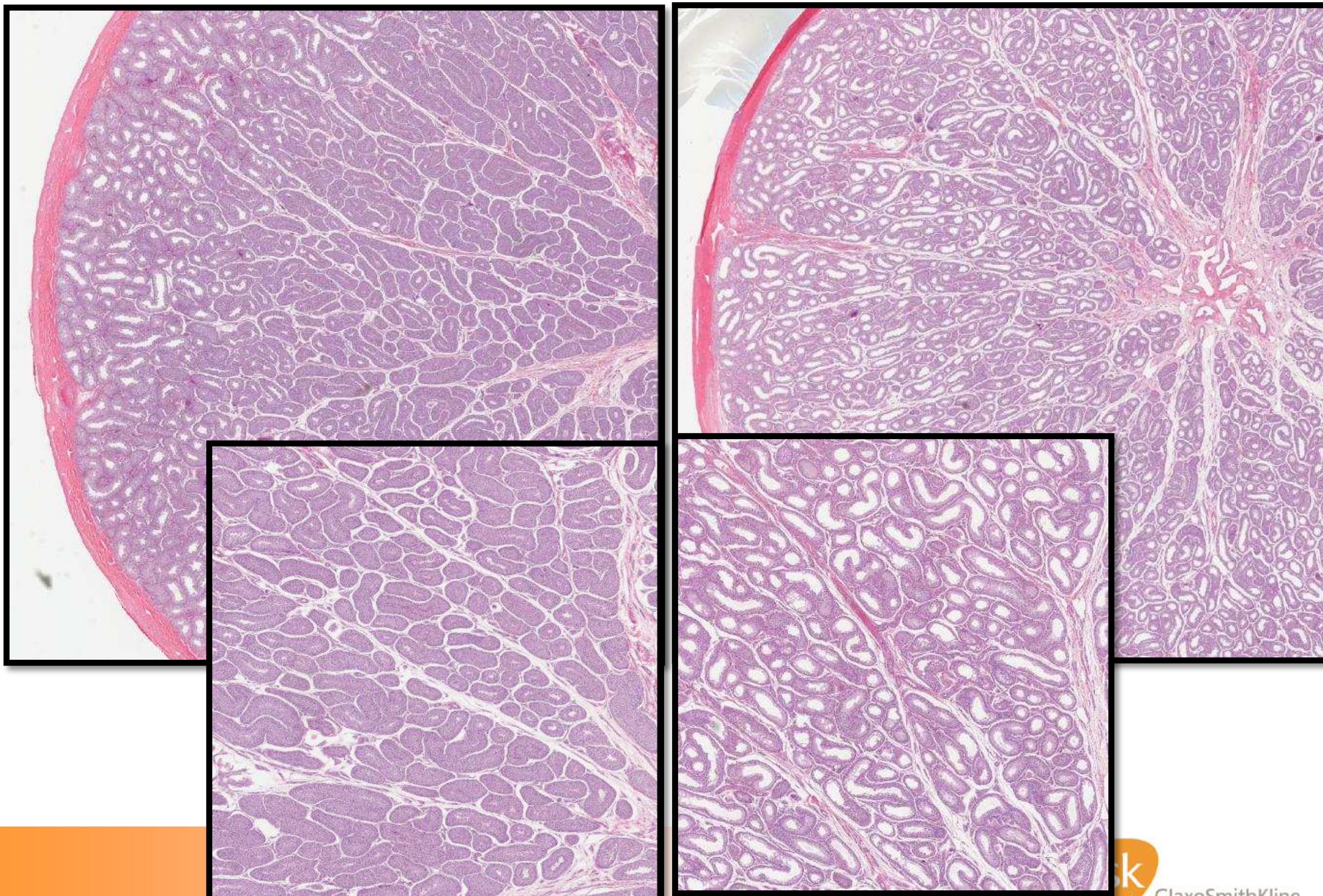
Histologic Changes in Ovary, Uterus, Vagina, and Mammary Gland of Mature Beagle Dogs Treated With the SERM Idoxifene

Sabine Rehm,* Henk A. Solleveld, Samm T. Portelli, and Patrick J. Wier

SERM

SERM

SERM



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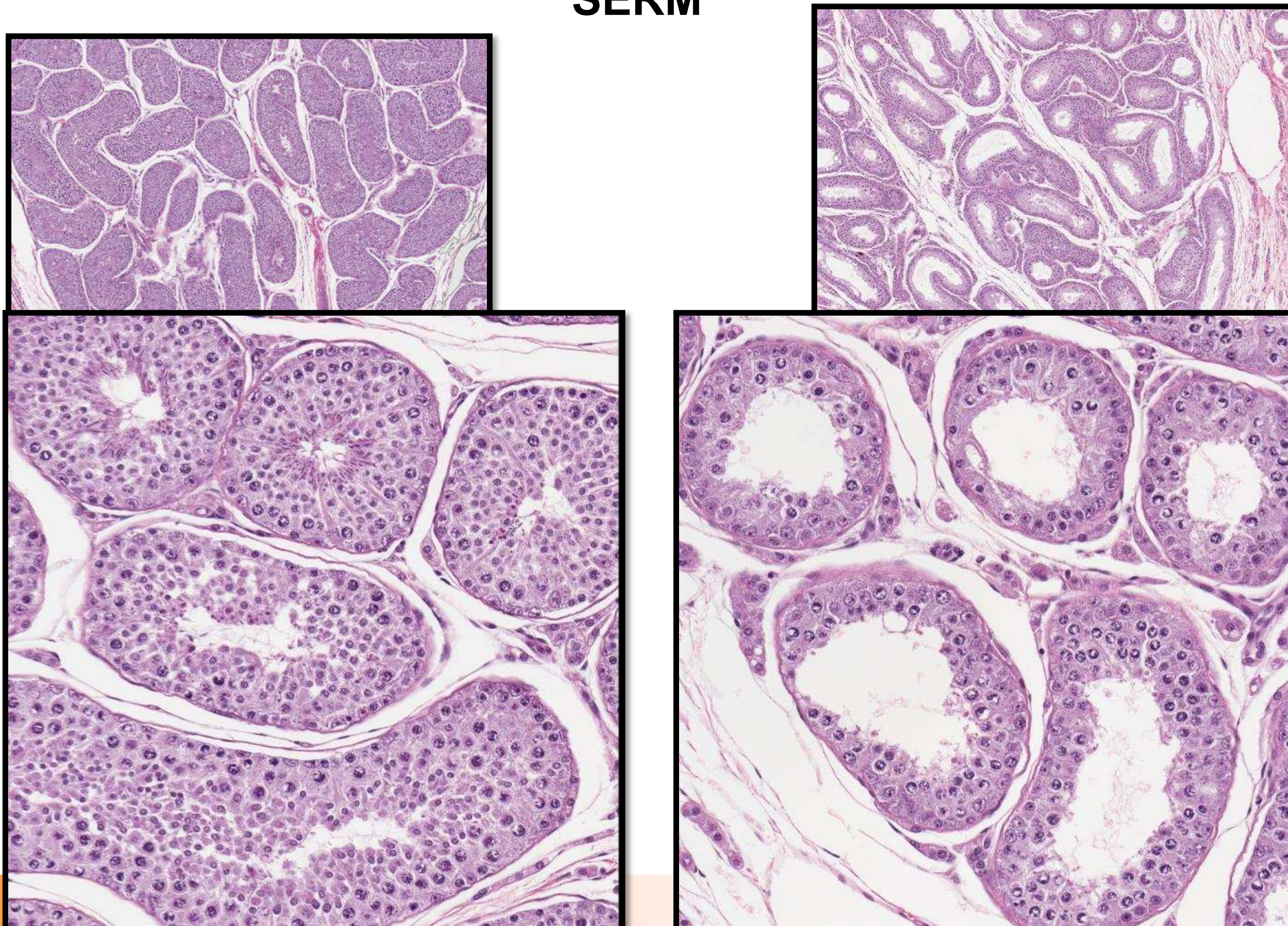

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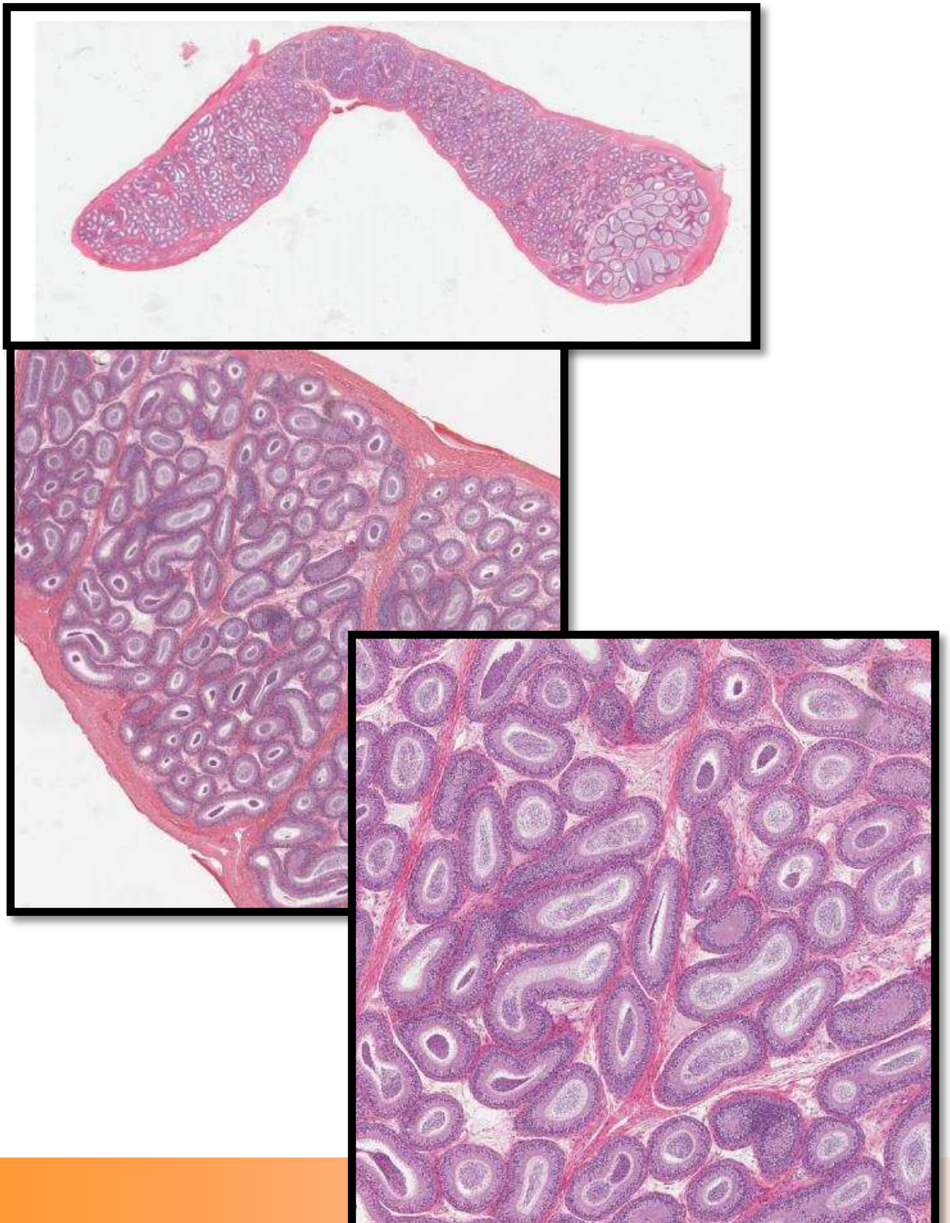
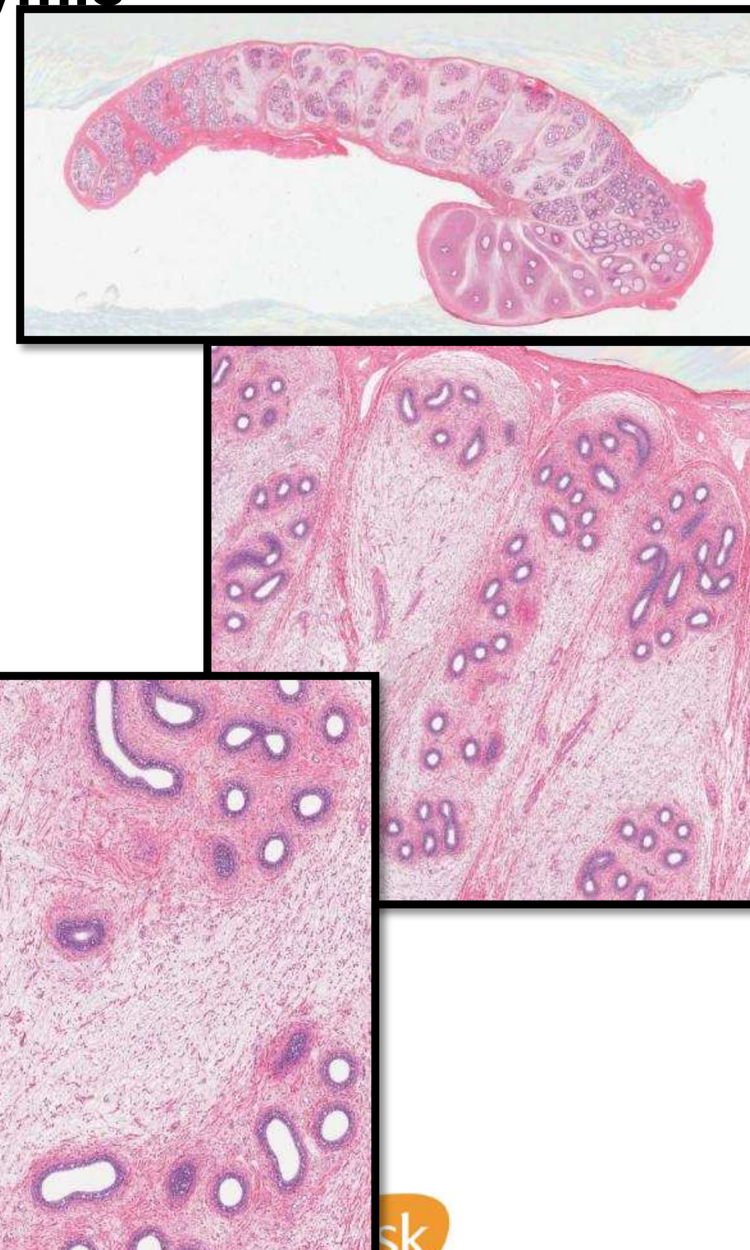
SERM – Epididymis





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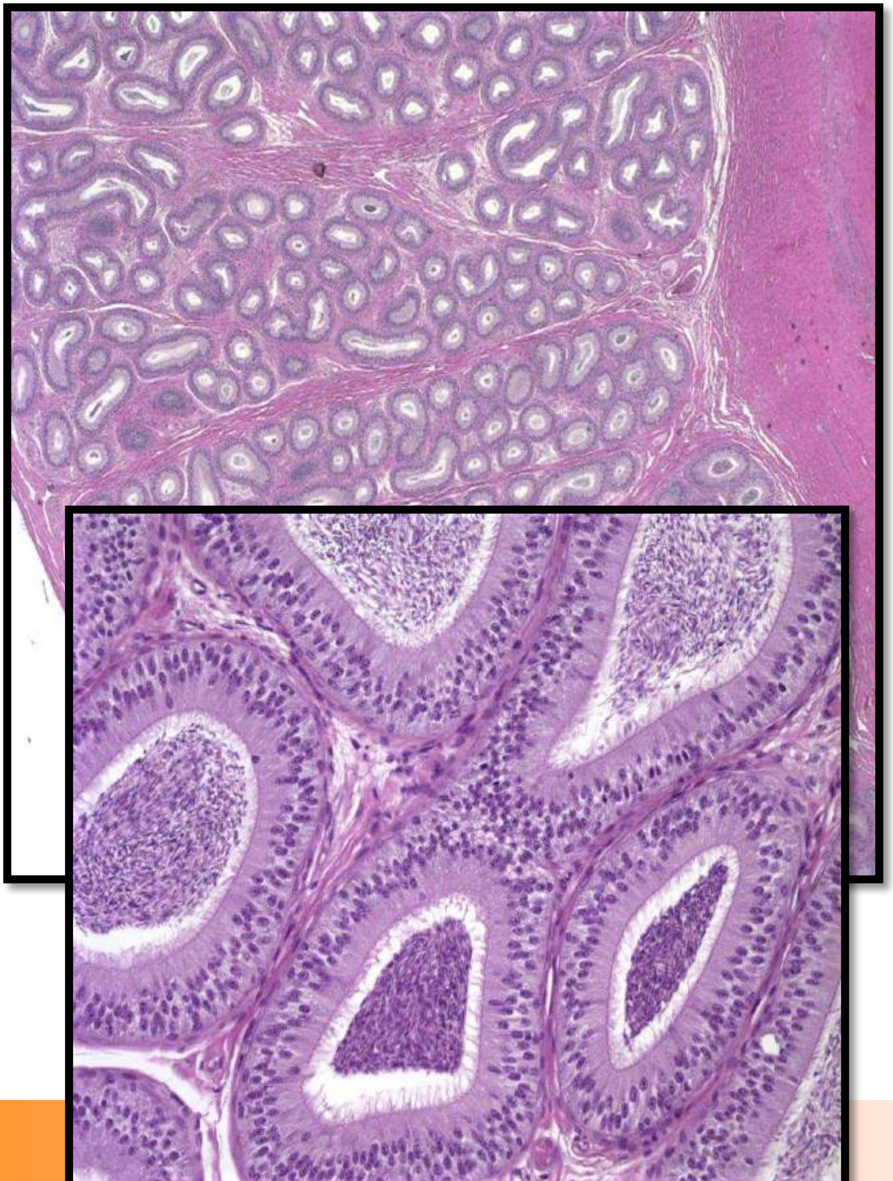
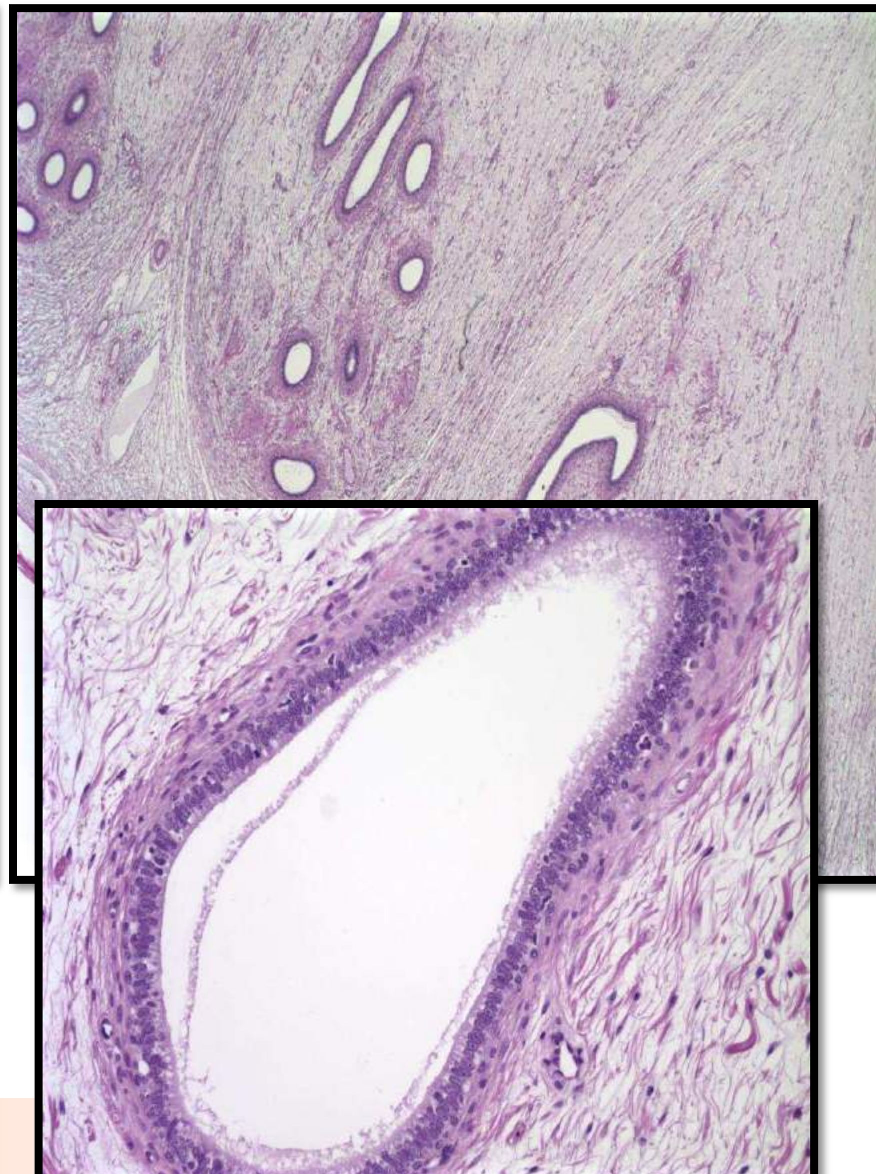
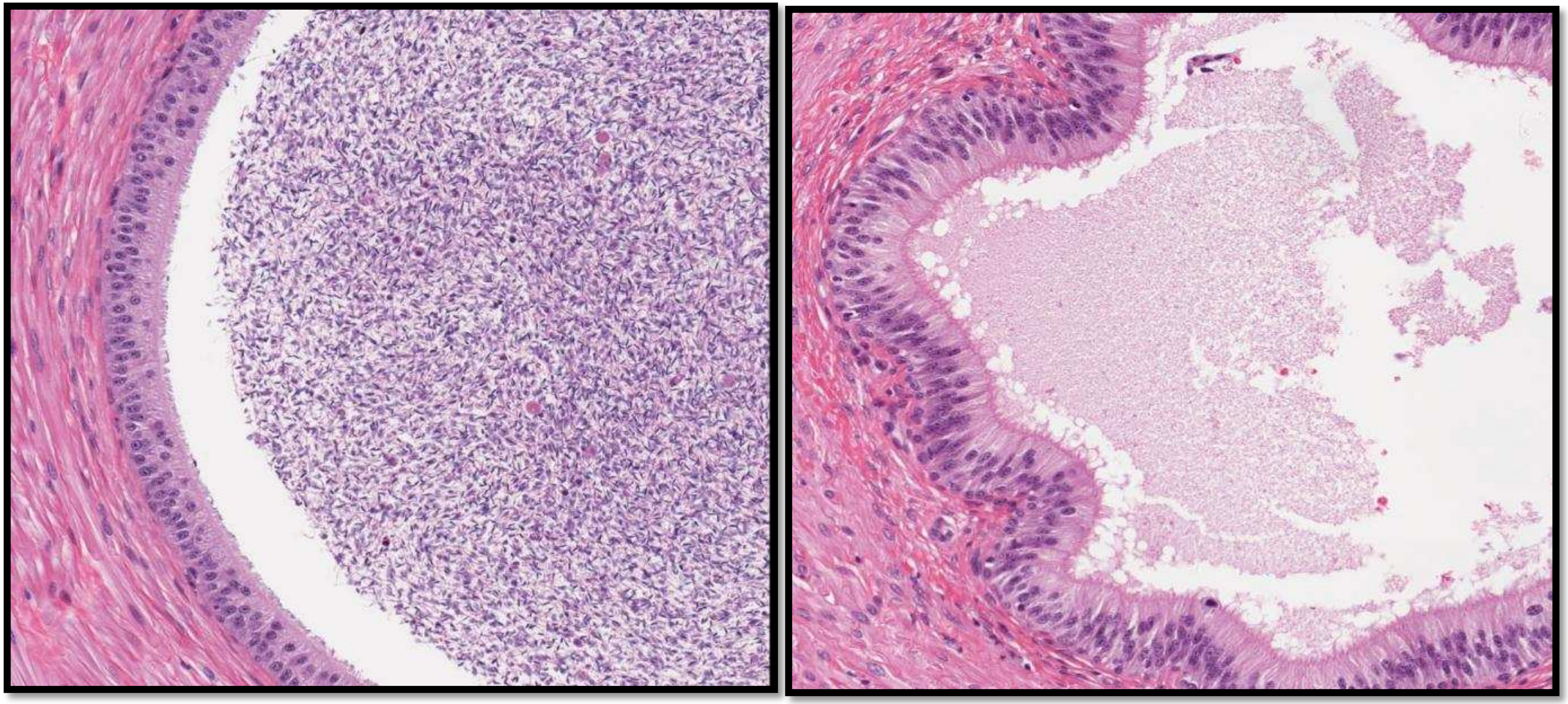




SERM

Control vs. High Dose



Species specificity

- **Rats and dogs frequently show different susceptibility**
- **May be due to differences in regulation of spermatogenesis, pharmacokinetic/metabolic factors, physiological differences**
- **Neither species is more or less relevant to man since spermatogenesis is basically the same in most mammals**

- *References on drug induced testicular effects noted in dogs, but not in rats*
  - *Losco et al (2007). Administration of an Antagonist of Neurokinin Receptors 1, 2, and 3 Results in Reproductive Tract Changes in Beagle Dogs, but Not Rats., Toxicologic Pathology, Vol. 35, No. 2, 310-322*
  - Gerson et al (1989). Animal safety and toxicology of simvastatin and related hydroxy-methylglutaryl-coenzyme A reductase inhibitors. Am J Med. Oct 16;87(4A):28S-38S.
  - MacDonald et al (1988). Preclinical evaluation of lovastatin. Am J Cardiol. Nov 11;62(15):16J-27J. Review.

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Evaluation of testicular toxicity Comprehensive Approach

- Immature and peripubertal dogs are confounding factors
- Mature dogs should be used (if possible)
- Background pathology is a major problem with adult dogs and is different from immaturity or peripuberty (Rehm 2000; Goedken et al 2008)
- Hypospermatogenesis –
  - 75% of dogs six to seven months of age
  - 10% in dogs over eleven months of age.
- Hypoplasia (atrophy)
  - 25%–40% of dogs under twelve months
  - 14%–17% in dogs twelve to thirty-six months old.

Evaluation of testicular toxicity
Comprehensive Approach

- Integrate the information
- Age of dogs
- Organ weights
- Epididymis:
  - sperm in the cauda - animal is mature
  - sloughed germ cells without sperm = probably peripubertal
  - Sloughed germ cells with sperm = possibly treatment related
- Know your species and know its background pathology

Evaluation of testicular toxicity
Few observations on evaluating dogs testes

- Absolute testicular weights are better
- Background lesions are more common in subcapsular areas
- Loss of spermatocytes in a few Stage VIII tubules (background lesion) mimics drug-related effect
- Observe mediastinum testes in conjunction with epididymis
- Conundrum of spontaneous vs. drug-related effect – are tubules in the same stage consistently affected?
- Spontaneous/background lesion of testes –
  - Spontaneous degenerative changes are uncommon in spermatids (round or elongate)
  - Loss of spermatocytes (usually in Stages VI/VIII to Stage I) with clear (punched out) spaces is common

Acknowledgements

- Rick Adler
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- S. Rehm
- The numerous fellow pathologists and colleagues who so willingly share their slides and experiences

Questions/Comments/Suggestions

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