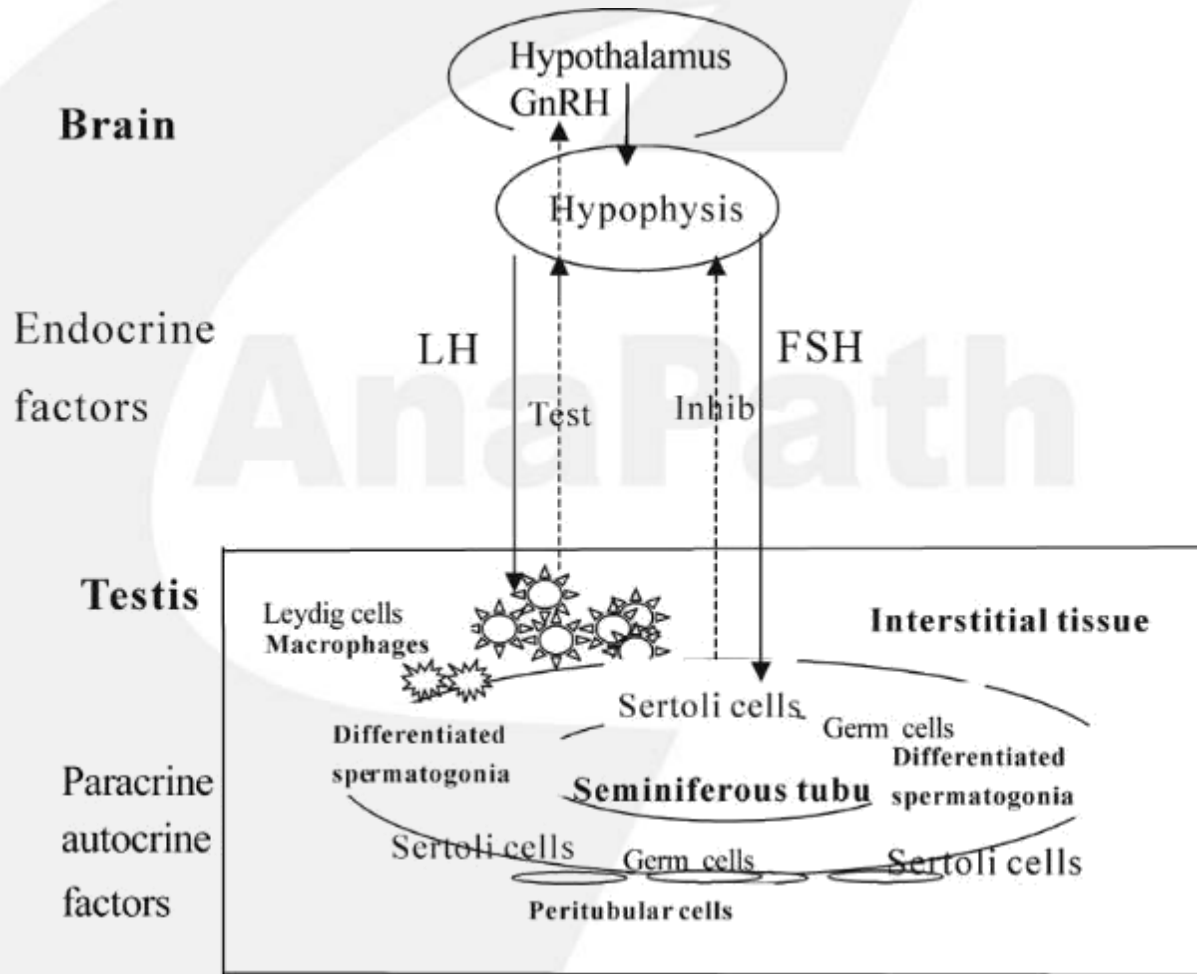


Spontaneous and Induced Lesions in the Male Reproductive System of Laboratory Animals

Klaus Weber, PhD, DVM, MSBiol
AnaPath GmbH, Switzerland

In Cooperation with
BSL Scientific Laboratories GmbH,
Planegg, Germany

Endocrine Regulation - Male



Major Differences

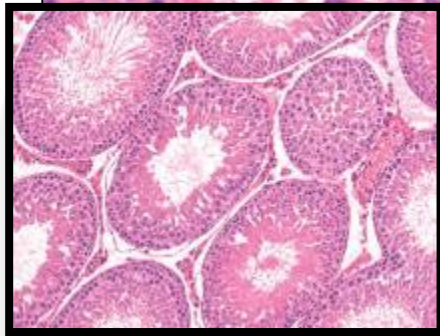
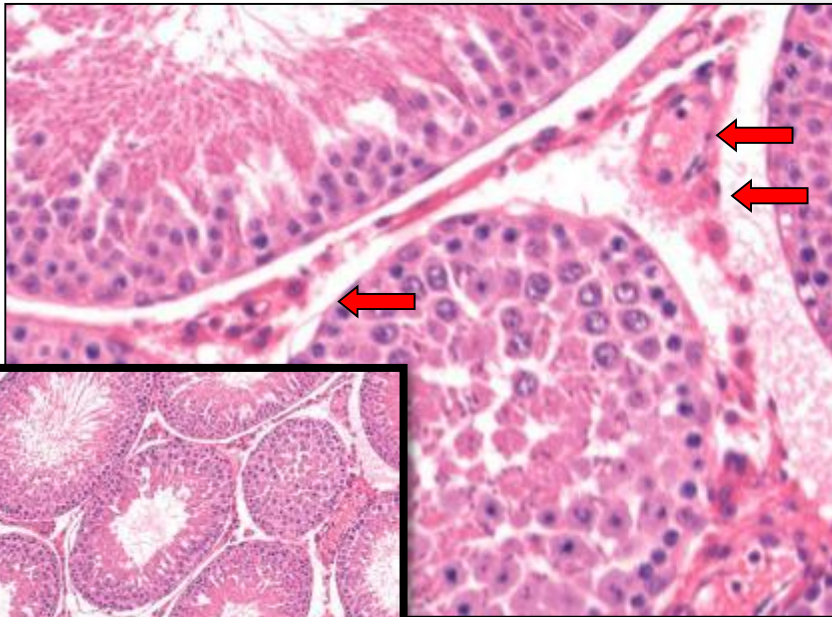
Tubular sections (transversal section):

- ✓ **Mouse: approx. 200**
- ✓ **Rat: 250-350**
- ✓ **Dog: 400-600**

Stages:

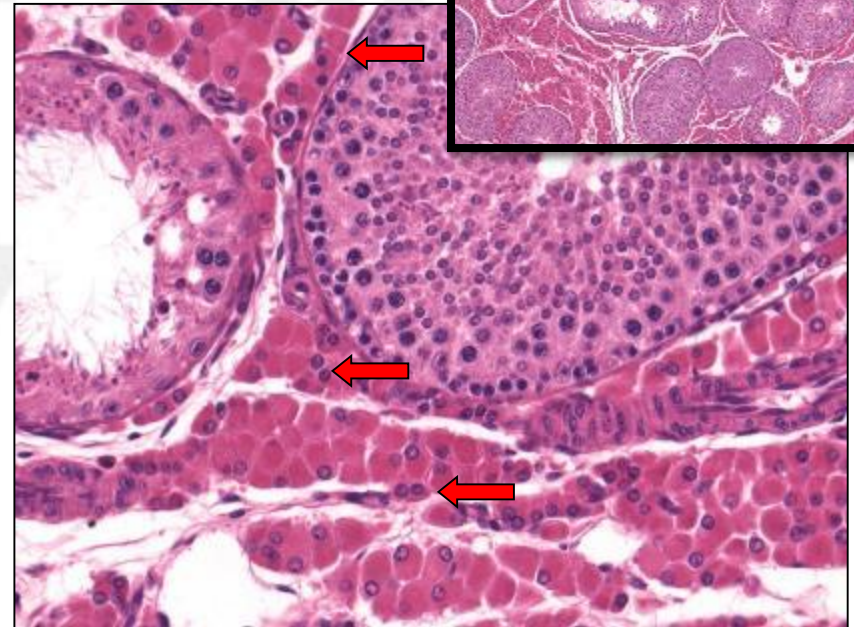
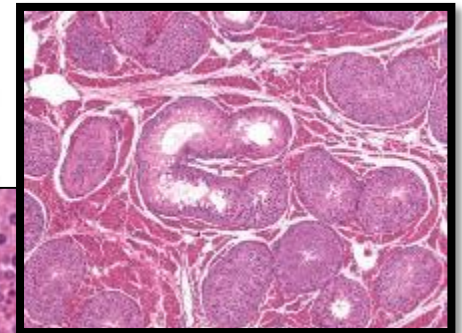
- ✓ **Dog: 8**
- ✓ **Mouse: 12**
- ✓ **Rat: 14**
- ✓ **Cynomolgus: 12**
- ✓ **Human: 6**

Major Differences in Testes of Laboratory Animals

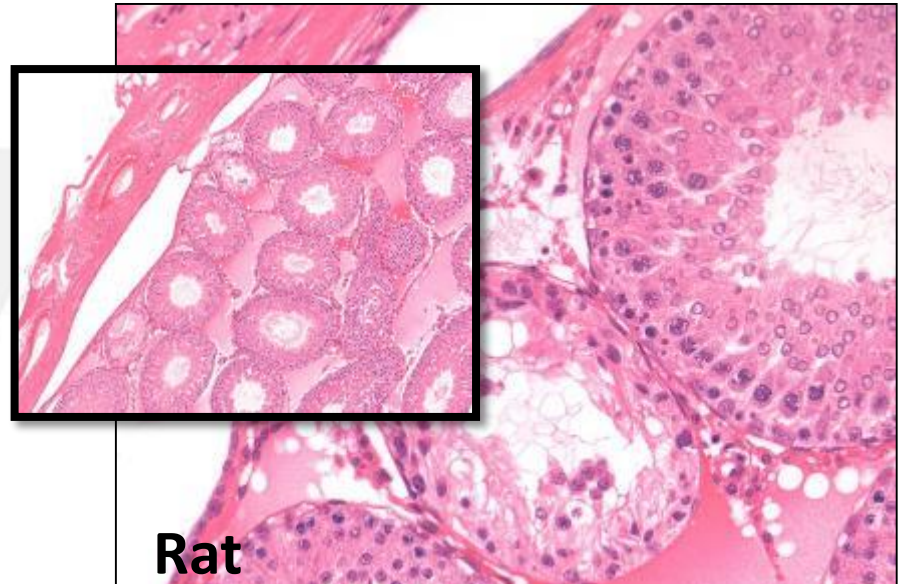
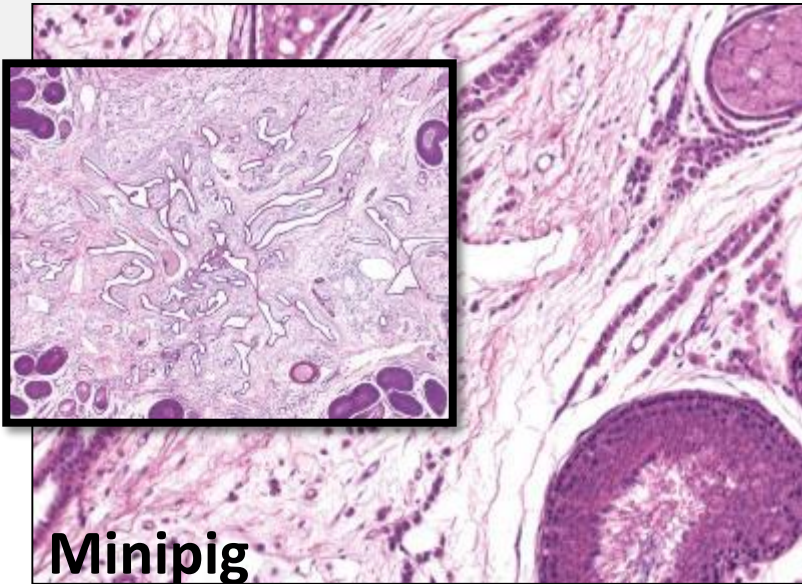
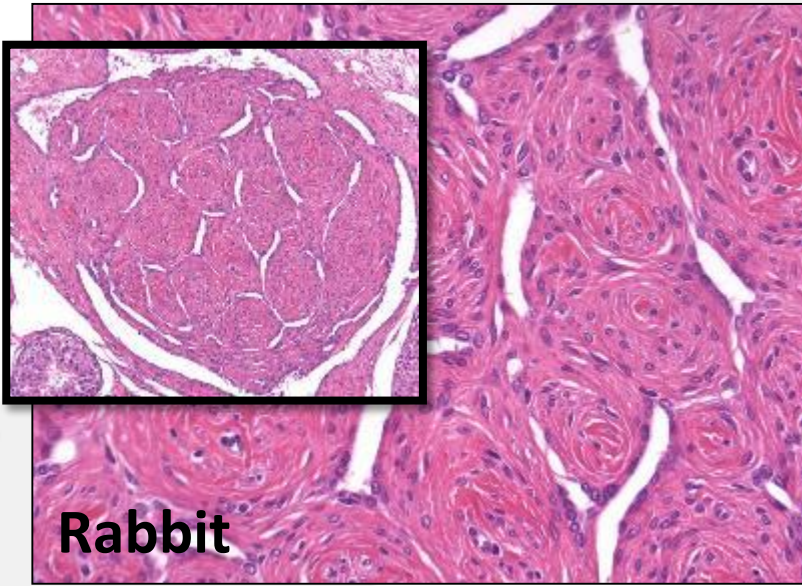


Rat:
Leydig cells,
normal amount in
young to
middle aged animals

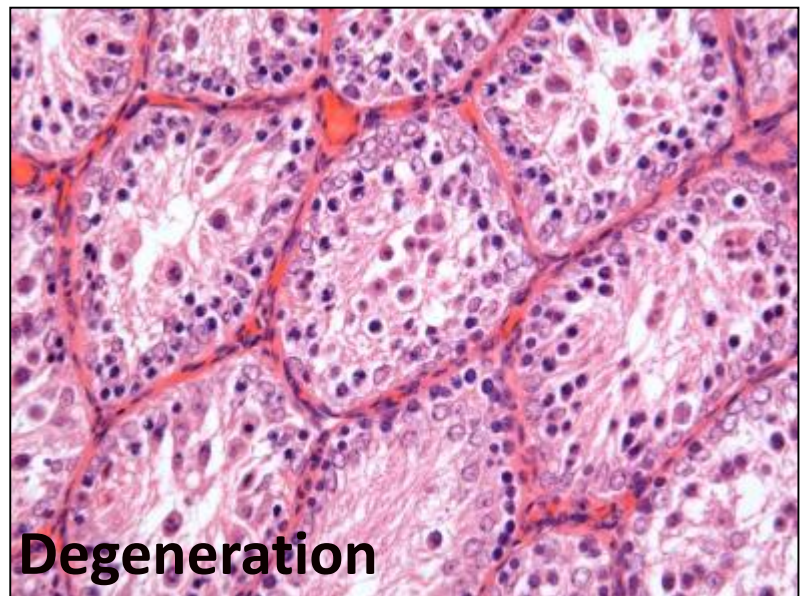
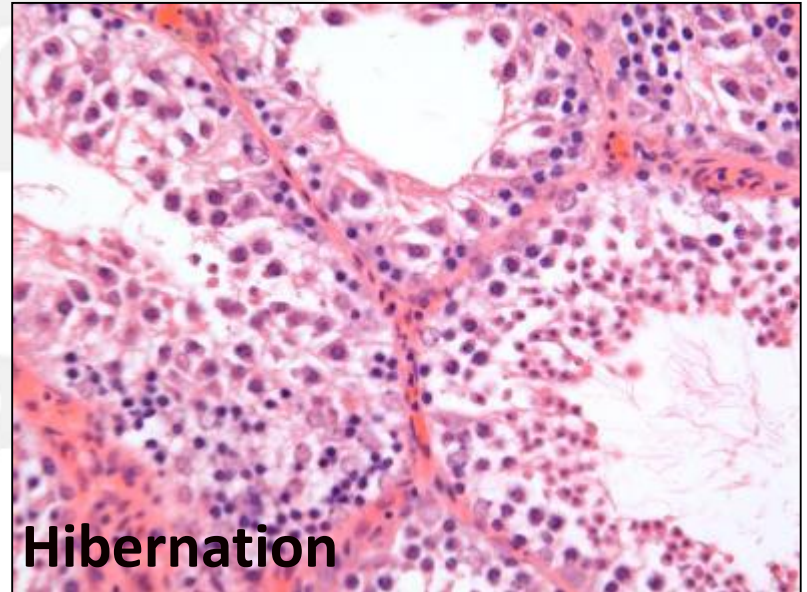
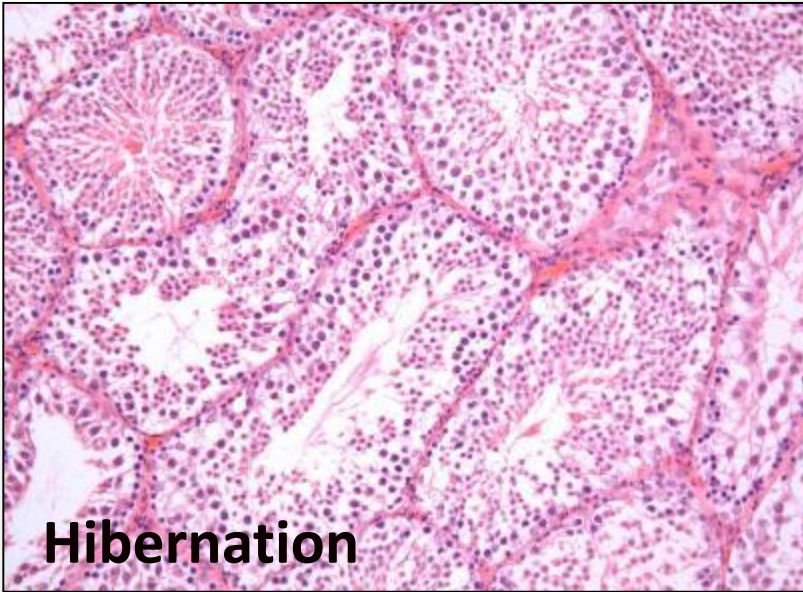
Pig:
Leydig cells,
normal amount in
young to middle
aged animals



Rete Testis



Hamster: Hibernation vs Degeneration



Study Conditions

- ✓ **Inhalation studies:**
over 1 hour inhalation time per day leading to testicular atrophy
- ✓ **Multigeneration studies by feeding:**
F1-Generation may exert testicular changes acquired as immature animals
- ✓ **Hamster studies:**
Performed in autumn/winter with hibernation status
- ✓ **Trauma: infusion studies**
- ✓ **Immunological conditions: beagle arteritis**

Any Guide?

Creasy DM. Evaluation of testicular toxicity in safety evaluation studies: the appropriate use of spermatogenic staging. Toxicol Pathol. 25:119-131

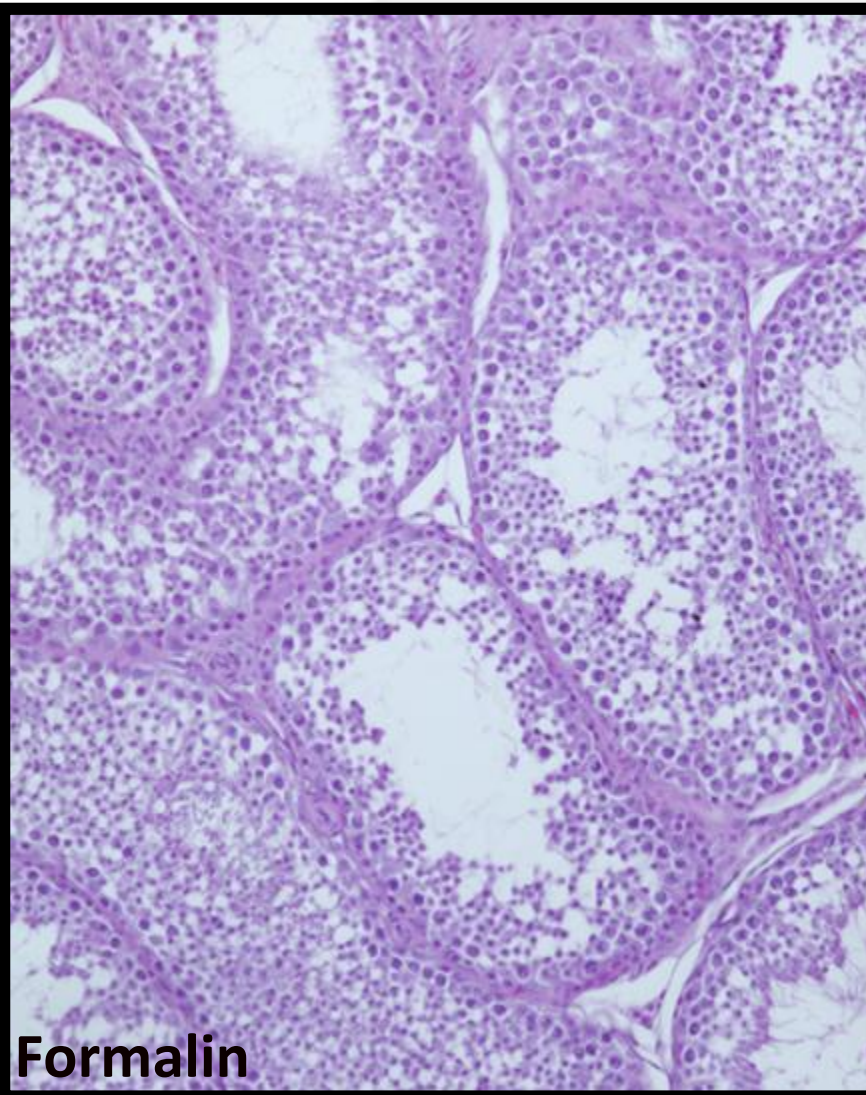
... regulatory guidelines ...**Unfortunately** this has been accompanied by a general **confusion regarding a practical approach** to undertaking such a detailed examination, particularly in respect to the **use of spermatogenic or tubular staging** to identify subtle disturbances in spermatogenesis.....

Histotechnique: Fixation

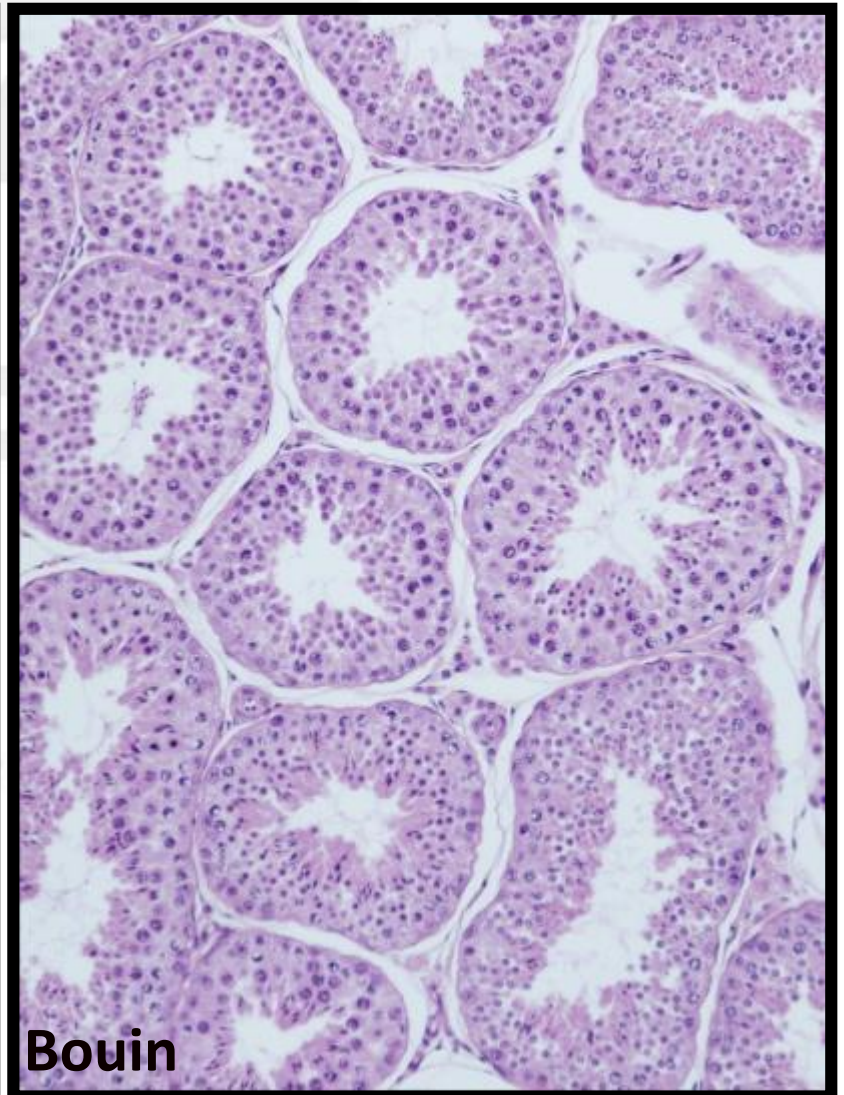
Latendresse JR, Warbritton AR, Jonassen H, Creasy DM.:Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. Toxicol Pathol. 2002, 30:524-533.

Most recent revisions of regulatory guidelines for testing effects of chemicals on reproduction **recommend Bouin's fluid** or a "comparable fixative" instead of formalin to preserve the morphologic detail of testes for histopathological evaluation. However.... Recently a **modified Davidson's fluid** has been reported as an **alternative** to BF to fix testes for routine histopathological examination

Fixation Issues



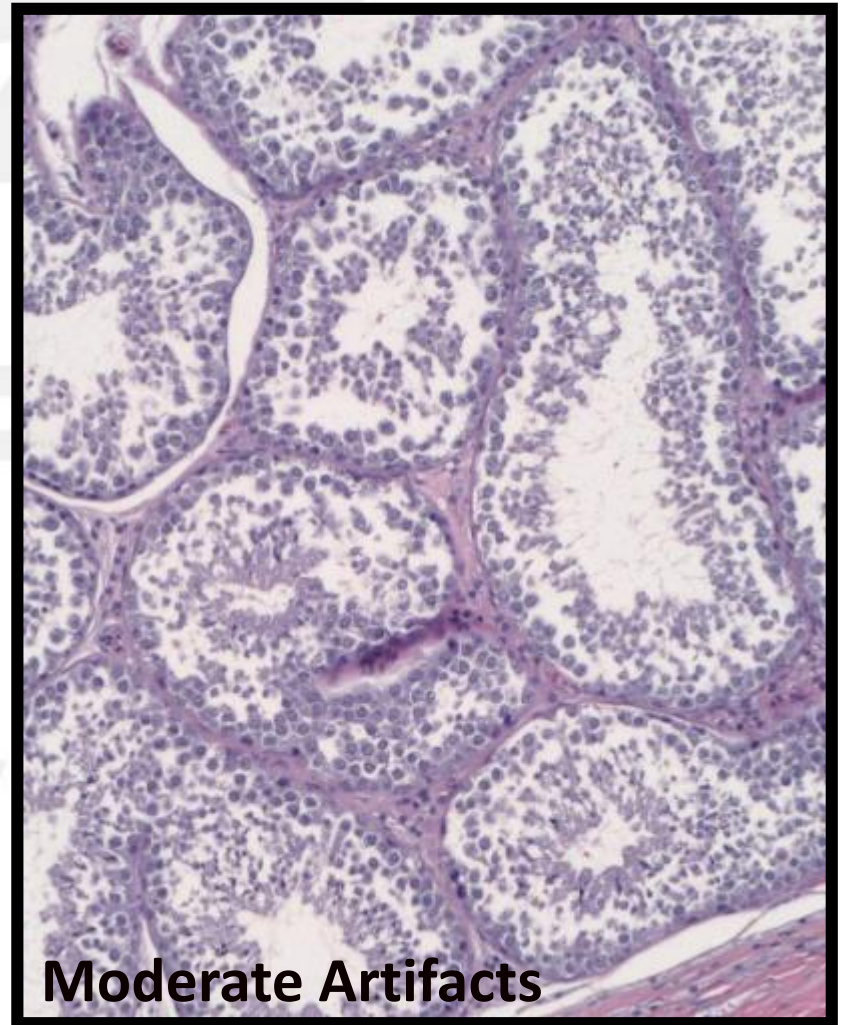
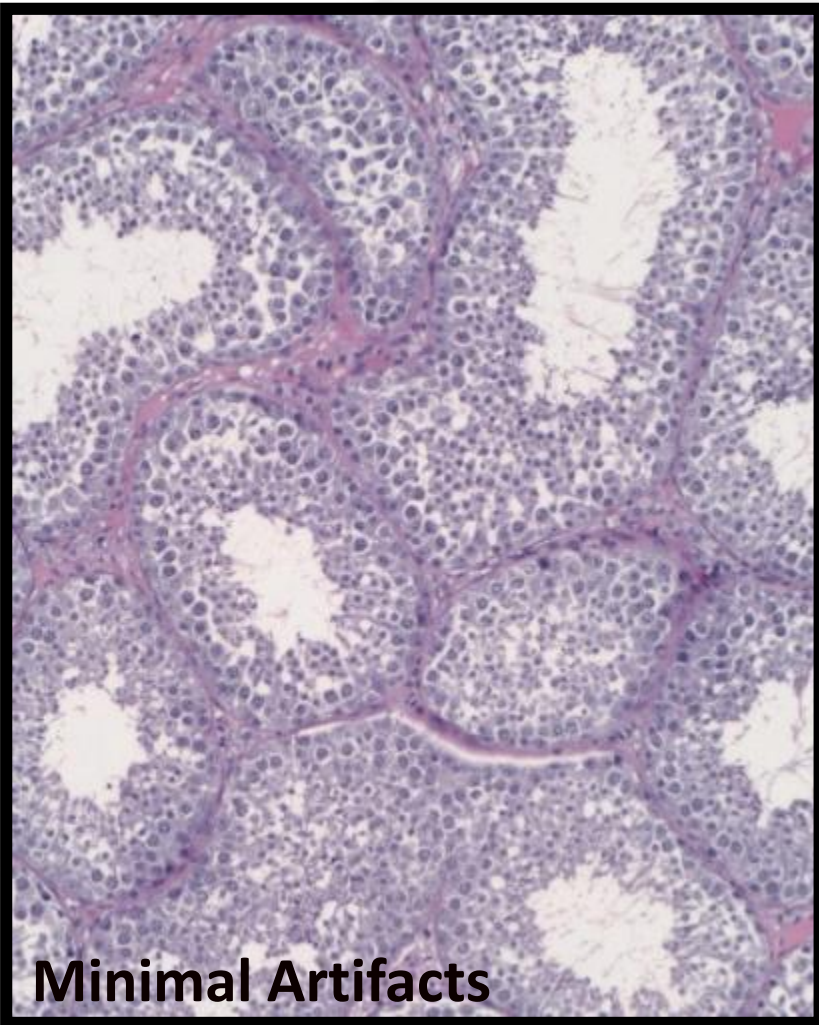
Formalin



Bouin

By J.Hardisty, EPL Inc

Fixation Issues



By J.Hardisty, EPL Inc

Autolysis

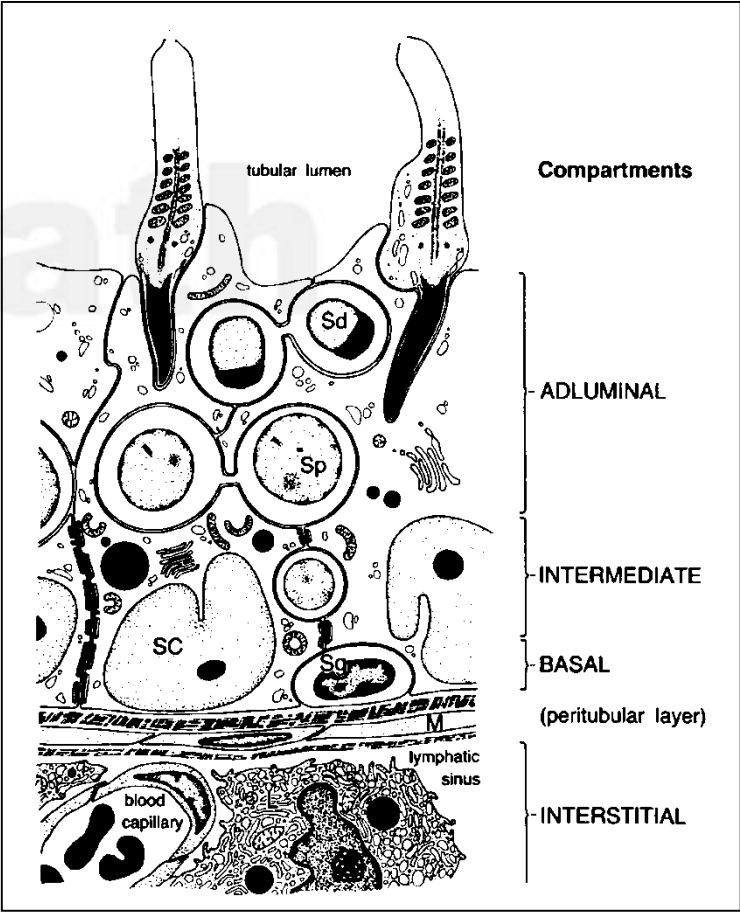
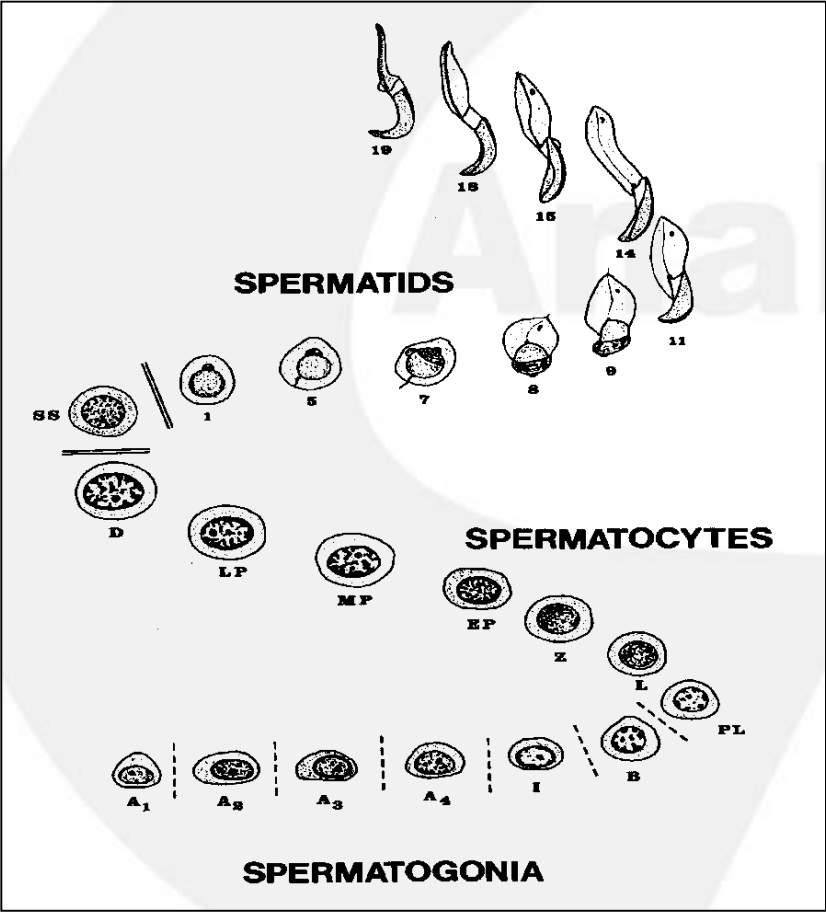
Bryant BH, Boekelheide K. Time-dependent changes in post-mortem testis histopathology in the rat. *Toxicol Pathol.* 2007, 35:665-671.

To **clarify** the contribution of spontaneous or autolytic post-mortem changes to testis histopathology...A **progressive decrease** in testis **weight** and **seminiferous tubule diameter** was observed, as well as **detachment of the seminiferous epithelium** from the basement membrane. As early as **12 hours postmortem**, there was observable **clumping and margination of chromatin** in Leydig cells, Sertoli cells, spermatogonia, spermatocytes, and step 7-10 spermatids; **extensive disintegration of Sertoli cells and residual bodies** by 24 hours postmortem; and **TUNEL positivity of Leydig cells** (by 36 hours postmortem) and **step 19 spermatids** (at 48 hours postmortem)....

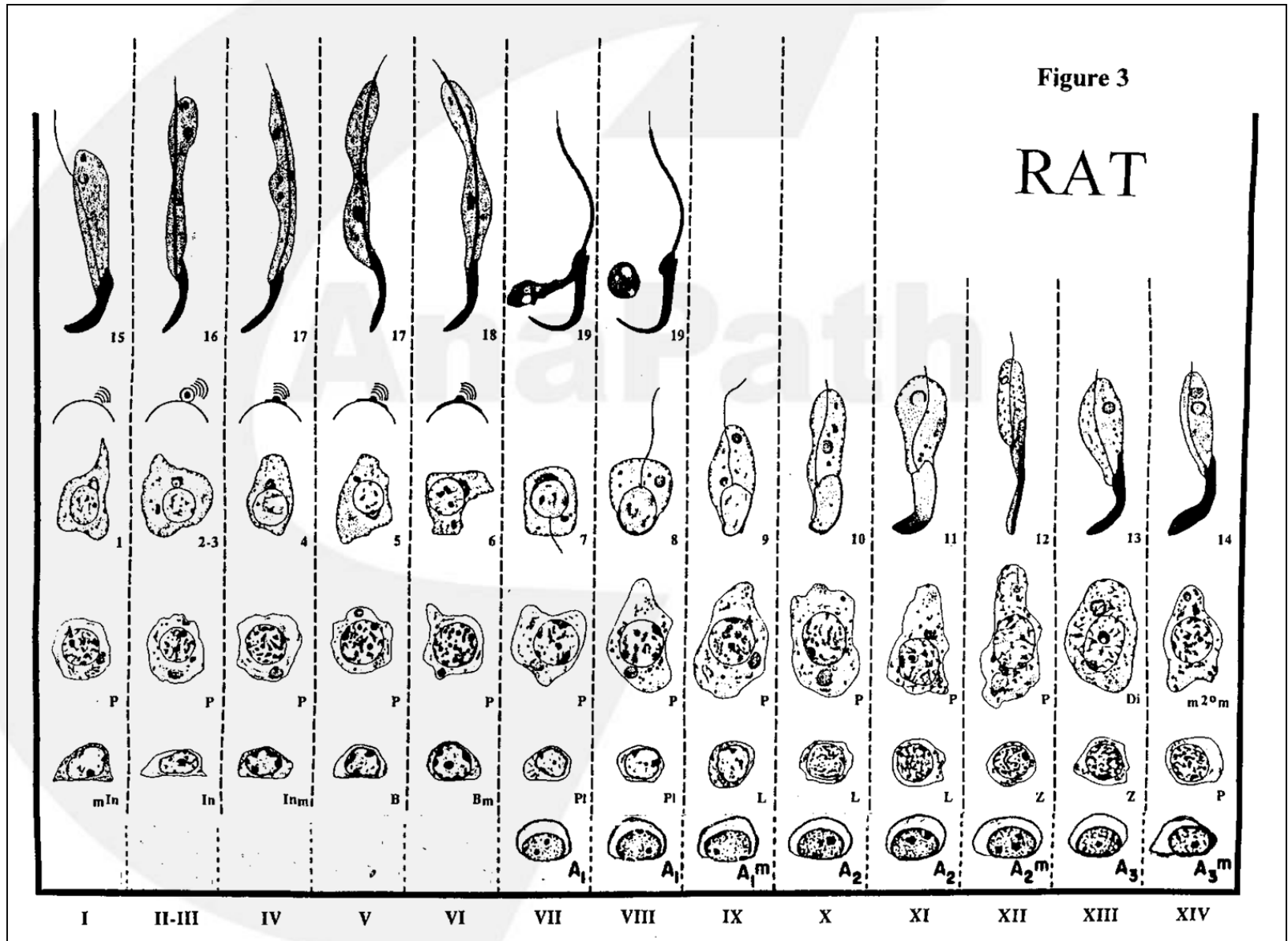
Sperm Development and Staging

Cycle: Developing Sperm

Stage:

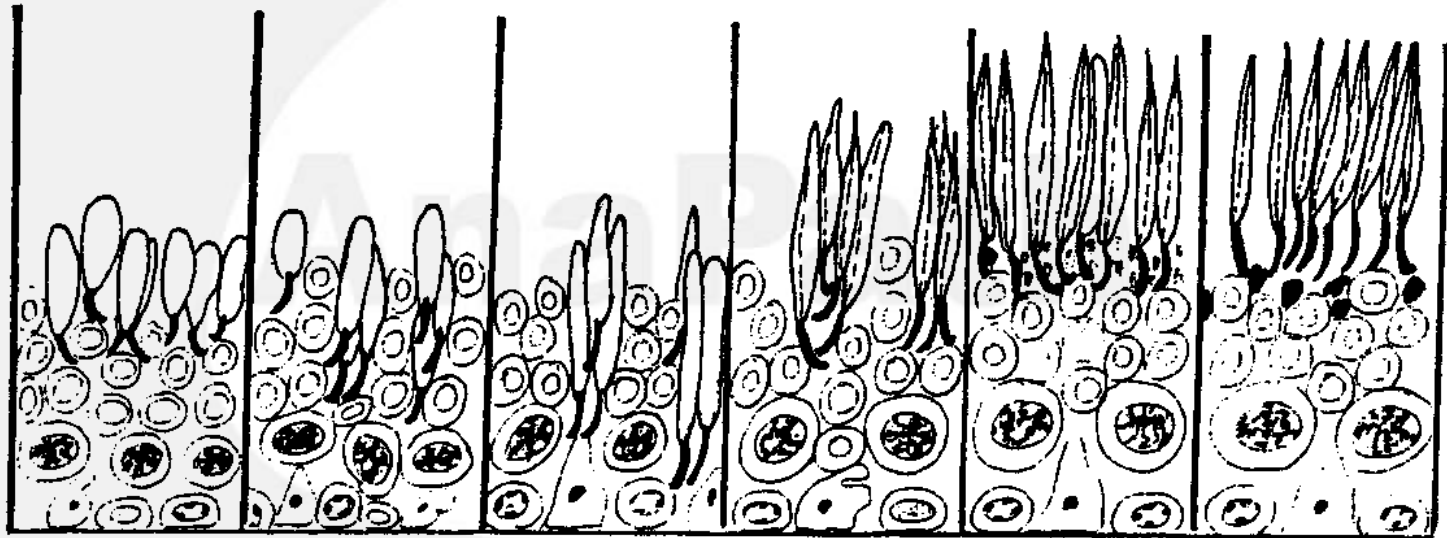


Stages: Example - Rats



Stages: Example - Rats

Depths of Spermatids During Cycle/Stages



Stage I	Stage II-III	Stage IV-V	Stage VI	Stage VII	Stage VIII
Sperm heads arranged in an irregular layer at lumen. Sperm tails short, fat and indistinct	Sperm heads start to descend in columns between round Sertoli cells. Sperm tails short, fat and indistinct	Sperm heads reach to the base of tubule and often make contact with Sertoli cell nucleus.	Sperm heads start to return to lumen. Tails thinner and longer than in stages II and III.	Sperm heads form an orderly layer at the lumen intermixed with discrete small cytoplasmic lobes. Tails long, thin and distinct. Often forming a whorl in the lumen	Sperm heads form an orderly layer at the lumen overlying large dense residual bodies which are starting to descend. Tails as in VII.

Staging

How to learn?

- ✓ Stage I-VII with elongated and round
- ✓ VIII-XIV no round spermatid
- ✓ VIII with spermia in lumen

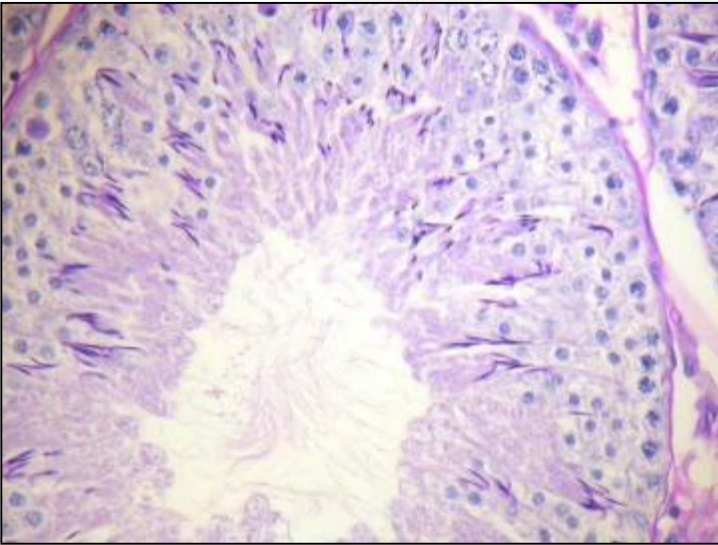
Qualitative staging:

- ✓ Cycle complete?
- ✓ All stages complete?
- ✓ Degeneration, necrosis, resorption, maturation arrest?

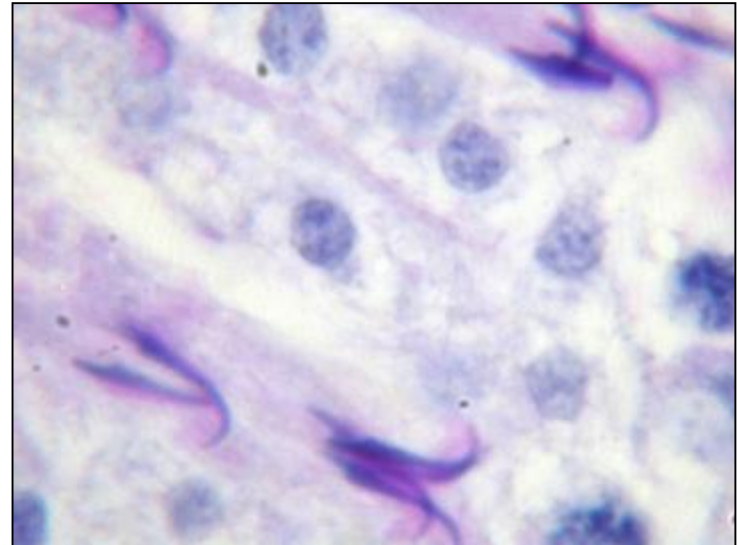
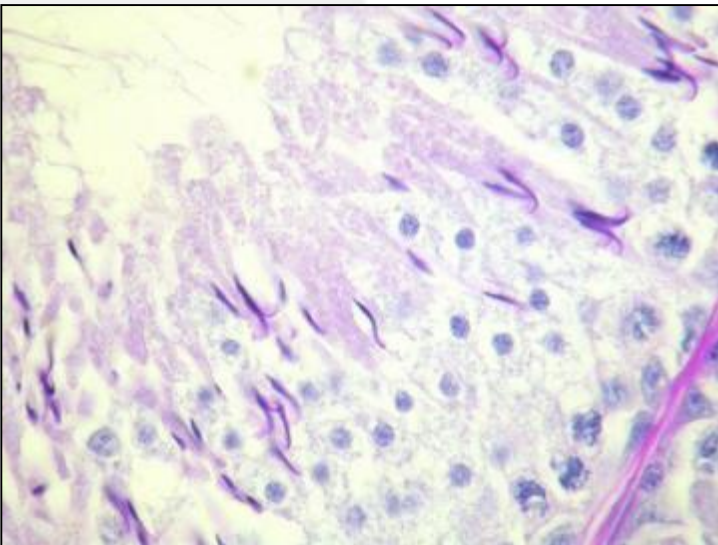
Quantitative staging:

- ✓ approx. 300-400 tubular sections per testes!
- ✓ for example counting stages II-III, V, VII, X
- ✓ **Is this necessary?**

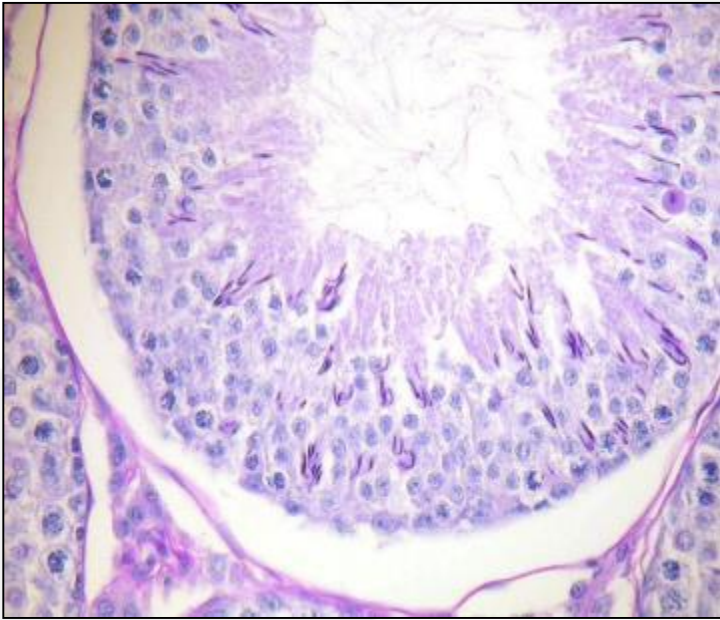
Stage I



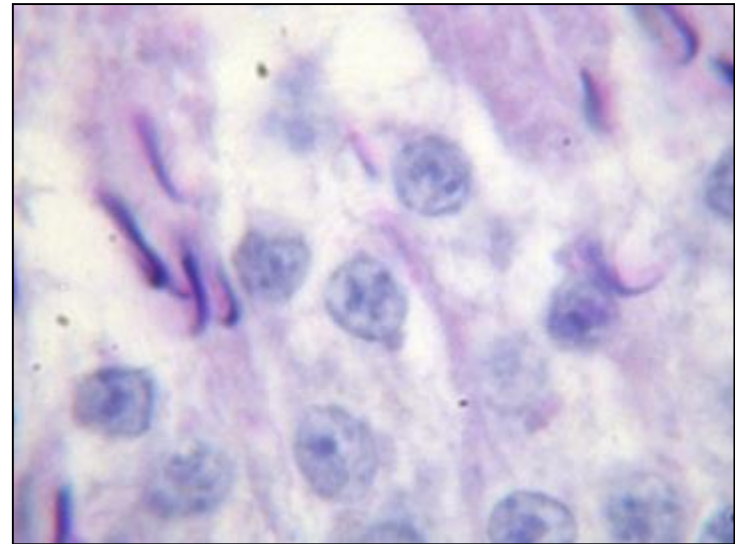
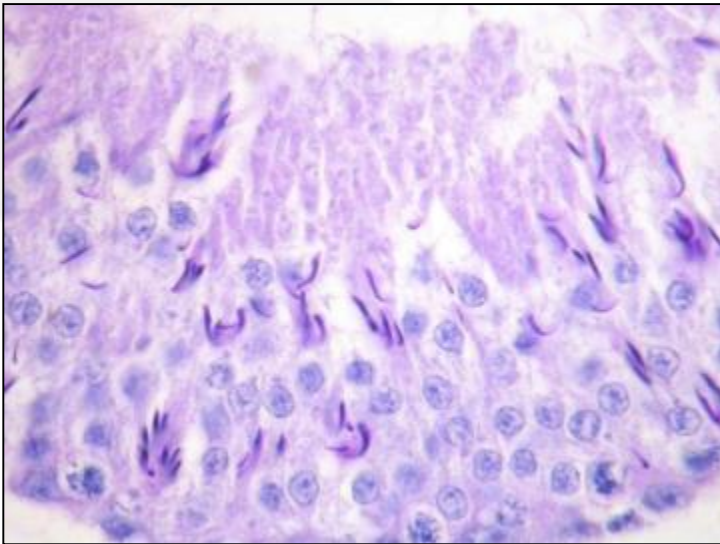
- ✓ Nucleus of elongated spermatids with well-formed hook
- ✓ Round nucleus of round spermatids
- ✓ No acrosome visible



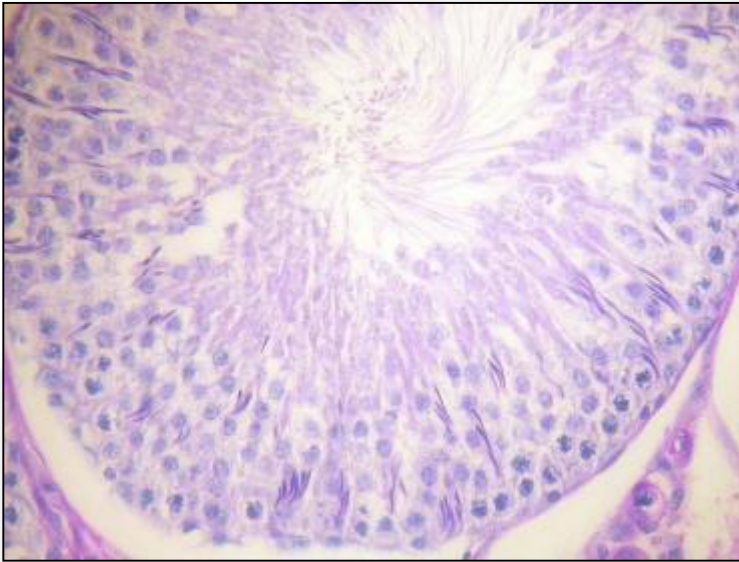
Stage II-III



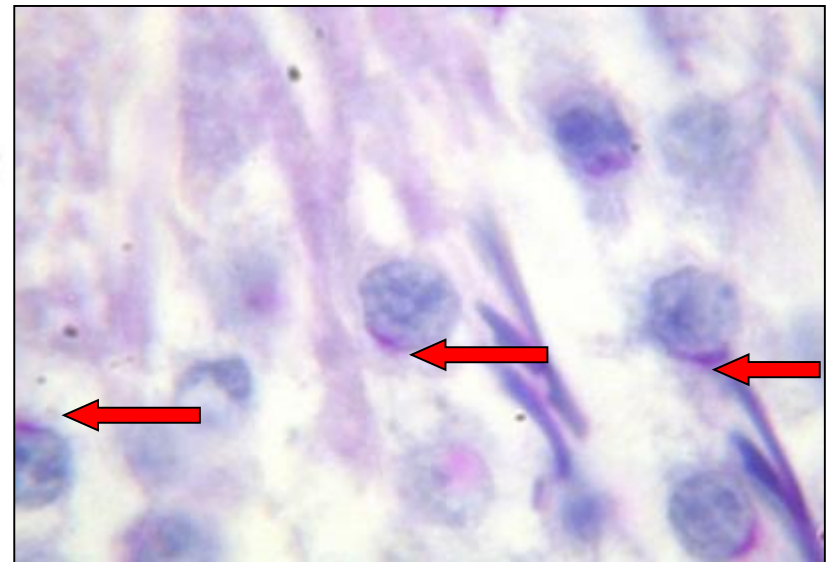
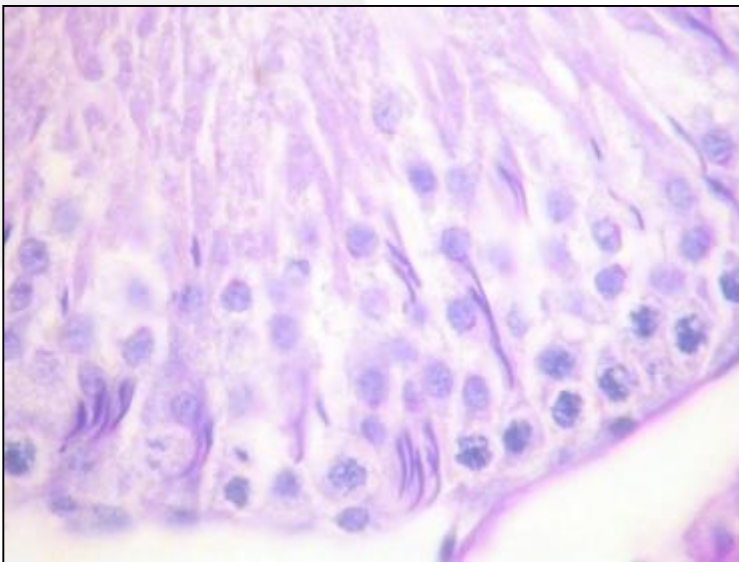
- ✓ Nucleus of elongated spermatids extending into deeper layers (between round spermatids)
- ✓ In round spermatids no clear acrosome but 1 or 2 small granula



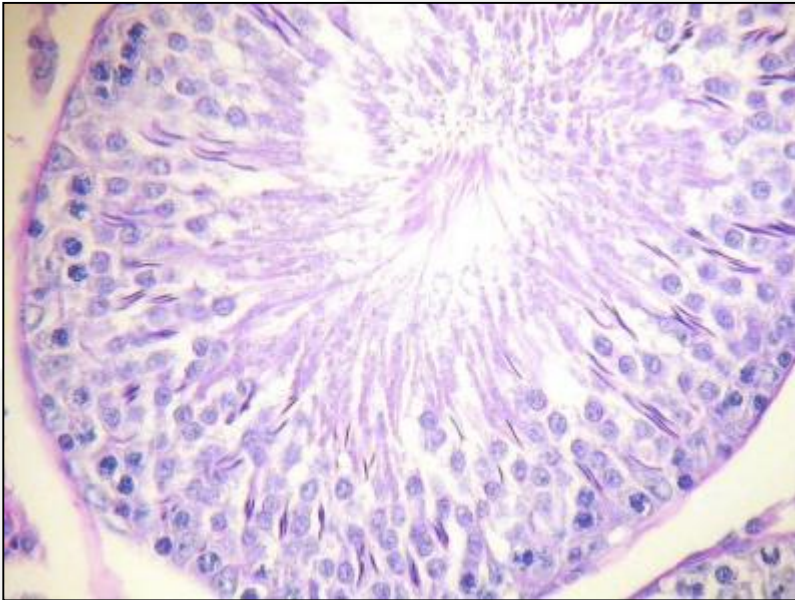
Stage IV



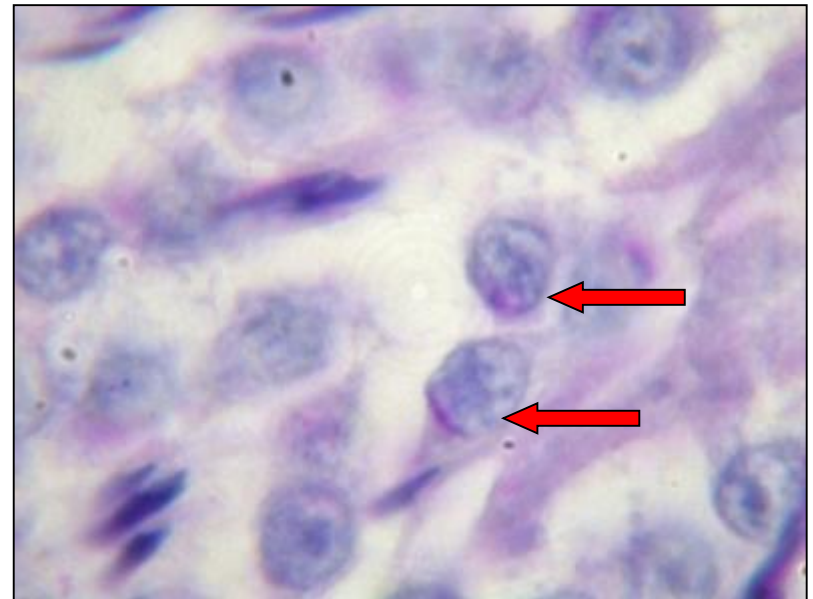
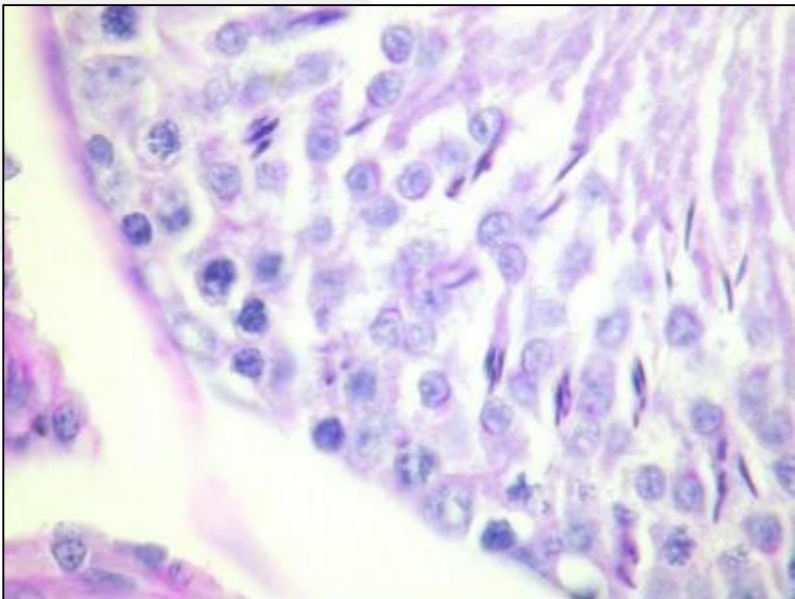
- ✓ Nucleus of elongated spermatids deep, near to basal lamina
- ✓ Acrosome on round spermatids covering approximately 30°



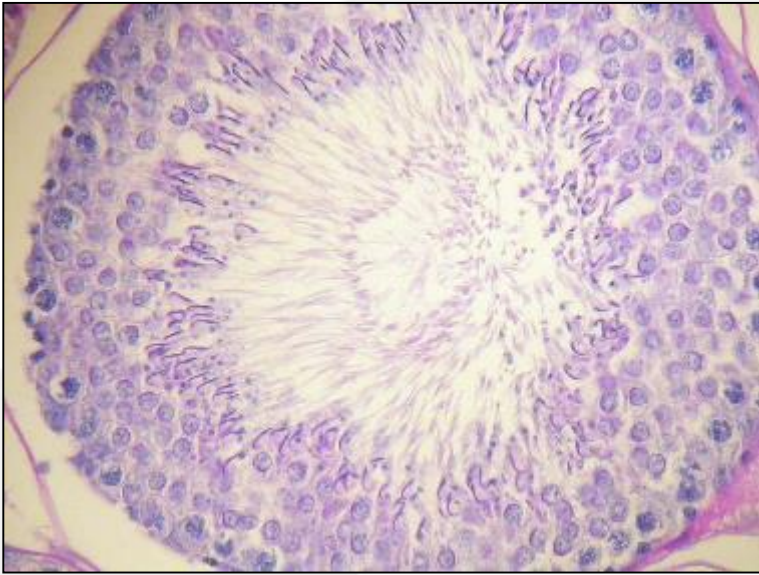
Stage V



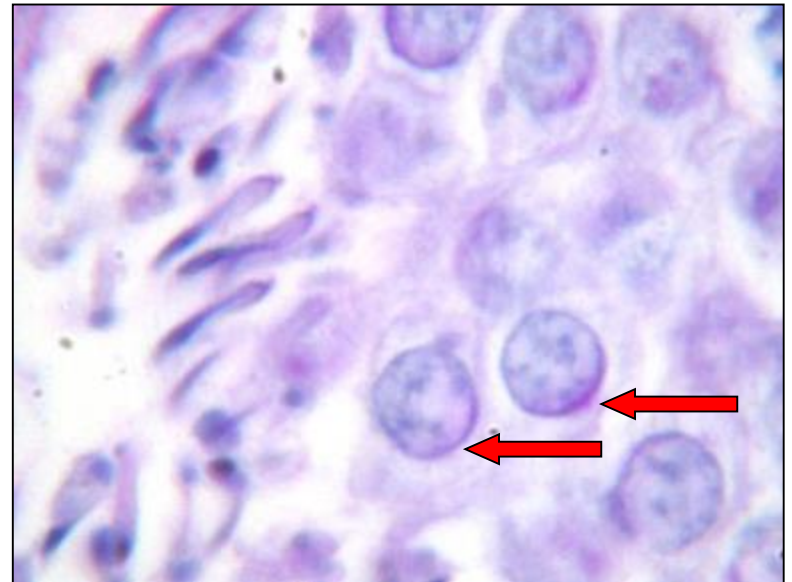
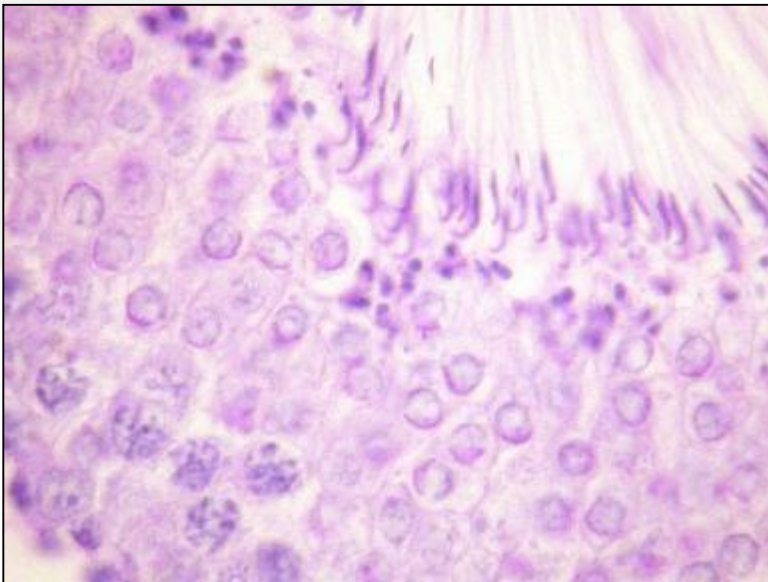
- ✓ Nucleus of elongated spermatids deep, but tending another one into luminal direction
- ✓ Acrosome on round spermatids covering approximately 45°



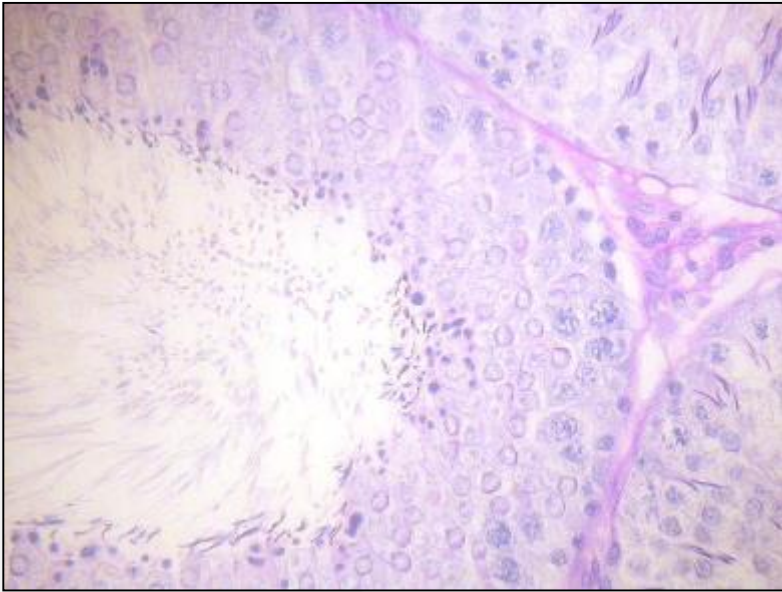
Stage VI



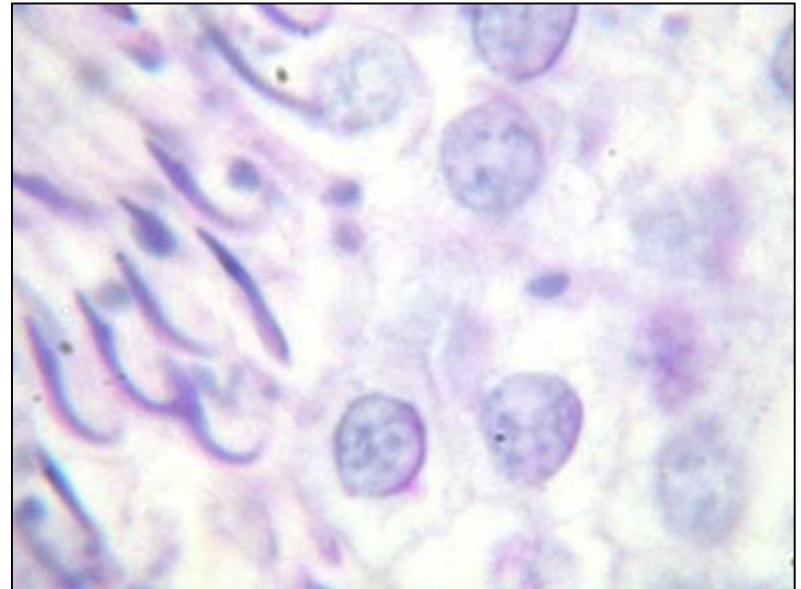
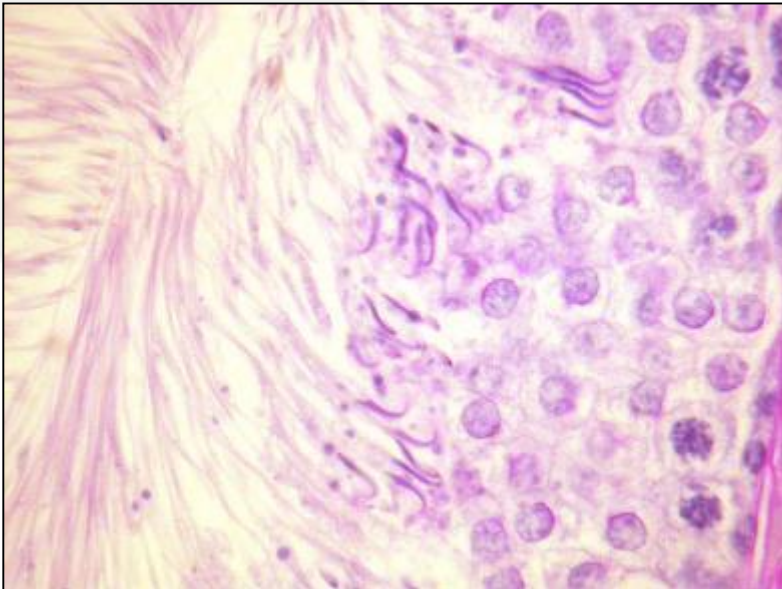
- ✓ Elongated spermatids on surface,
- ✓ Acrosome on round spermatids covering approximately 80°



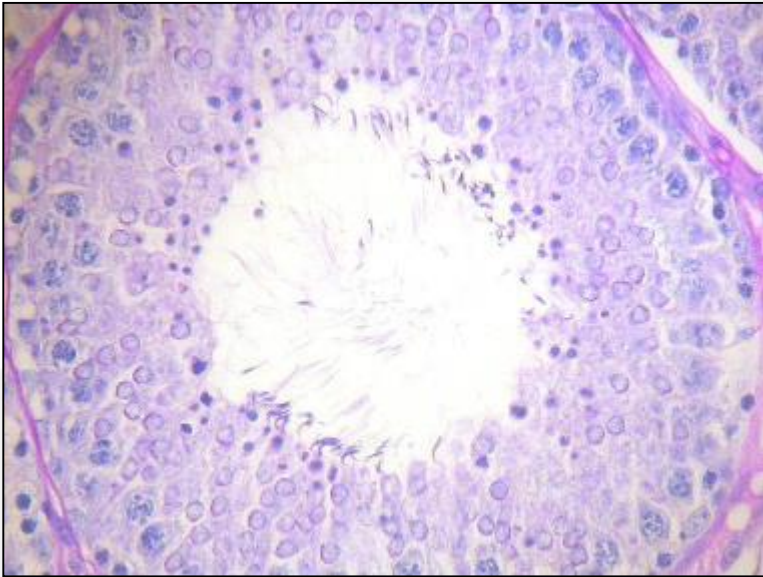
Stage VII



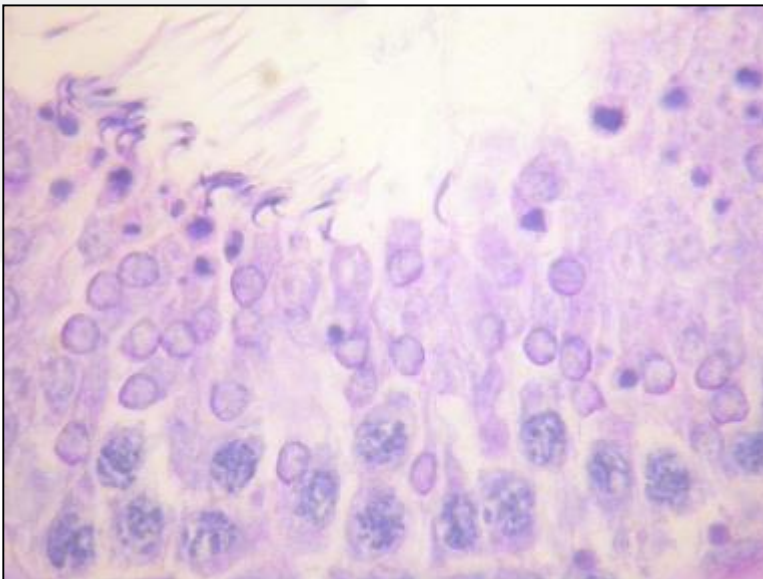
- Spermia on surface**
- ✓ **Residual bodies**
- ✓ **Acrosome/nucleus of round spermatids is round**



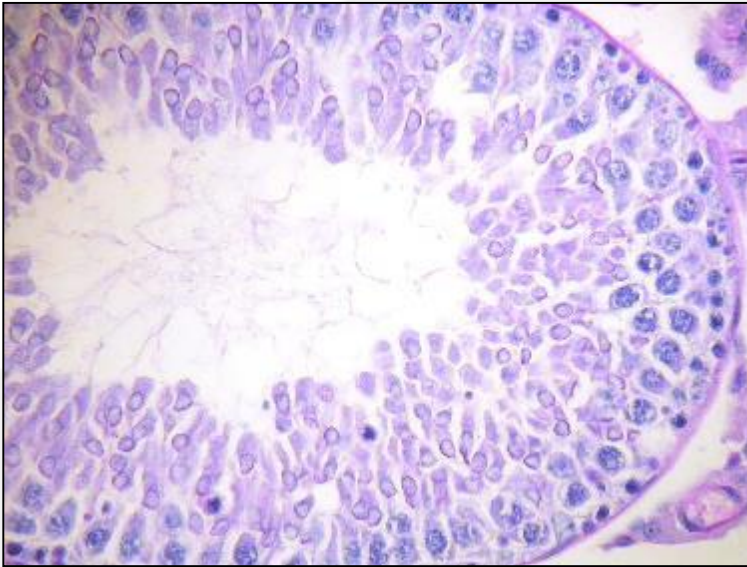
Stage VIII



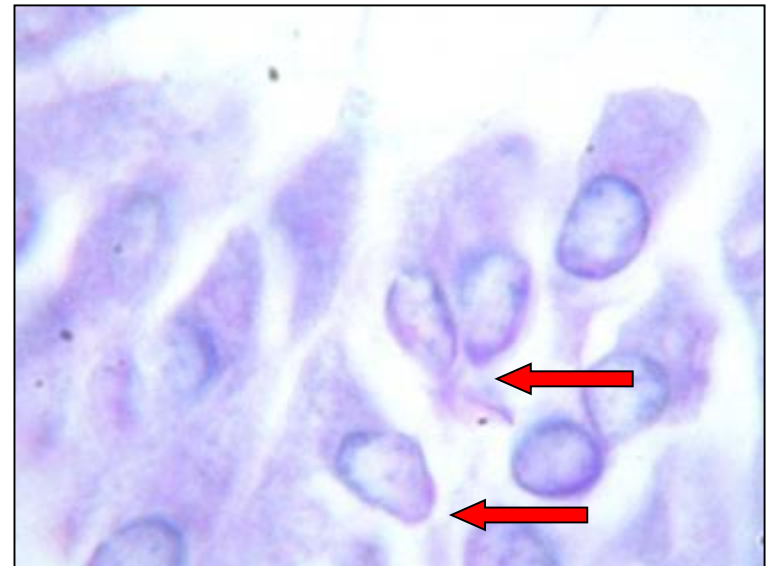
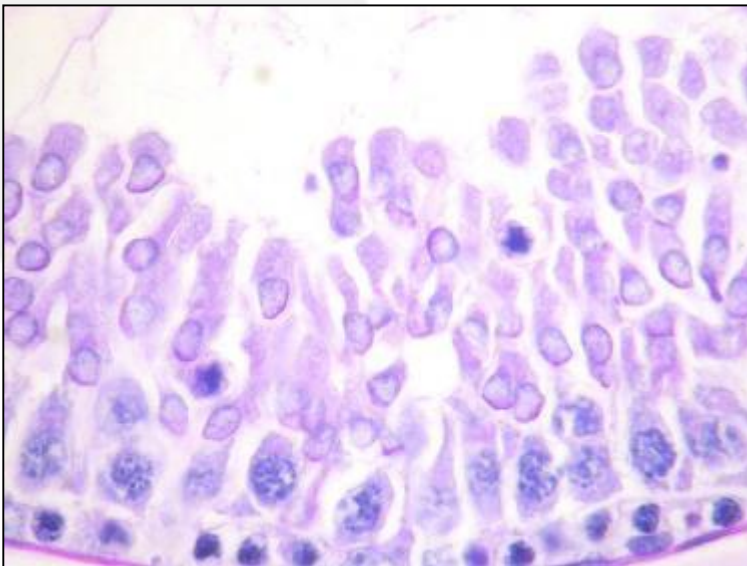
- ✓ Only a few spermia
- ✓ Residual bodies often resorbed
- ✓ Acrosome of elongated spermatids ,umbrella'-like on round nucleus



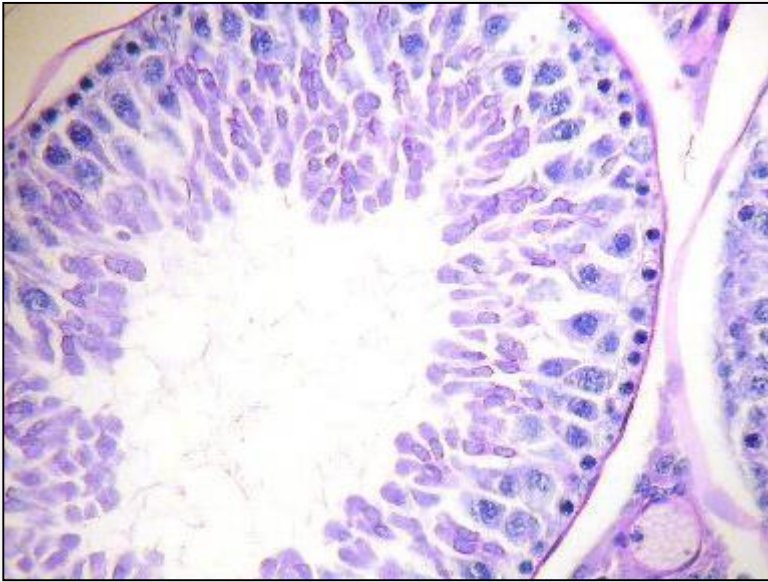
Stage IX



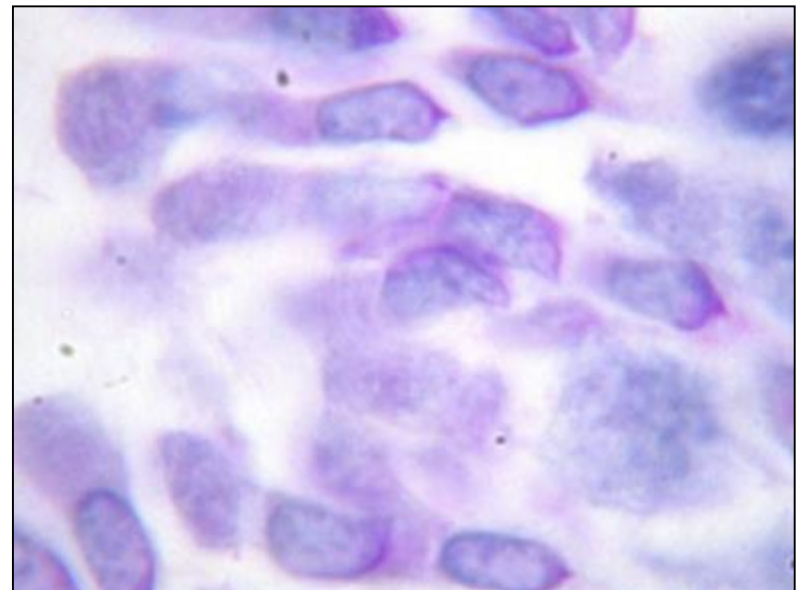
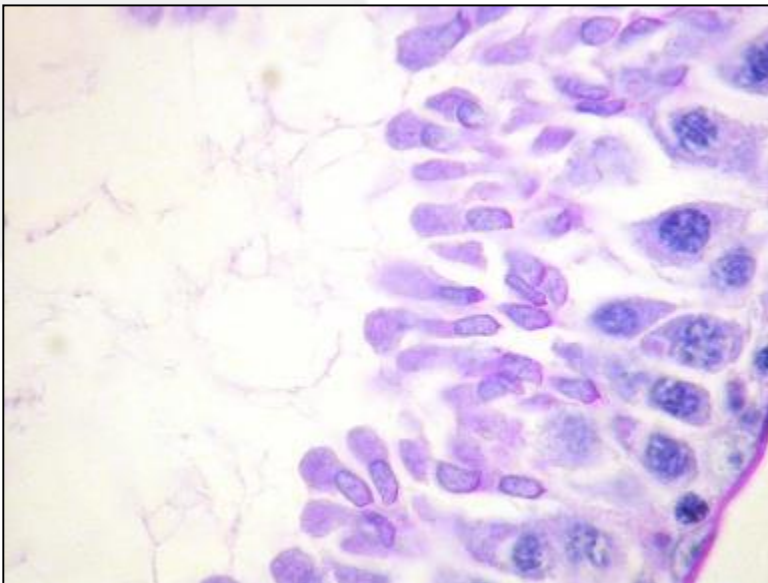
- ✓ No spermatids
- ✓ Nucleus of elongated spermatids tend to form elipsoids
- ✓ Tail is forming



Stage X



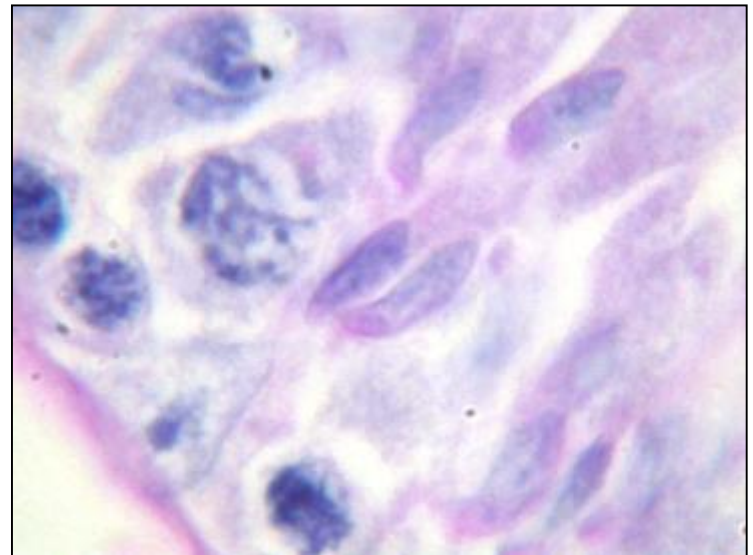
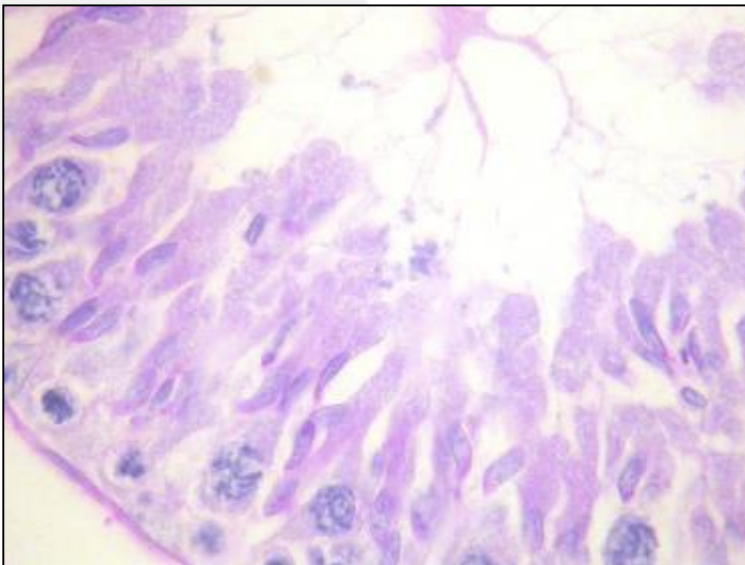
- ✓ Nucleus of elongated spermatids tends to form an angle
- ✓ Condensation of karyoplasma



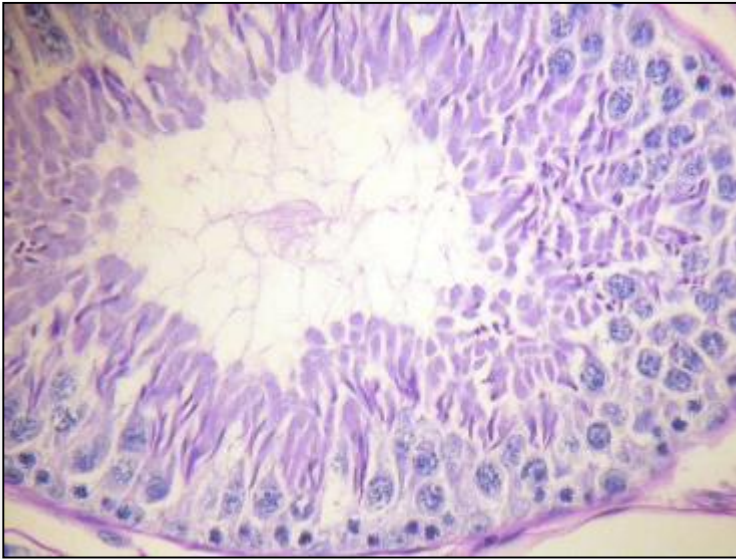
Stage XI



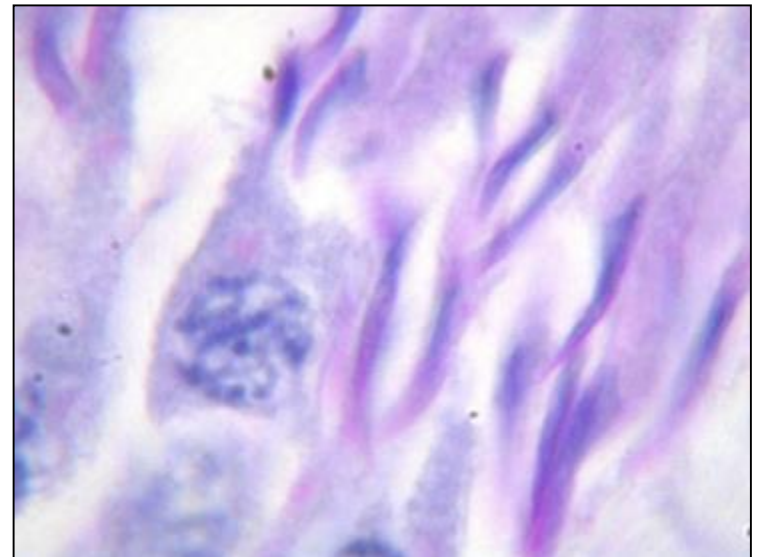
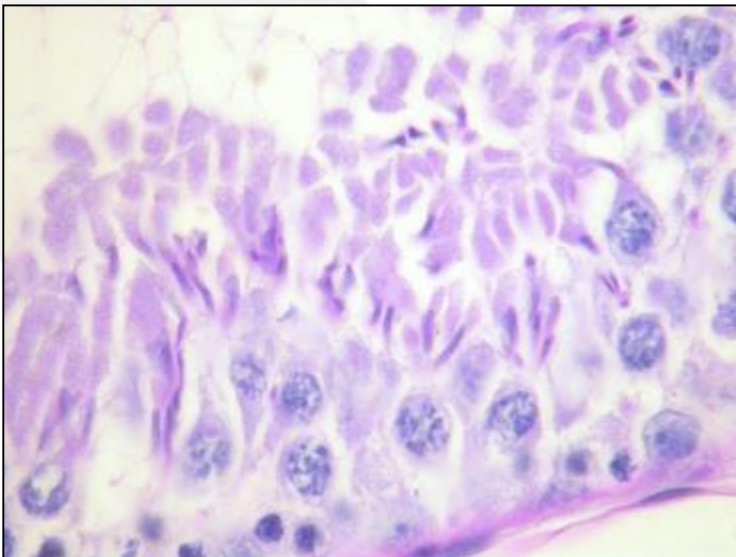
- ✓ Nucleus of elongated spermatids tend to become rectangular with pronounced condensation
- ✓ Large round p-spermatocytes



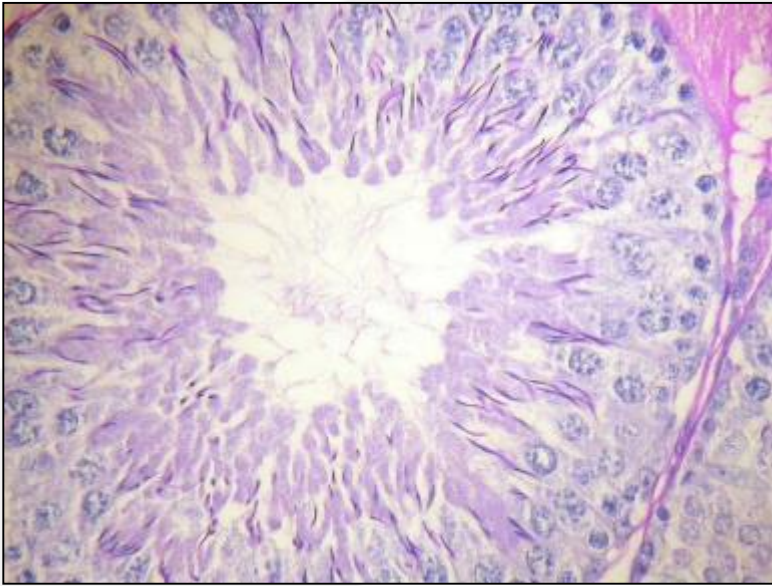
Stage XII



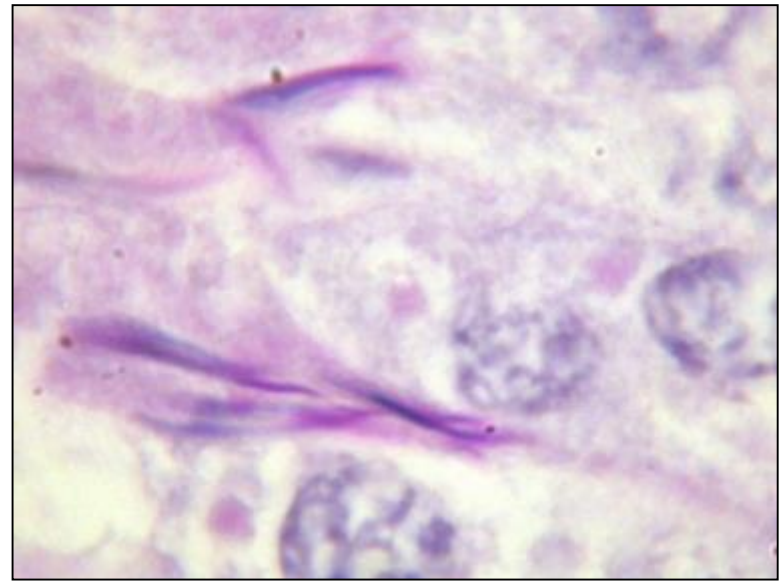
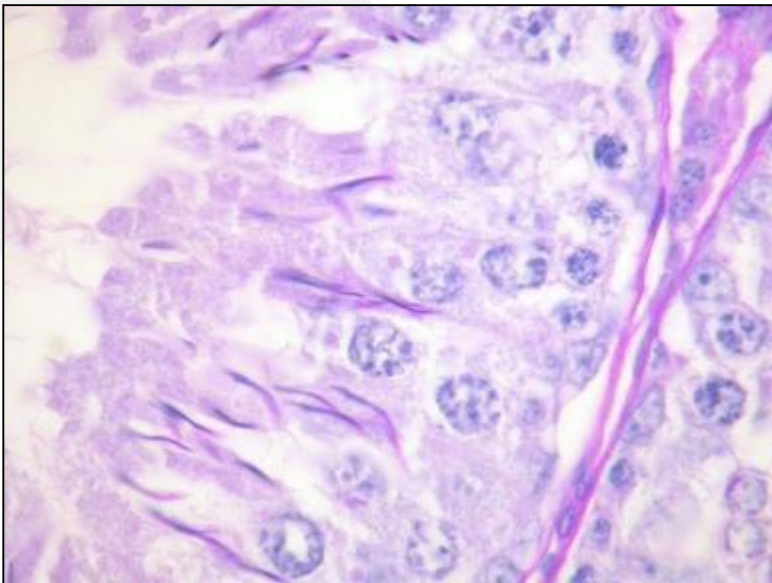
- ✓ Nucleus of elongated spermatids elongated and more condensed
- ✓ Acrosome vesicle large in round and large p-spermatocytes



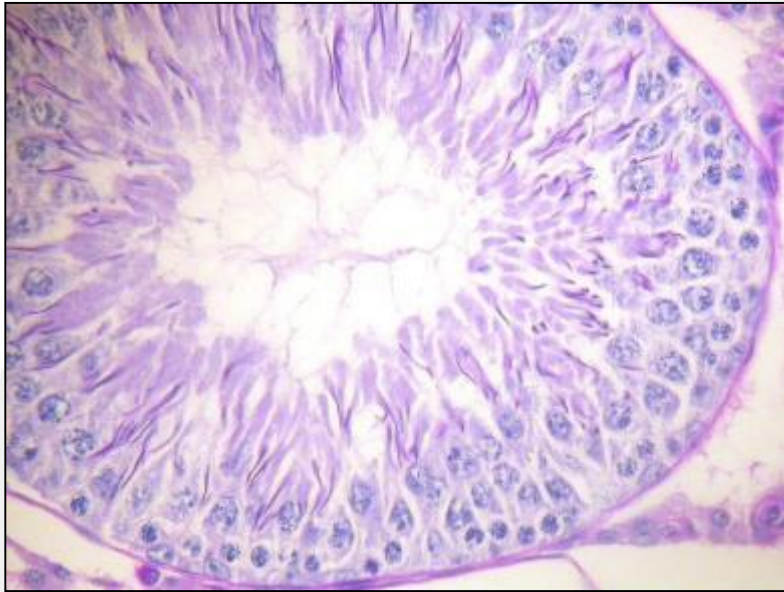
Stage XIII



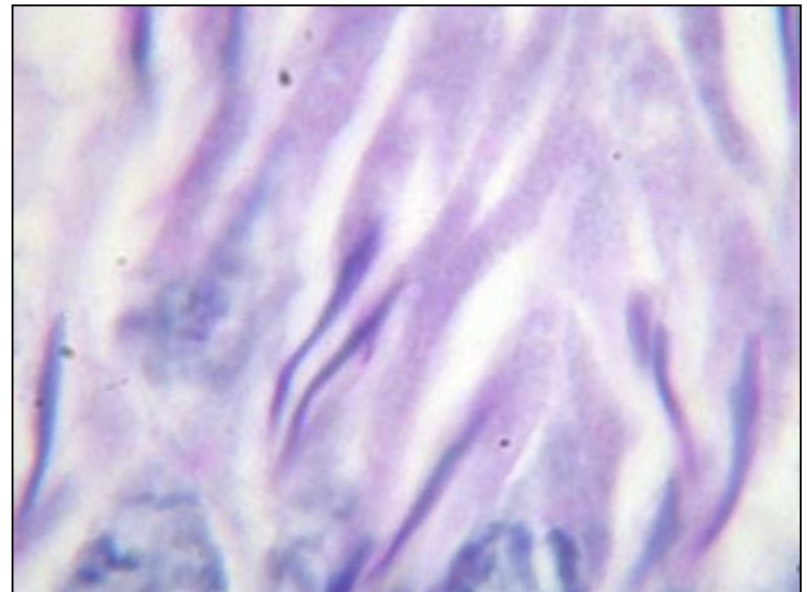
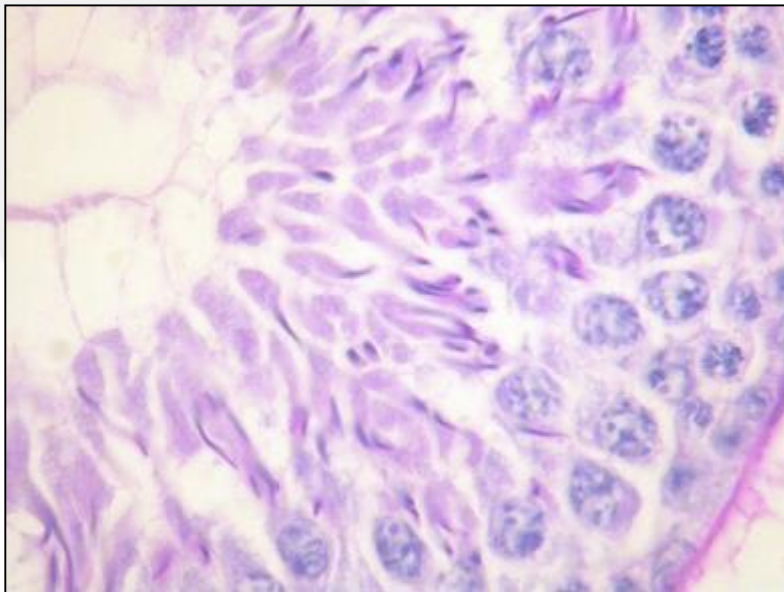
- ✓ Nucleus of elongated spermatids is forming hook-like head
- ✓ Acrosome vesicle in large round Di-spermatocytes



Stage XIV

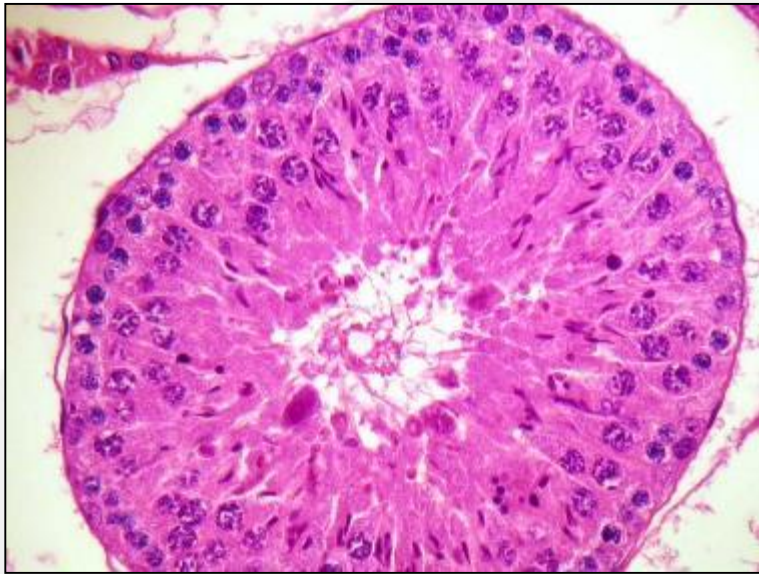


Nucleus of elongated spermatids completely condensed



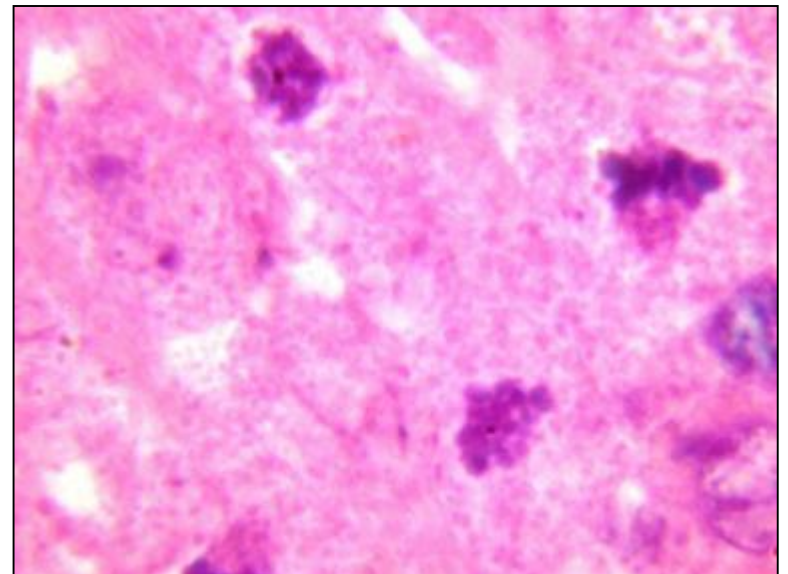
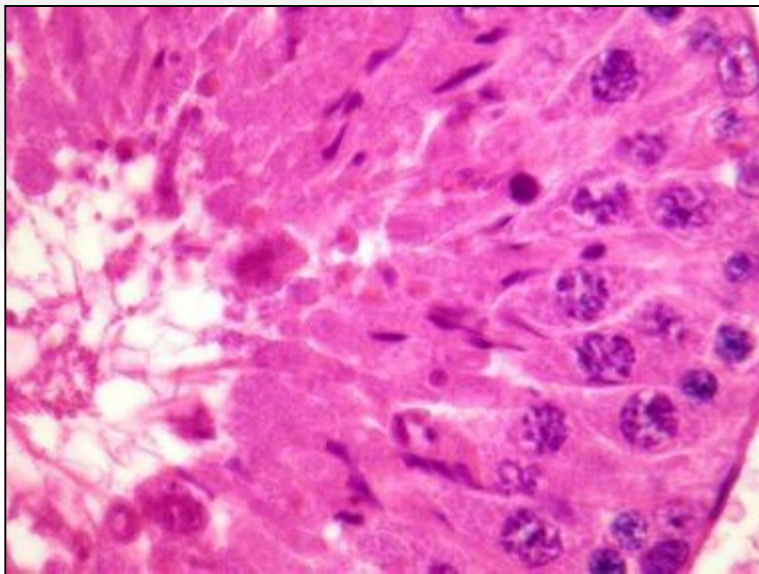
Path

Staging: How to use?

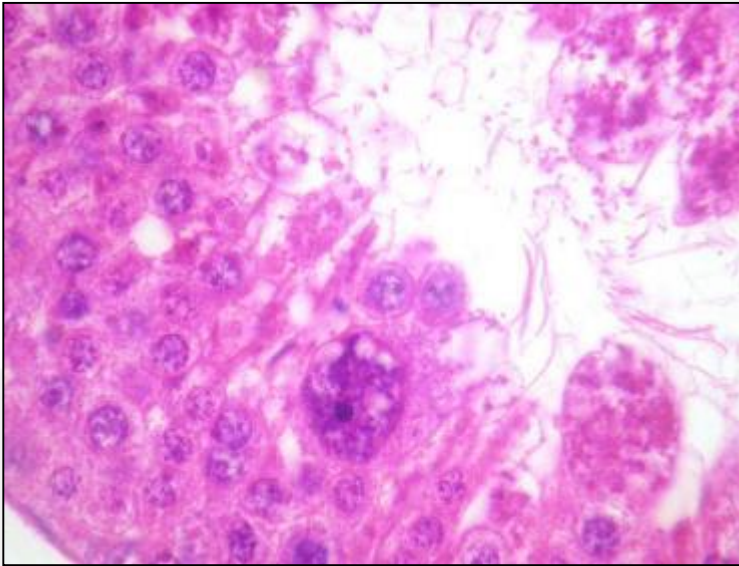


**A case:
28-Day Study
Terminal sacrifice**

Necrosis!

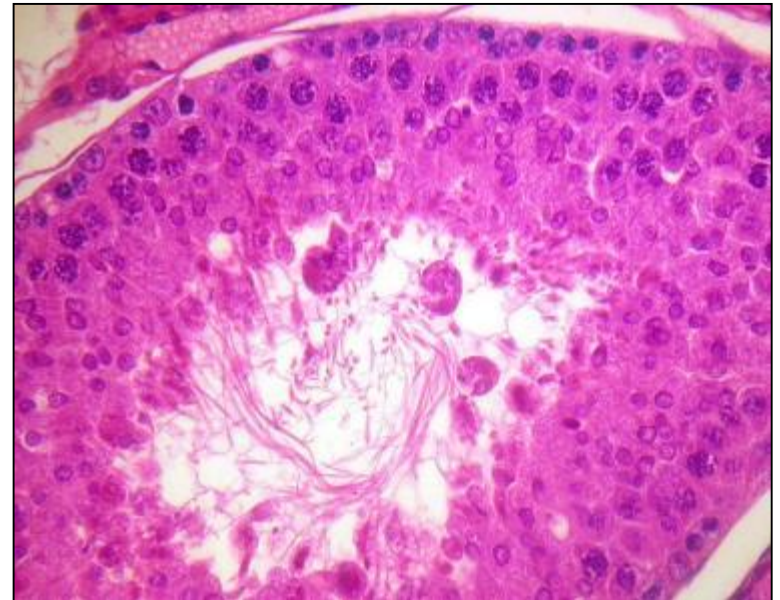
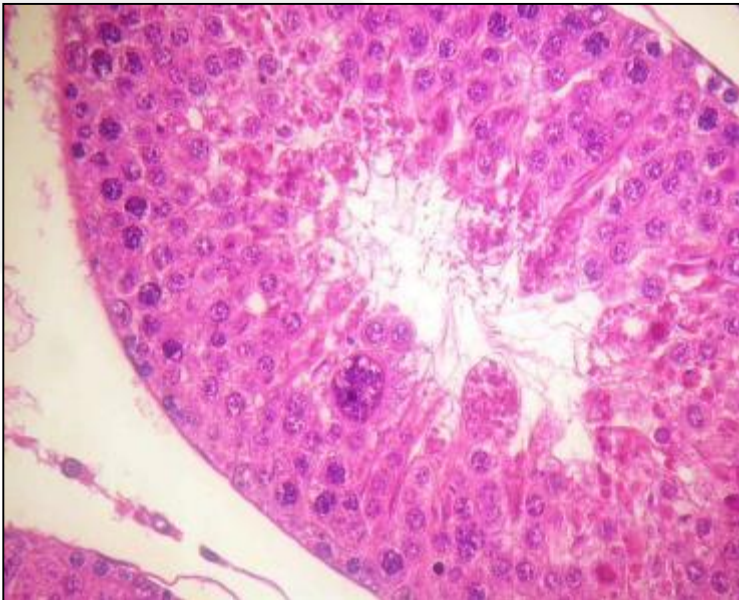


Staging: How to use?

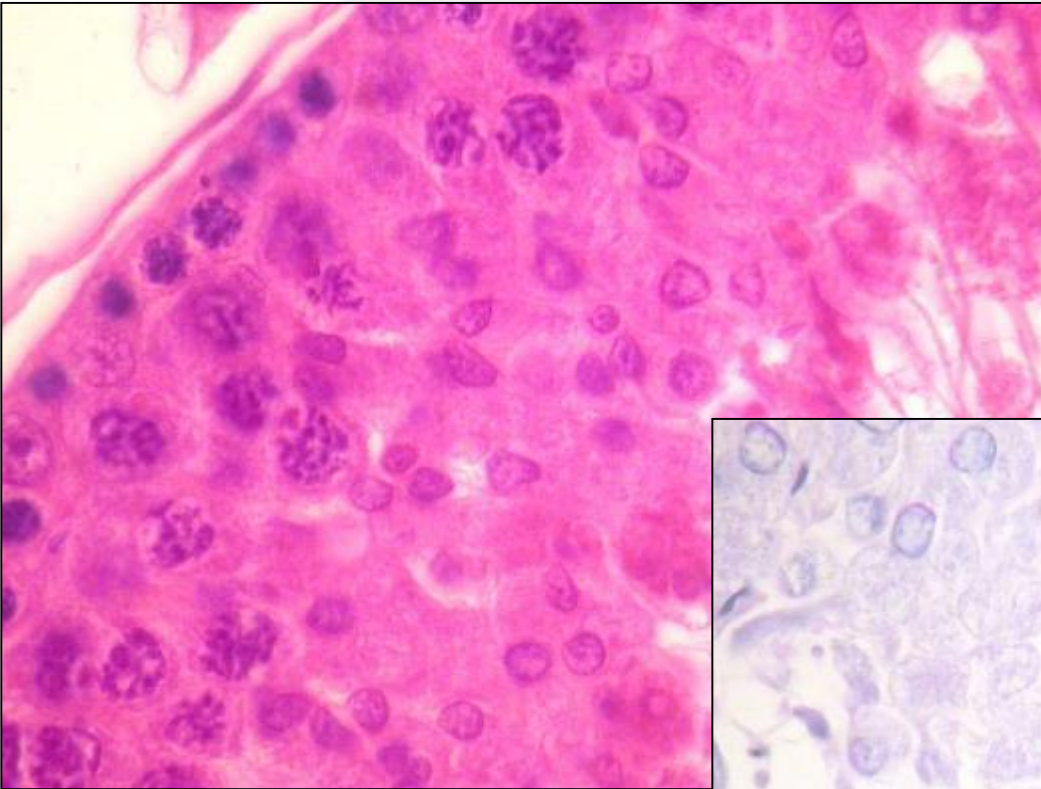


Necrosis and formation of giant cells!

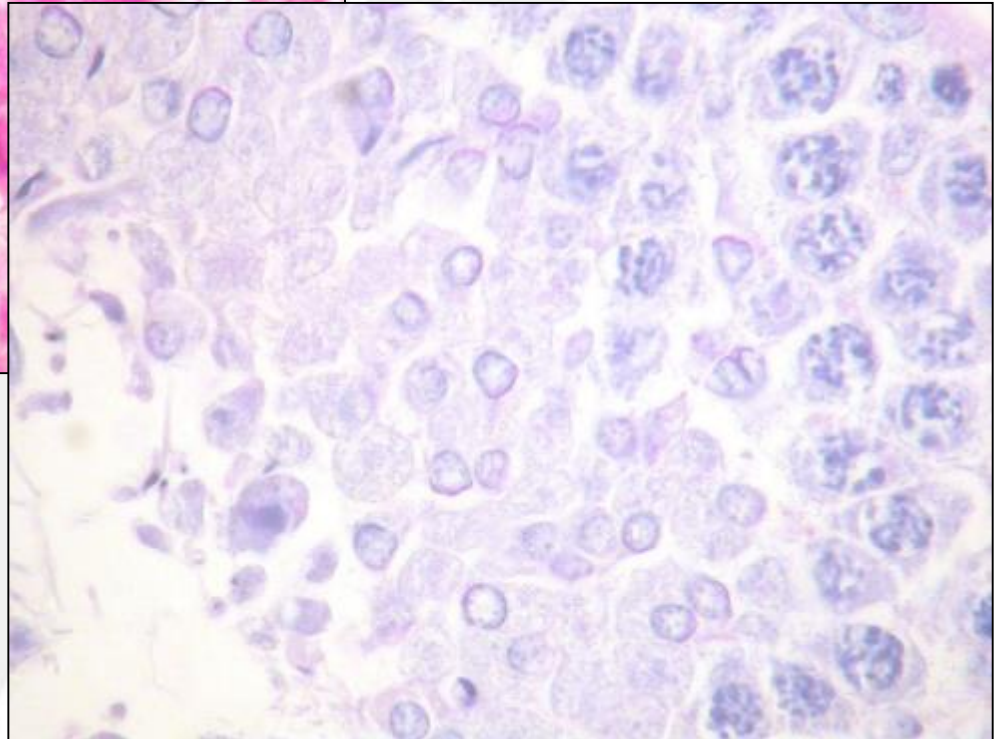
Which population???



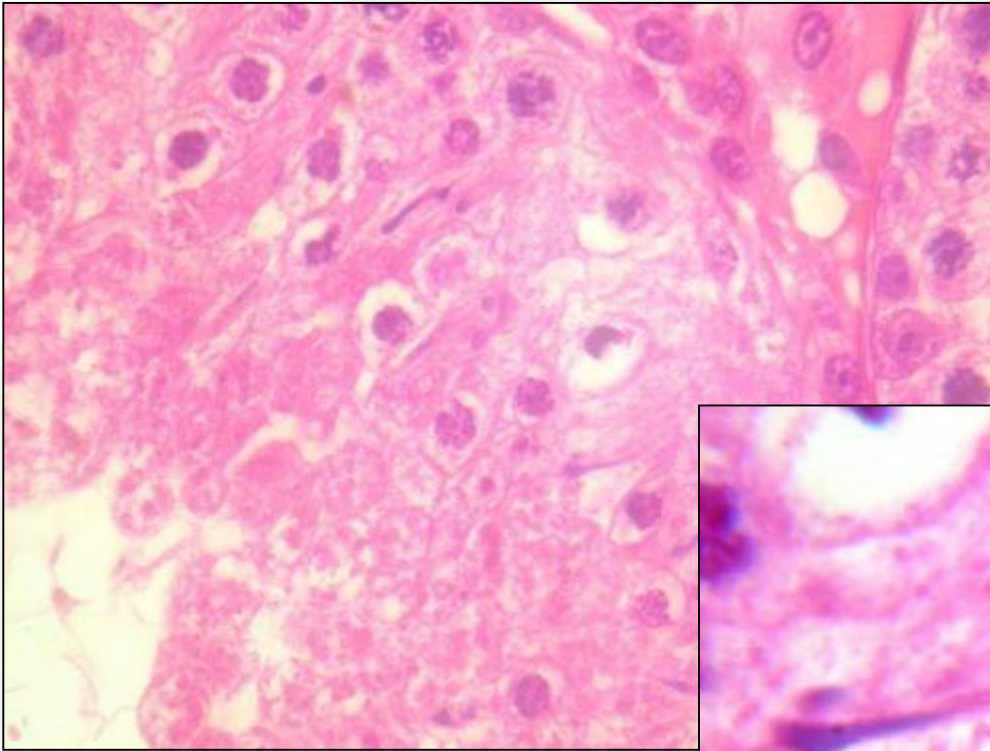
Staging: How to use?



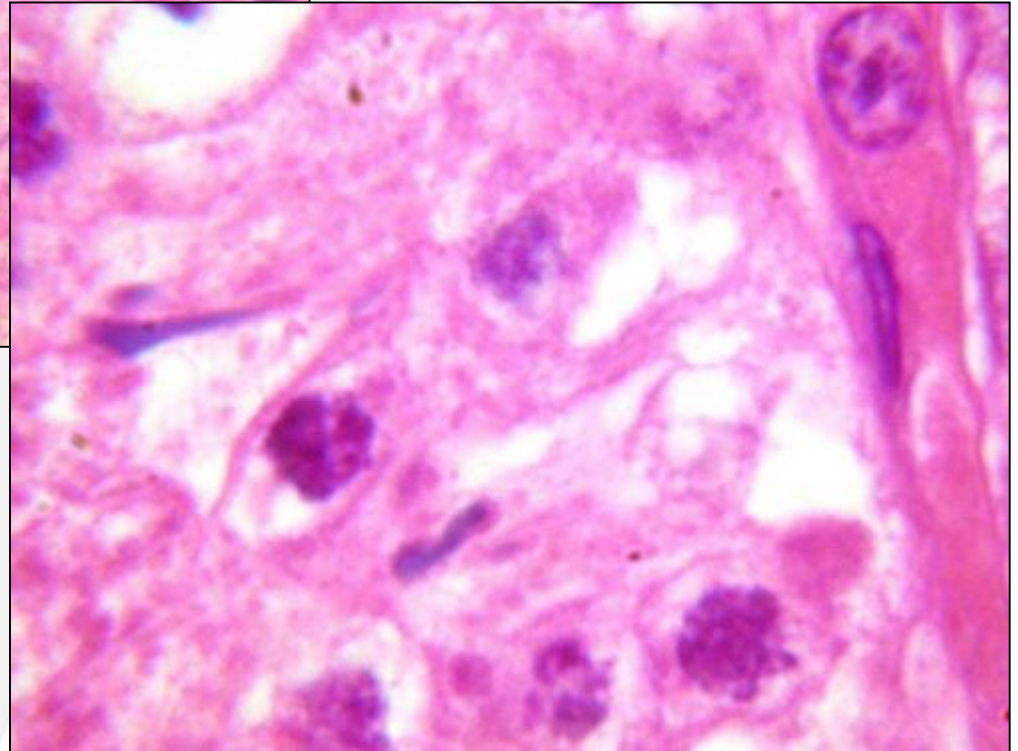
**Elongated Spermatids
in stage VII and VIII
with resorption!**



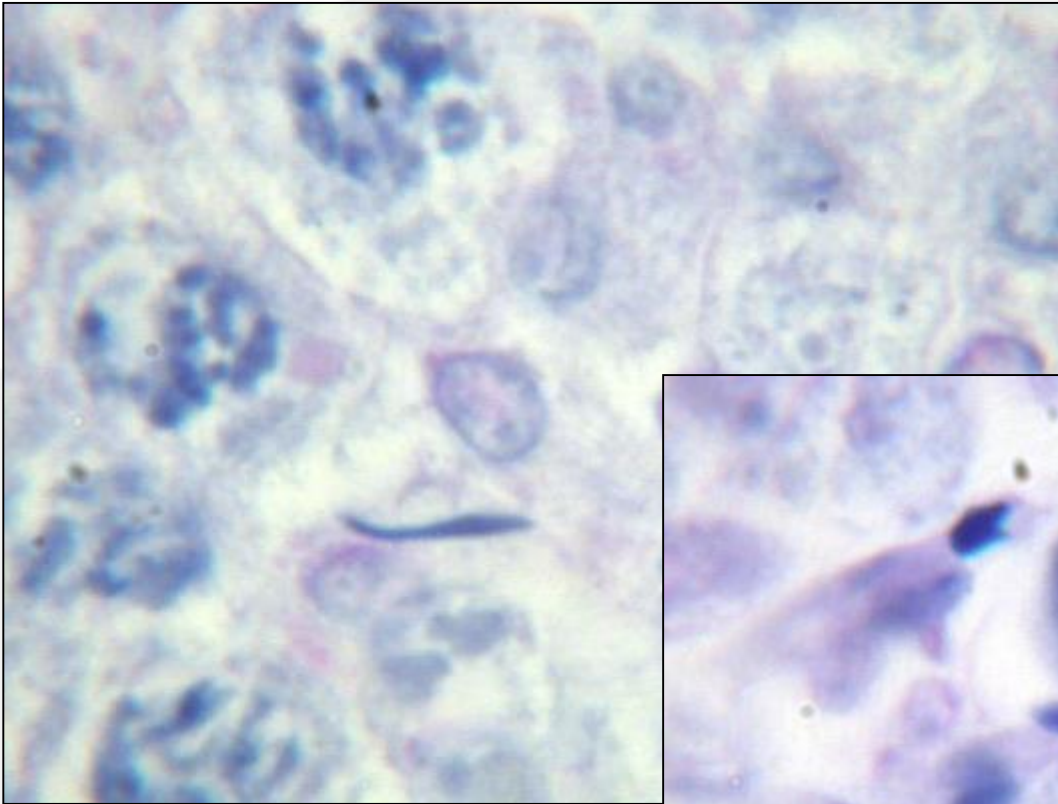
Staging: How to use?



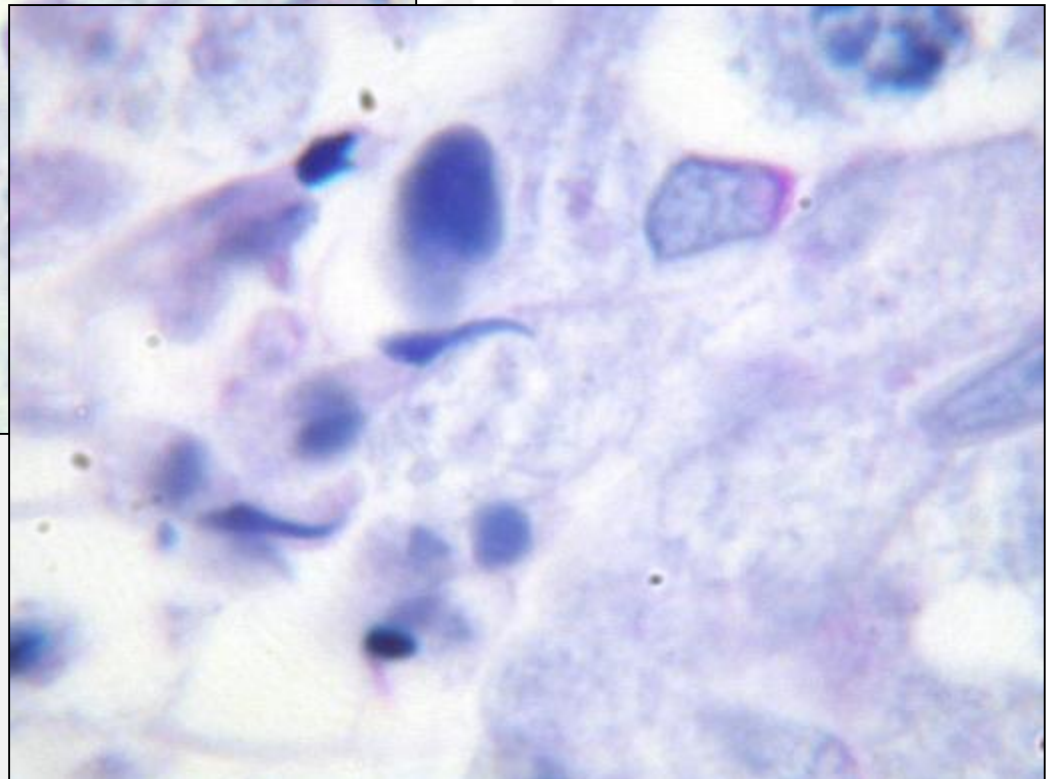
**Also necrosis in
round spermatids!
Stage?
Maturation arrest?**



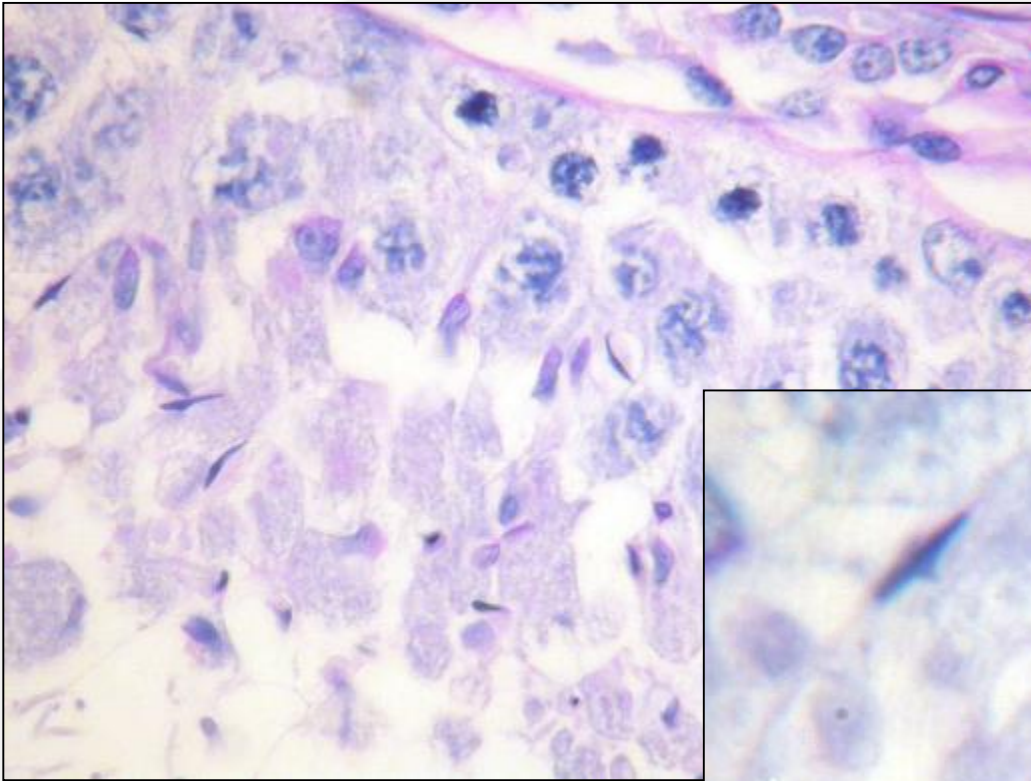
Staging: How to use?



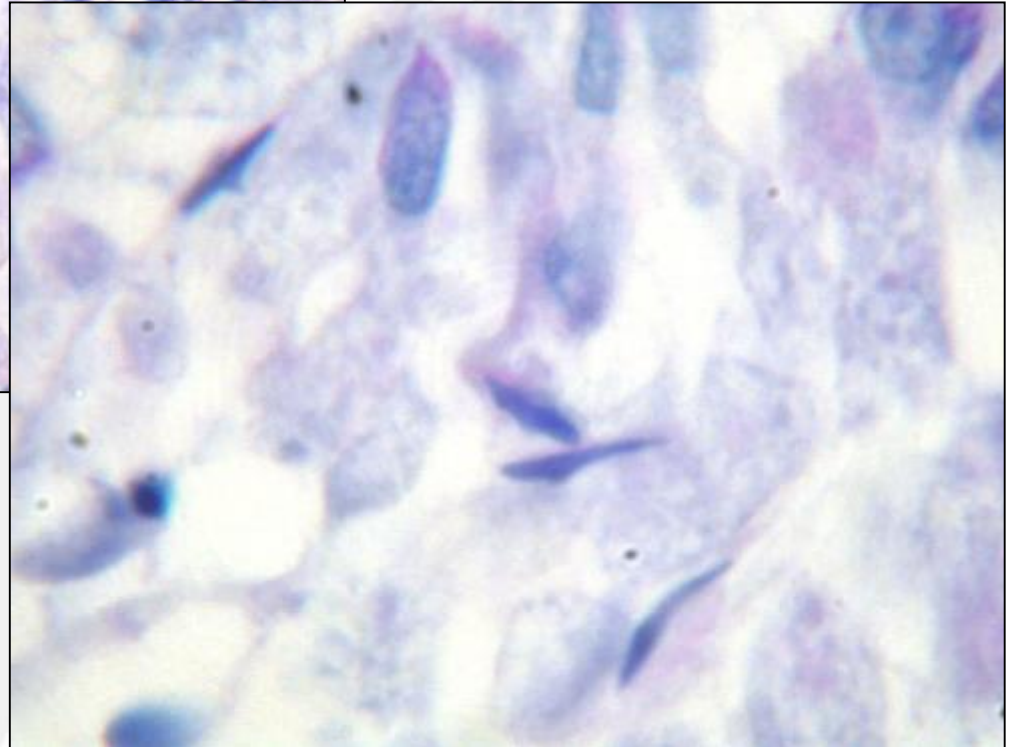
**Maturation arrest
in stage IX!**



Staging: How to use?



**Maturation arrest
in stage X!**



Other : Staging

Dog:

Acrosome ruptures during fixation

Cynomolgous:

In most toxicity studies immature

Human:

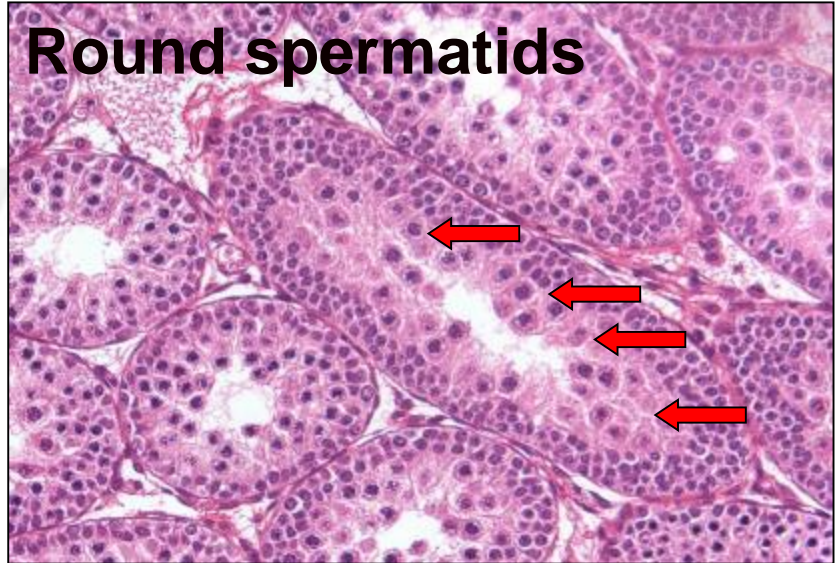
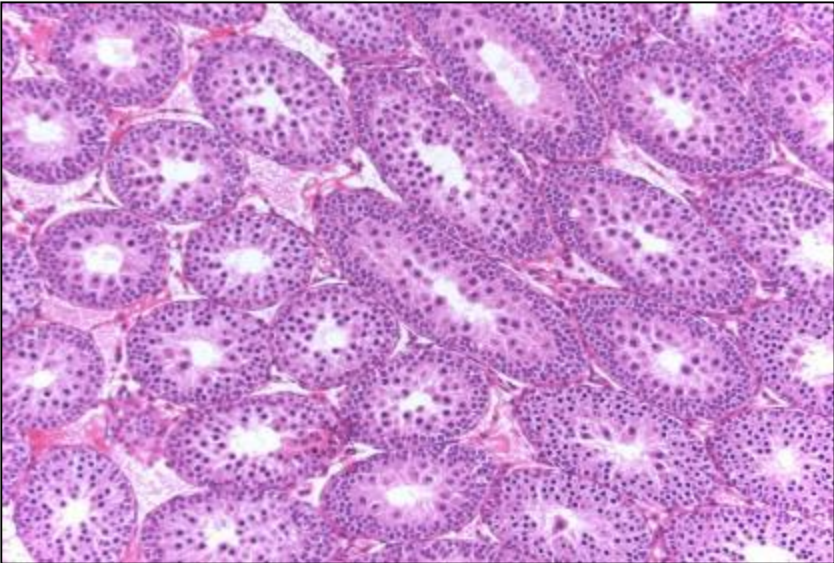
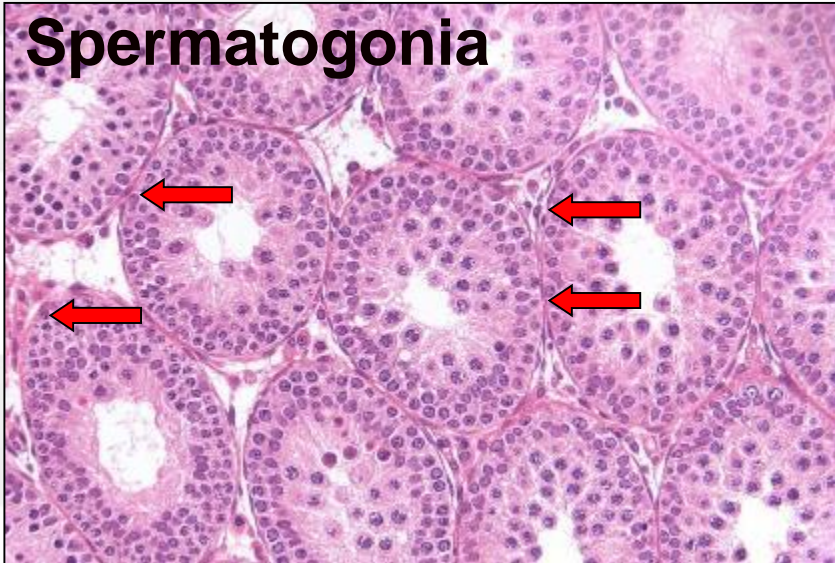
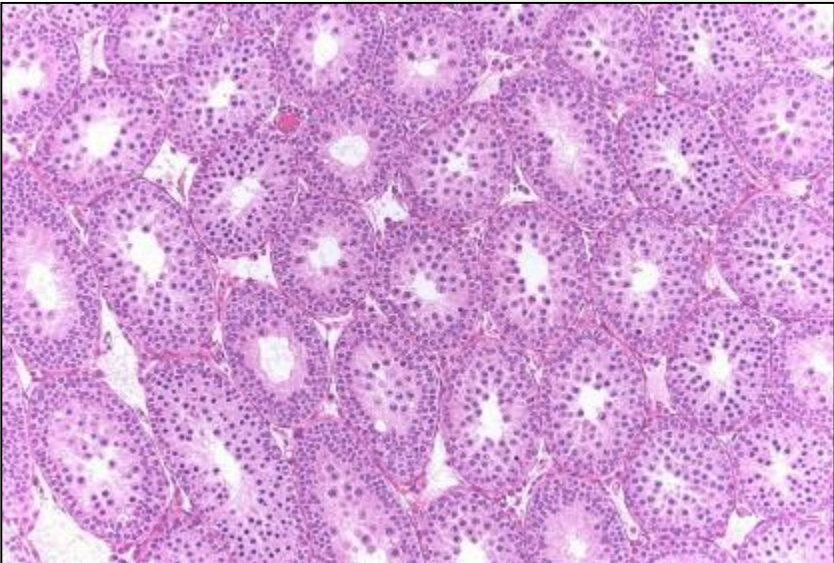
Approximately 4 stage on one tubular section due to spiral arrangement



Maturation

AnnaPath

Immaturity: Rat



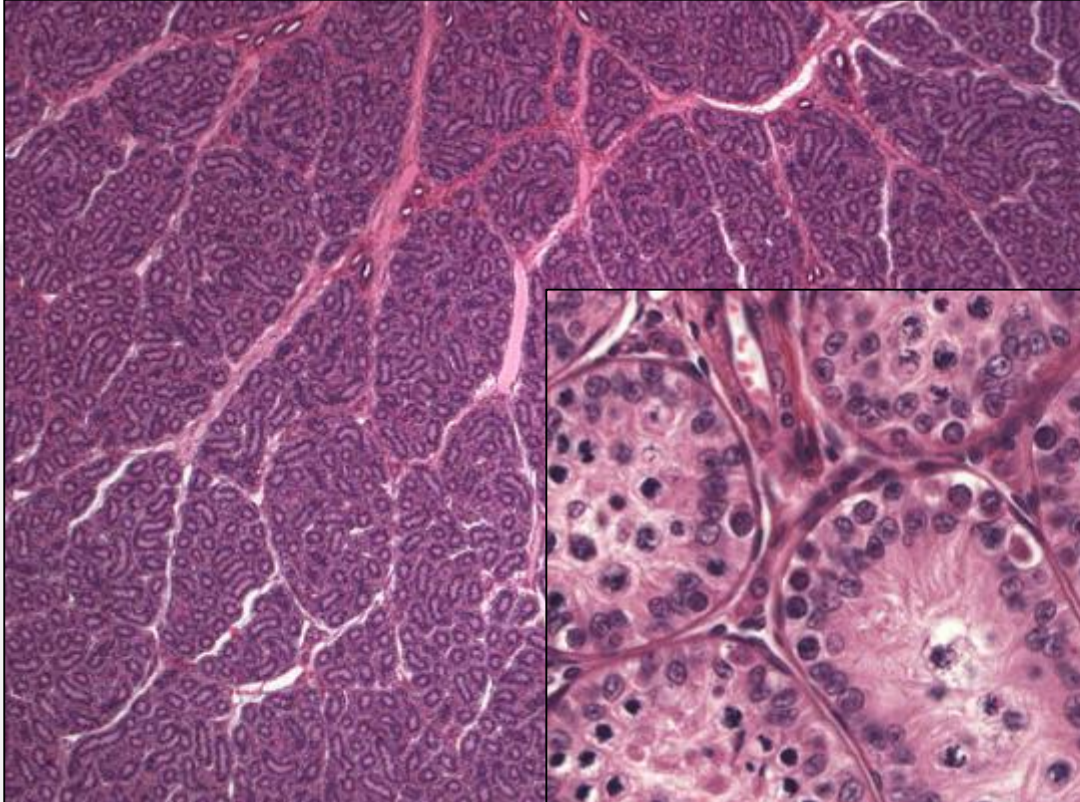
Maturation

- Haruyama E, Suda M, Ayukawa Y, Kamura K, Mizutsumi M, Ooshima Y, Tanimoto A (2012): Testicular Development in *Cynomolgus*. [Toxicol Pathol.](#) 2012 May 18. [Epub ahead of print]

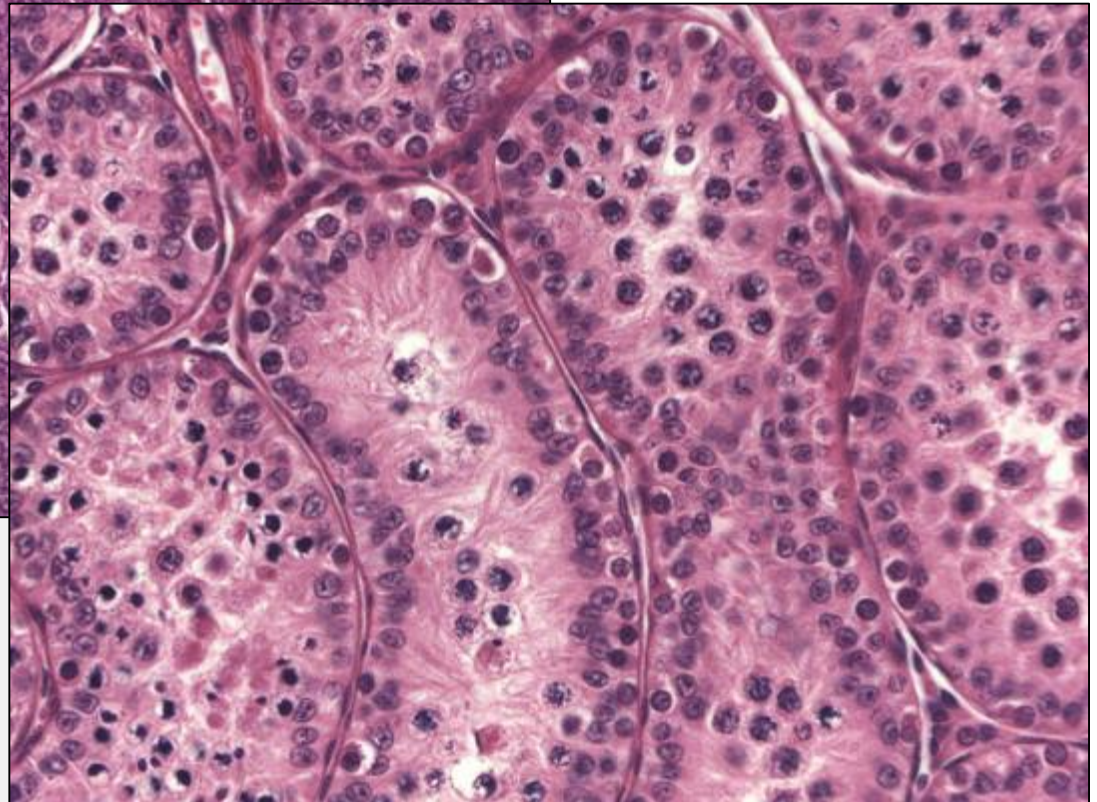
AnaPath

Maturation - Testes

Completely immature

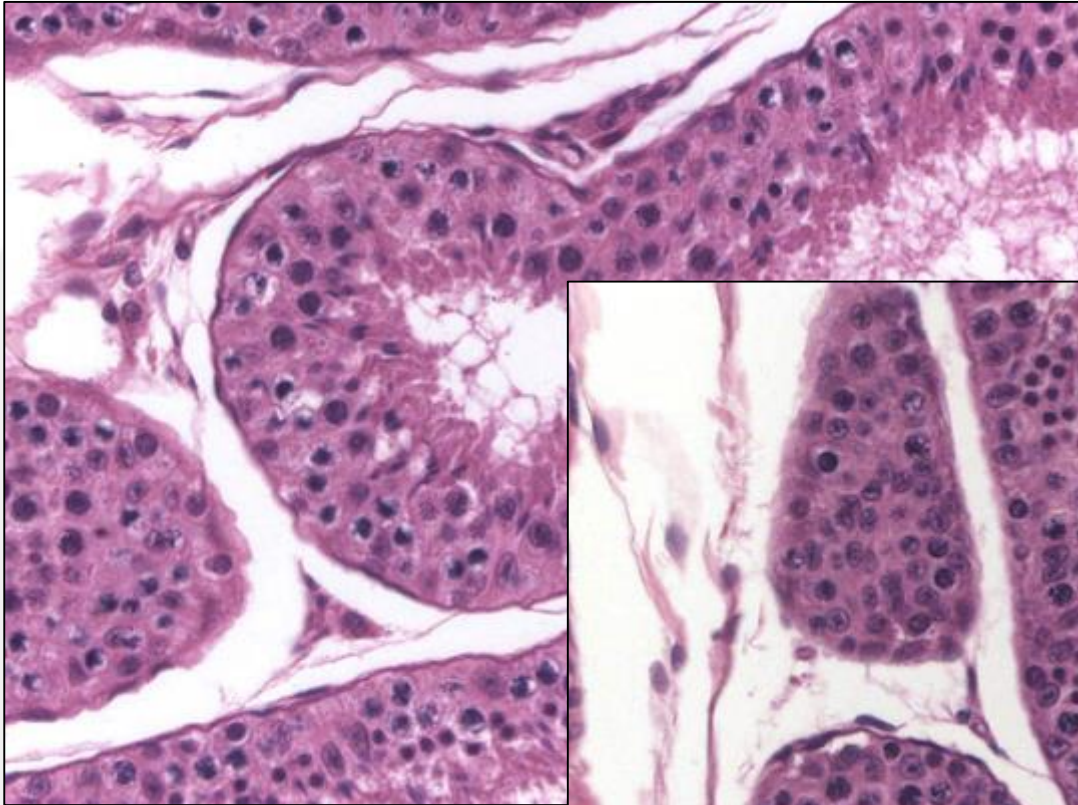


Round spermatogonia

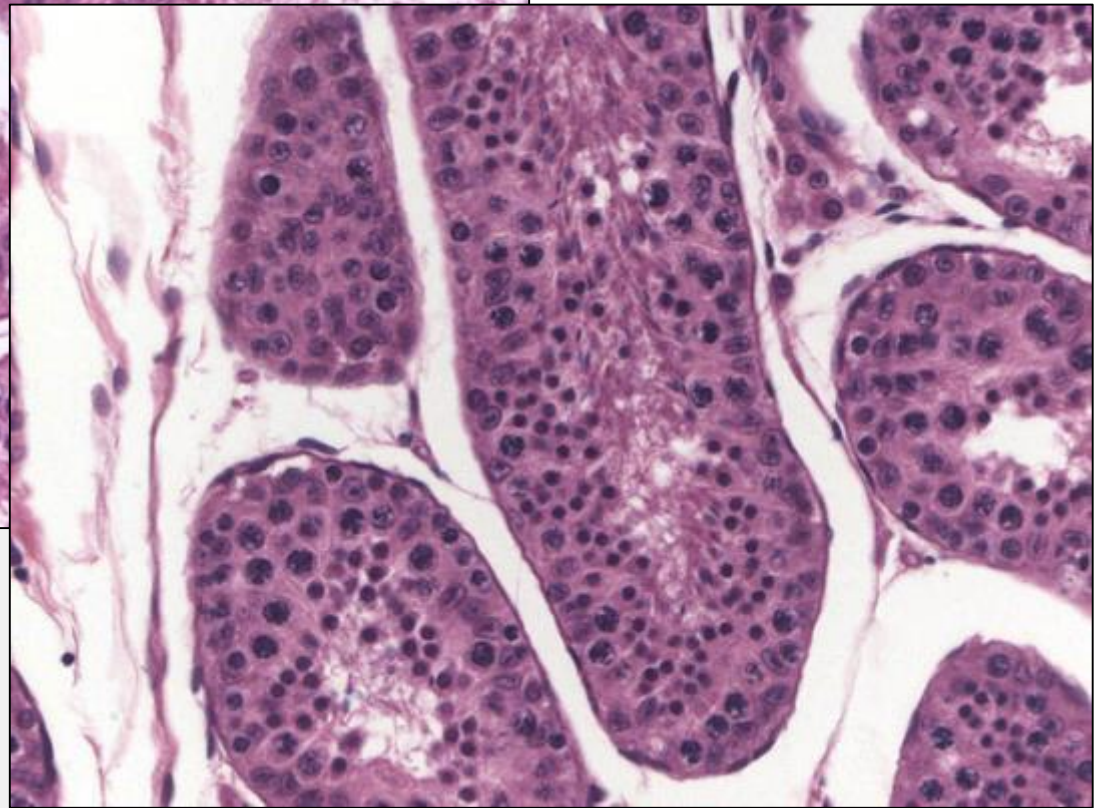


Maturation - Testes

Elongated spermatogonia

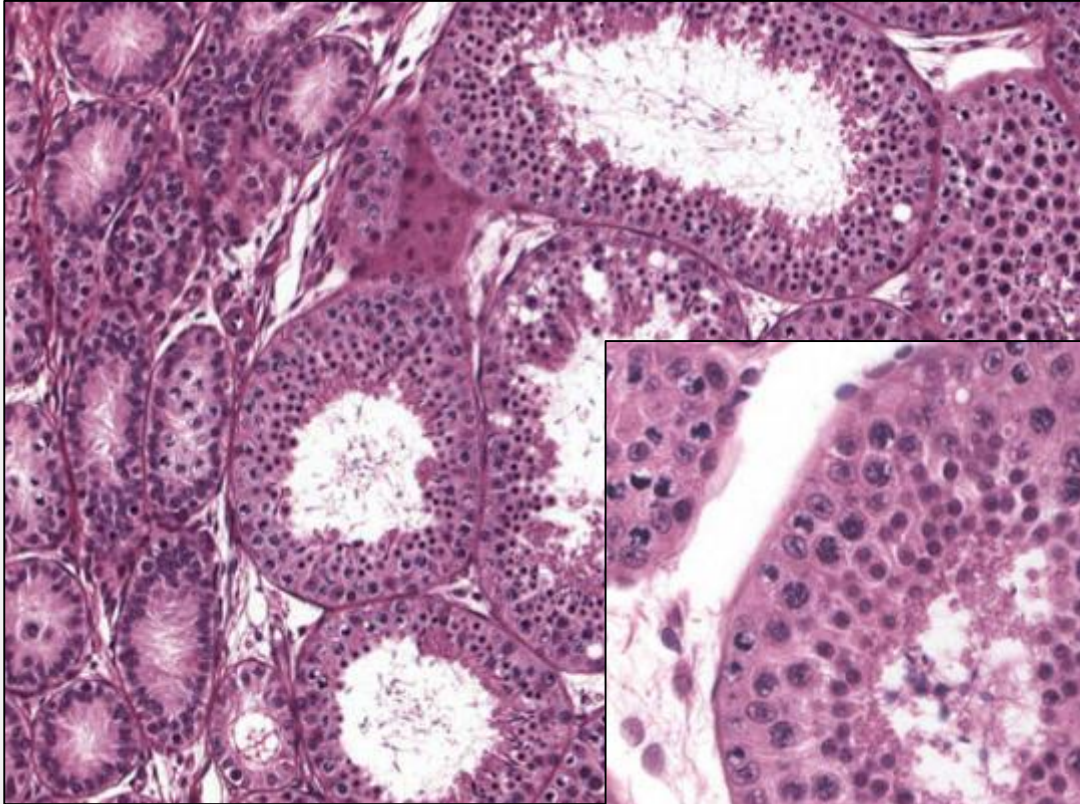


Incomplete maturation

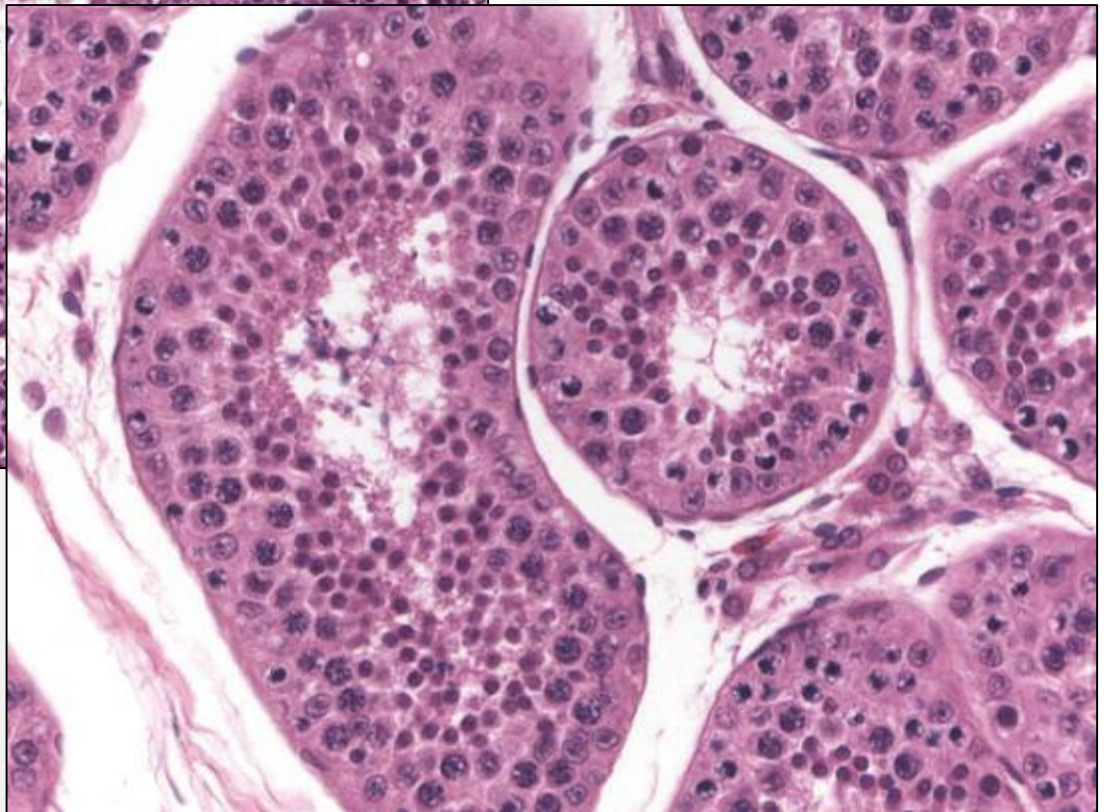


Maturation - Testes

Focal maturity

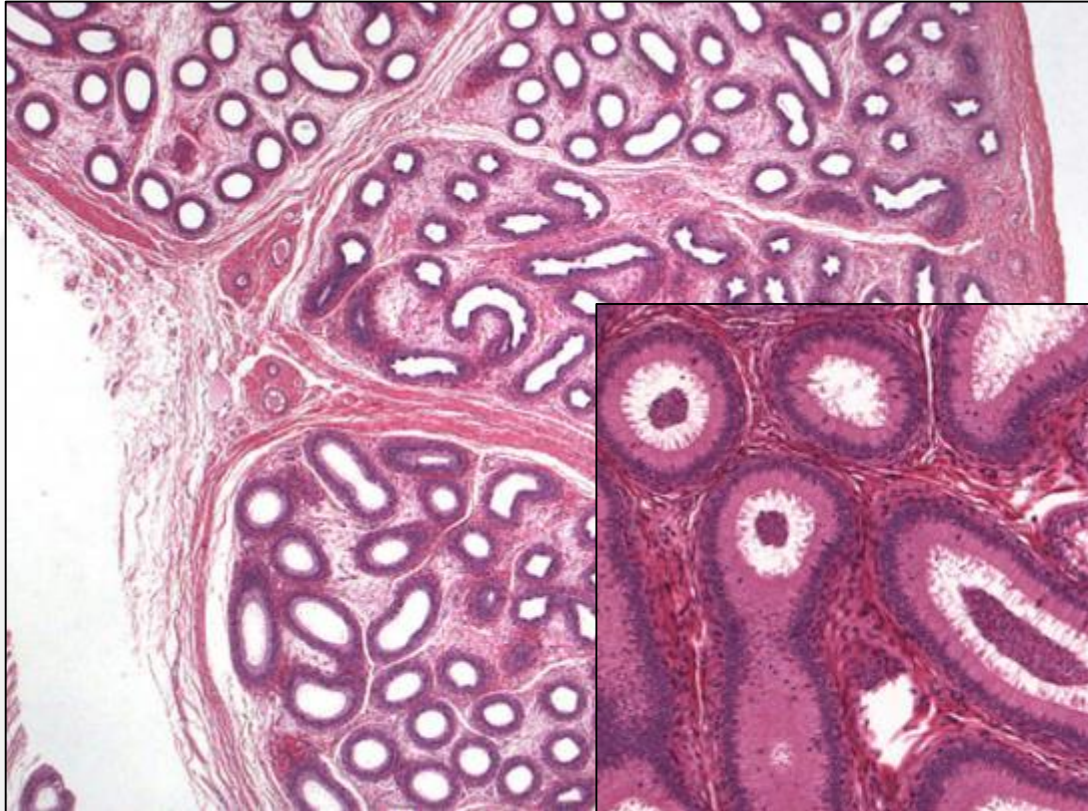


Mature

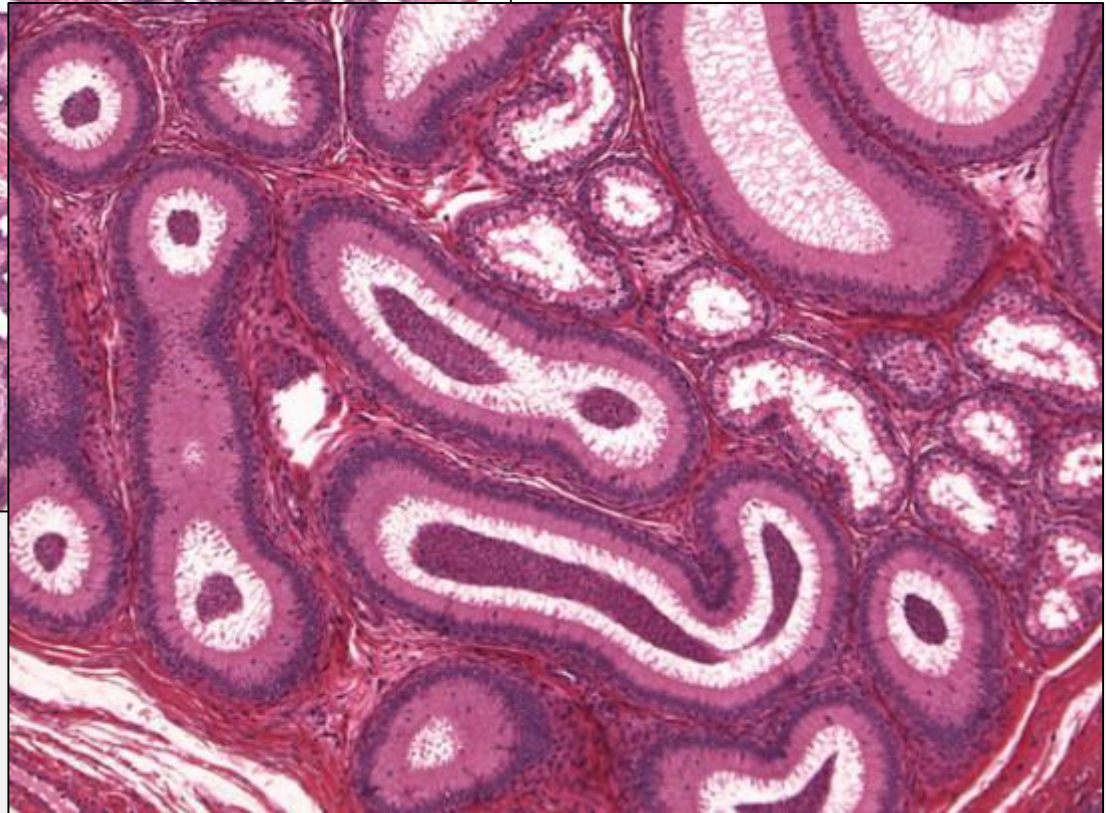


Maturation - Epididymides

Completely immature

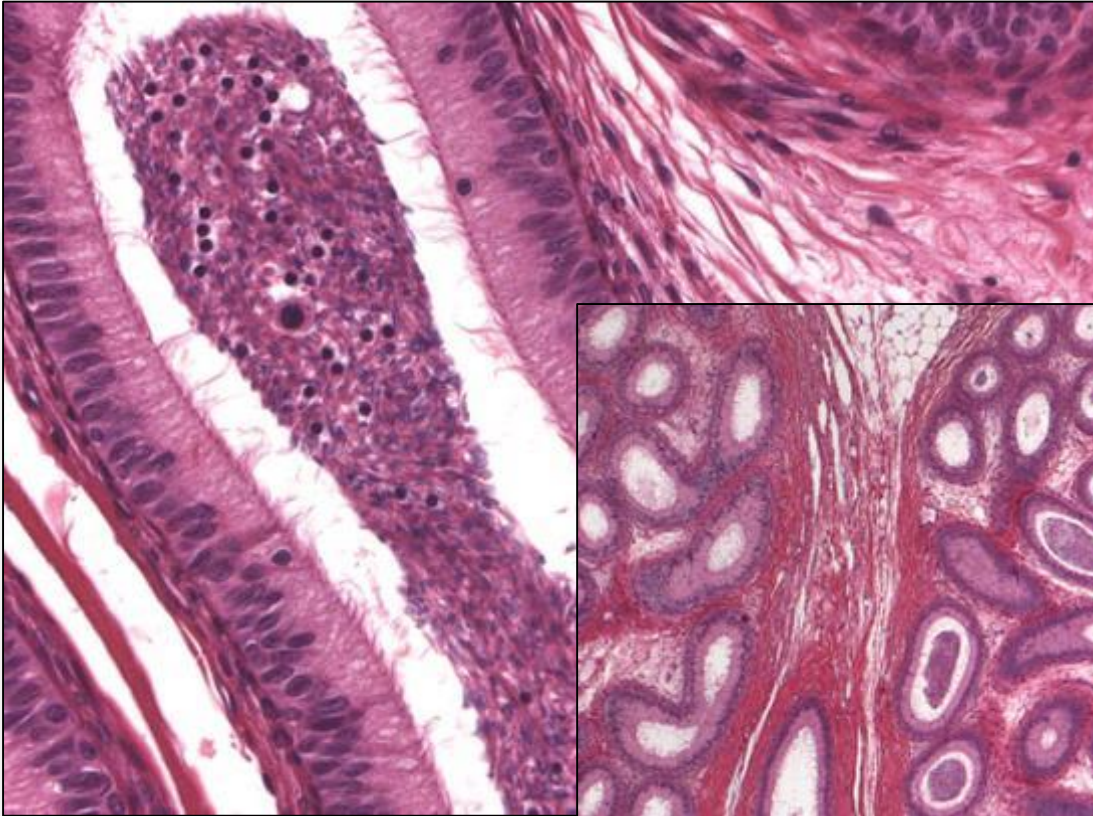


First spermia

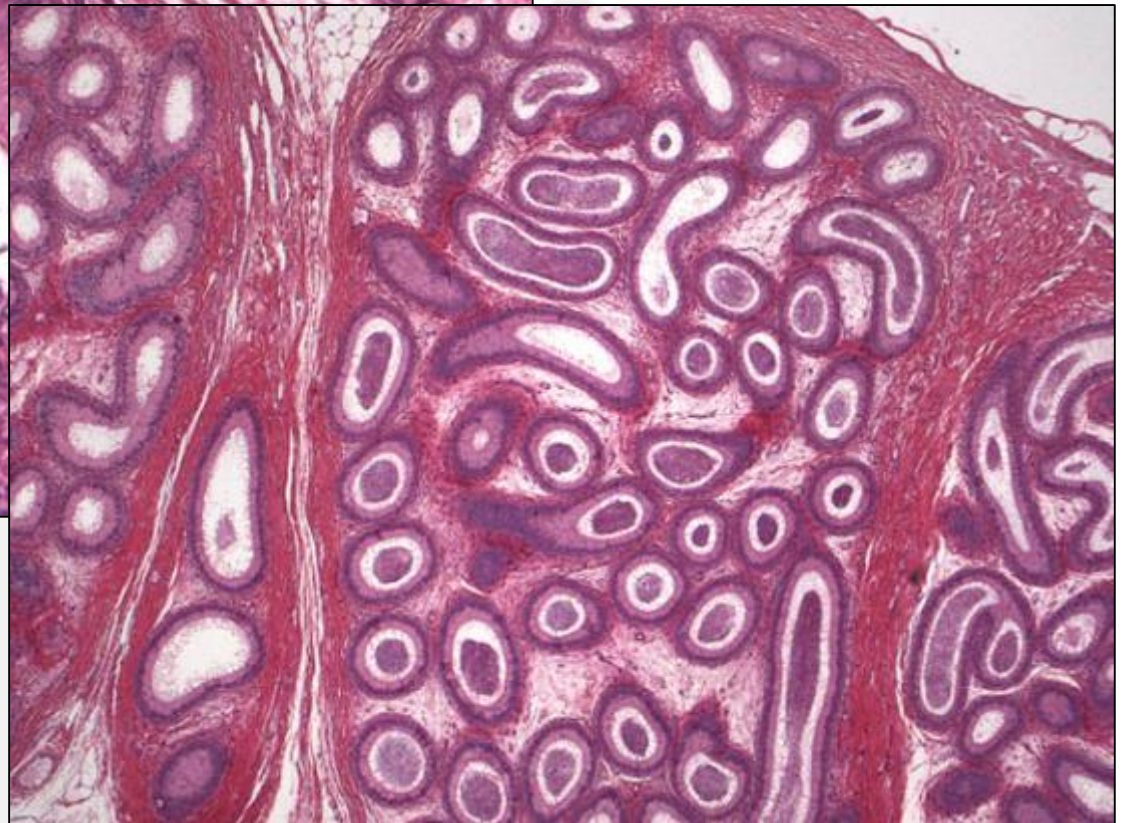


Maturation - Epididymides

Mature but still a lot of detritus



Mature





Lesions
AnaPath

Testes - Spontaneous lesions: RccHan™ : WIST (4-Week)

Male	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Numbers of rats examined	2077					
Immature	1	0.05	0.06	1.12	0.00	20.00
Hypoplastic tubules	1	0.05	0.06	1.12	0.00	20.00
Cellular debris	11	0.53	0.28	2.79	0.00	40.00
Congestion	1	0.05	0.03	0.56	0.00	10.00
Sertoli cell vacuolation	112	5.39	6.22	19.76	0.00	100.00
Hypospermatogenesis	2	0.10	0.09	1.25	0.00	20.00
Interstitial edema	4	0.19	0.19	2.37	0.00	40.00
Apoptosis	5	0.24	0.28	2.79	0.00	40.00
Sperm stasis	3	0.14	0.13	1.58	0.00	20.00
Tubular degeneration	83	4.00	4.22	10.03	0.00	100.00
Edema	1	0.05	0.03	0.56	0.00	10.00
Giant spermatidic cells	19	0.91	0.98	4.76	0.00	40.00
Mononuclear cell foci	5	0.24	0.31	2.48	0.00	20.00
Spermatid retention	2	0.10	0.09	1.25	0.00	20.00
Leydig cell hyperplasia	2	0.10	0.13	1.58	0.00	20.00

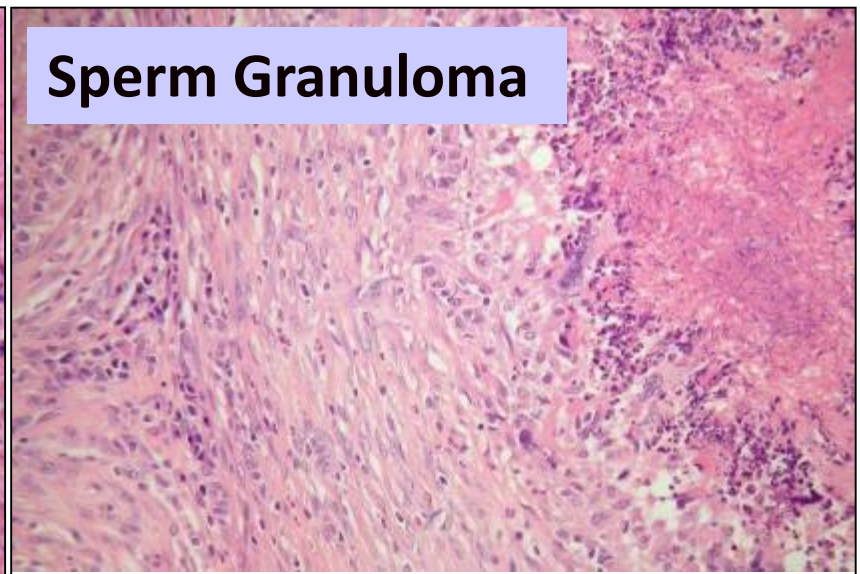
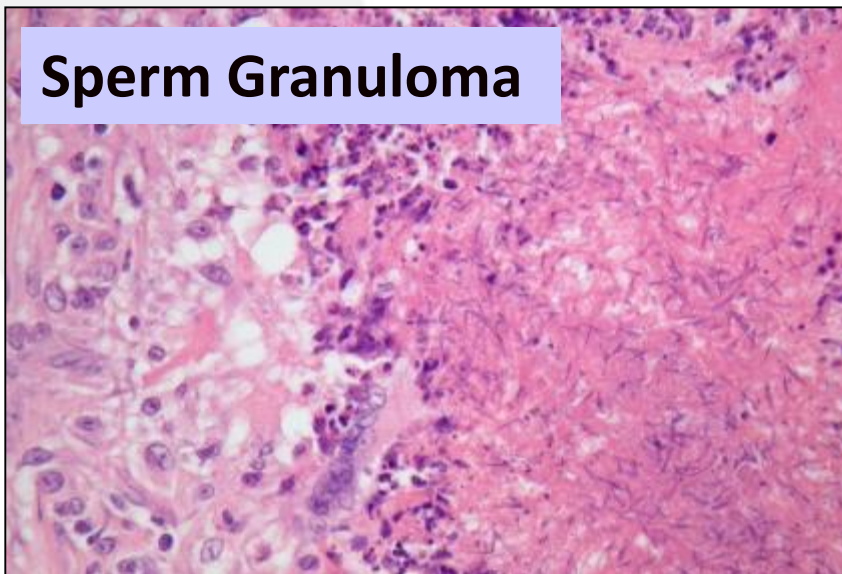
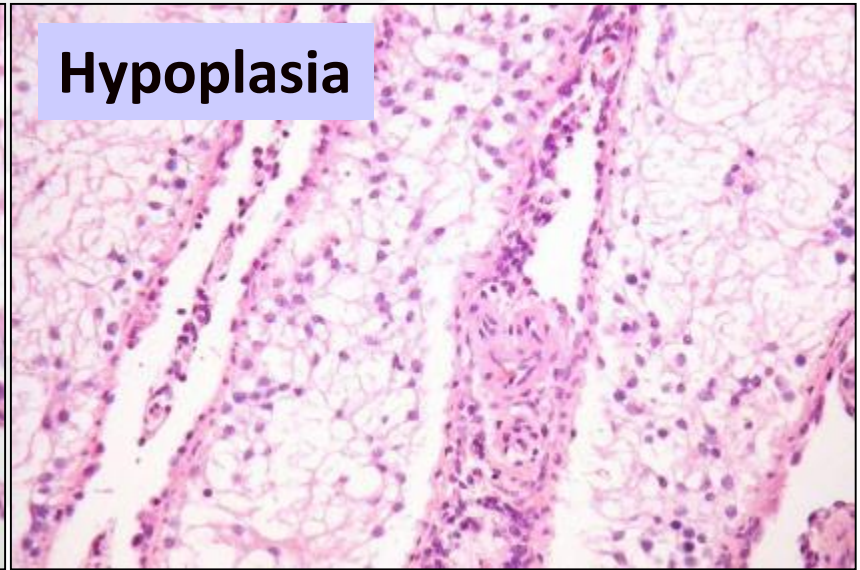
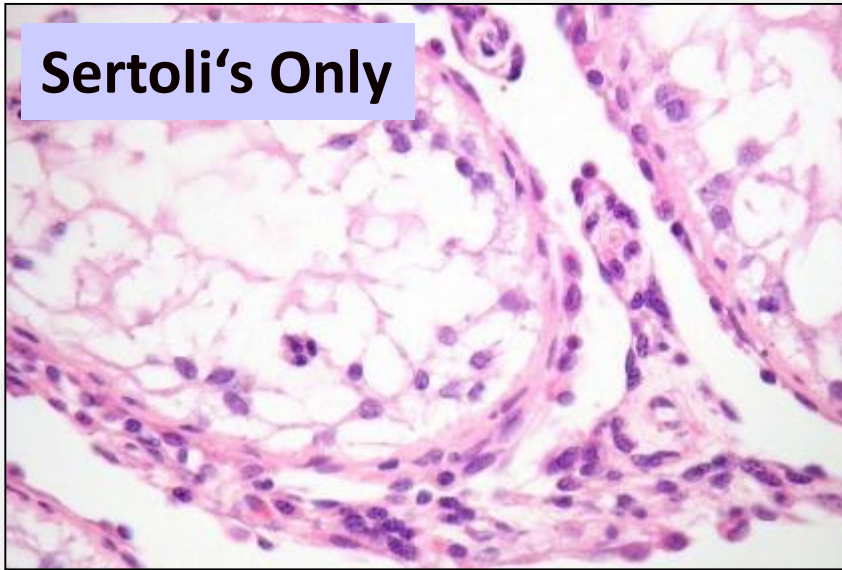
Testes - Spontaneous lesions: RccHan™ : WIST (13-Week)

Male	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Numbers of rats examined	1510					
Tubular ectasia	1	0.07	0.08	0.89	0.00	10.00
Mineralization	1	0.07	0.08	0.89	0.00	10.00
Anomaly	1	0.07	0.08	0.89	0.00	10.00
Hypoplastic testis	3	0.20	0.24	1.99	0.00	20.00
Hemorrhage	1	0.07	0.05	0.59	0.00	6.67
Cellular debris	3	0.20	0.26	2.97	0.00	33.33
Mononuclear cell foci	4	0.26	0.21	2.38	0.00	26.67
Multinuclear giant cell	3	0.20	0.24	1.53	0.00	10.00
Hyospermiogenesis	1	0.07	0.04	0.45	0.00	5.00
Tubular degeneration	83	5.50	5.69	9.20	0.00	50.00
Sertoli cell vacuolation	18	1.19	1.39	5.99	0.00	40.00
Spermatid retention	5	0.33	0.40	2.65	0.00	20.00
Granuloma	1	0.07	0.08	0.89	0.00	10.00
Leydig cell hyperplasia	3	0.20	0.20	1.83	0.00	20.00
Edema	3	0.20	0.24	1.53	0.00	10.00

Testes - Spontaneous lesions: RccHan™ : WIST (104-Week, Non-Neo.)

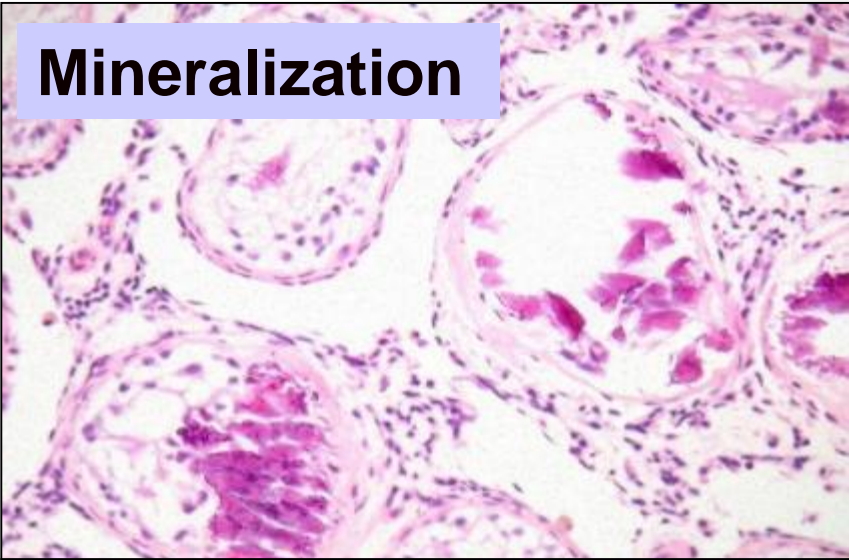
Males	Total n	Total n %	Mean %	STDEV %	MIN %	MAX %
Numbers of rats examined	3826					
Dilation/rete testis	1	0.03	0.04	0.27	0.00	2.00
Capsular dilatation	2	0.05	0.03	0.23	0.00	1.67
Spermatocele	18	0.47	0.48	1.12	0.00	5.00
Congestion	21	0.55	0.56	2.28	0.00	15.71
Hemorrhage	5	0.13	0.16	0.49	0.00	2.00
Edema	196	5.12	4.59	8.66	0.00	37.37
Vascular hyalinosis	3	0.08	0.08	0.33	0.00	1.43
Amyloidosis	1	0.03	0.04	0.27	0.00	2.00
Mineralization	164	4.29	4.34	3.78	0.00	13.13
Angiopathy	7	0.18	0.19	1.37	0.00	10.00
Oligospermia	5	0.13	0.13	0.54	0.00	3.00
Aspermia	16	0.42	0.57	2.09	0.00	12.00
Maturation arrest	3	0.08	0.08	0.44	0.00	2.86
Sperm stasis	49	1.28	1.32	3.56	0.00	20.00
Giant cells	18	0.47	0.49	1.18	0.00	4.29
Tubular degeneration	851	22.24	21.91	15.14	0.00	99.00
Mononuclear cell foci	5	0.13	0.13	0.56	0.00	2.86
Granuloma(s)	18	0.47	0.50	2.06	0.00	14.49
Periarteritis/arteritis	330	8.63	8.42	8.71	0.00	40.58
Inflammation	1	0.03	0.04	0.27	0.00	2.00
Fibrosis	4	0.10	0.10	0.43	0.00	2.02

Spontaneous lesions - Testes: RccHan™:WIST

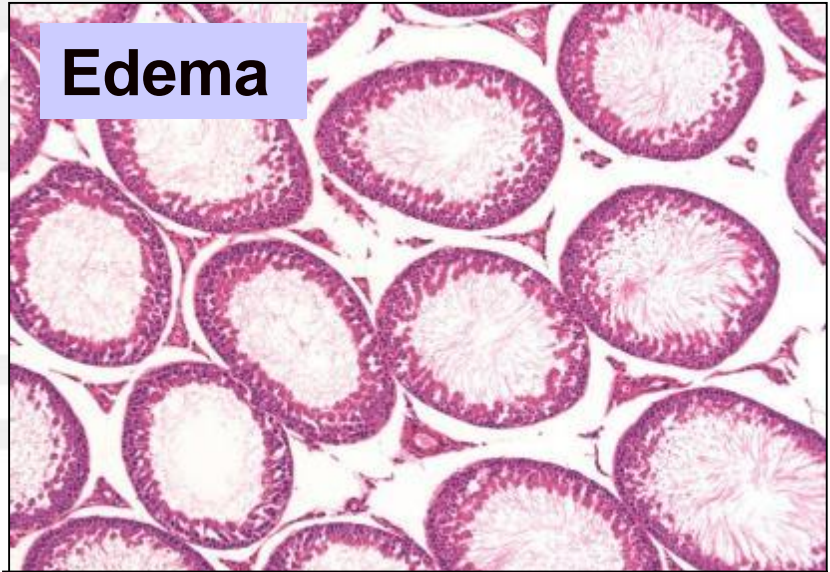


Spontaneous Lesions - Testes: RccHan™:WIST

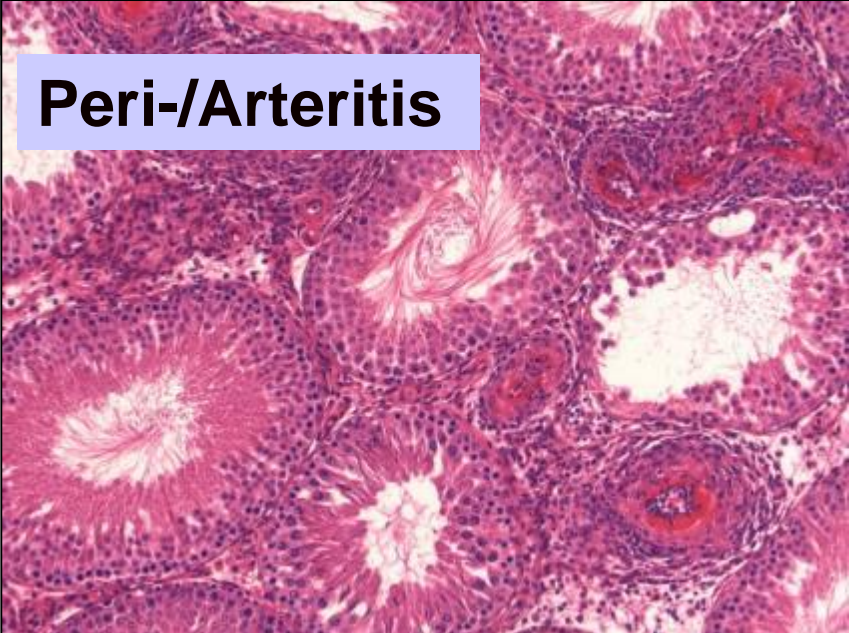
Mineralization



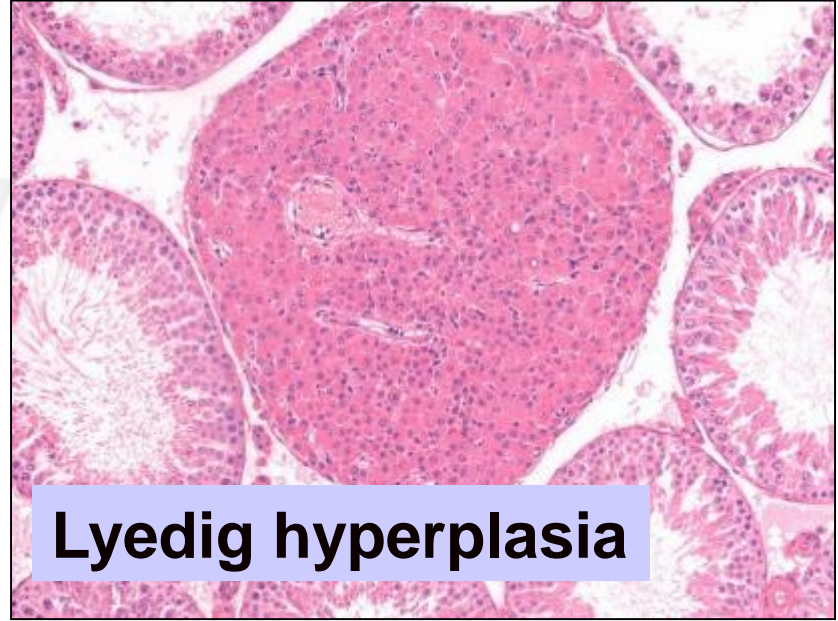
Edema



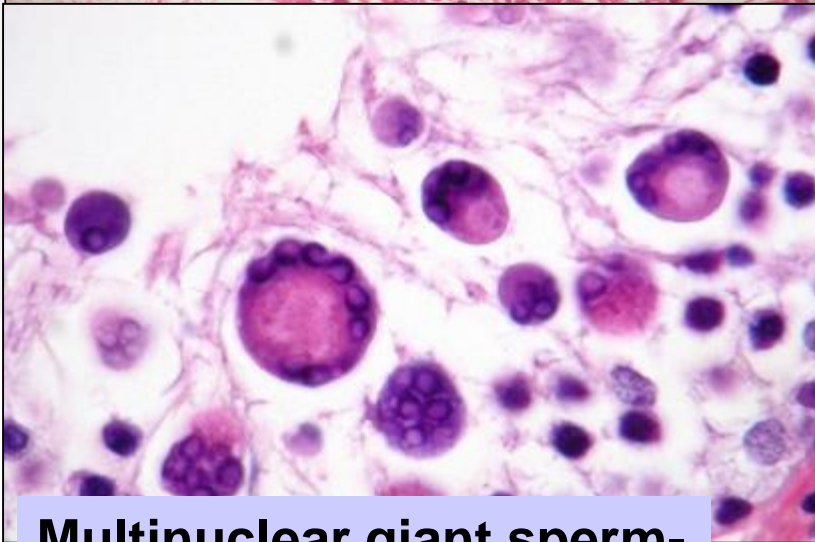
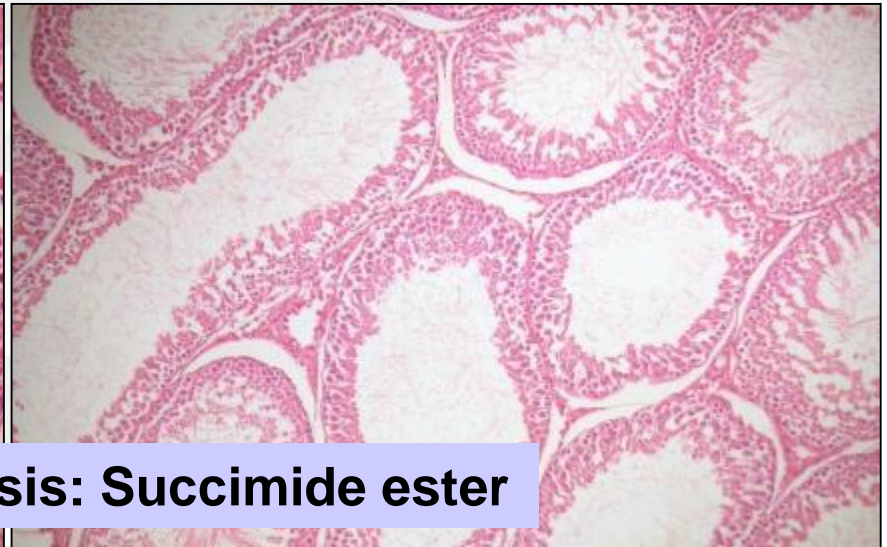
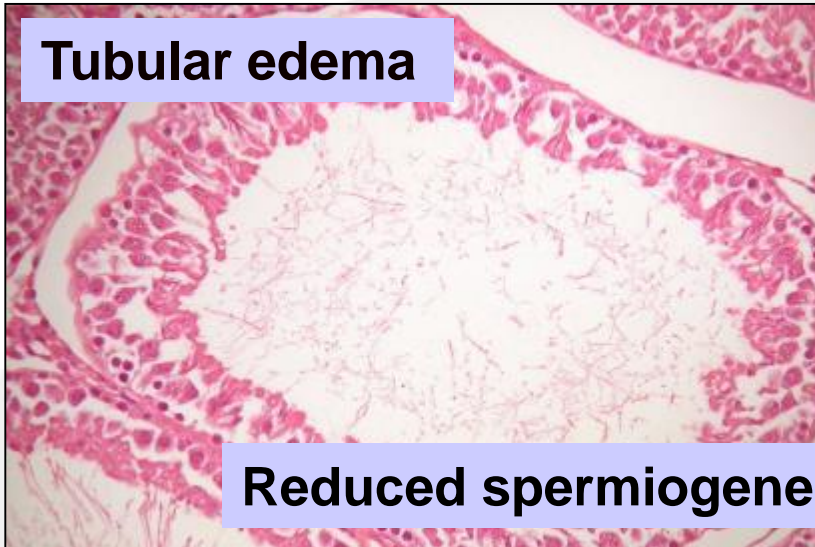
Peri-/Arteritis



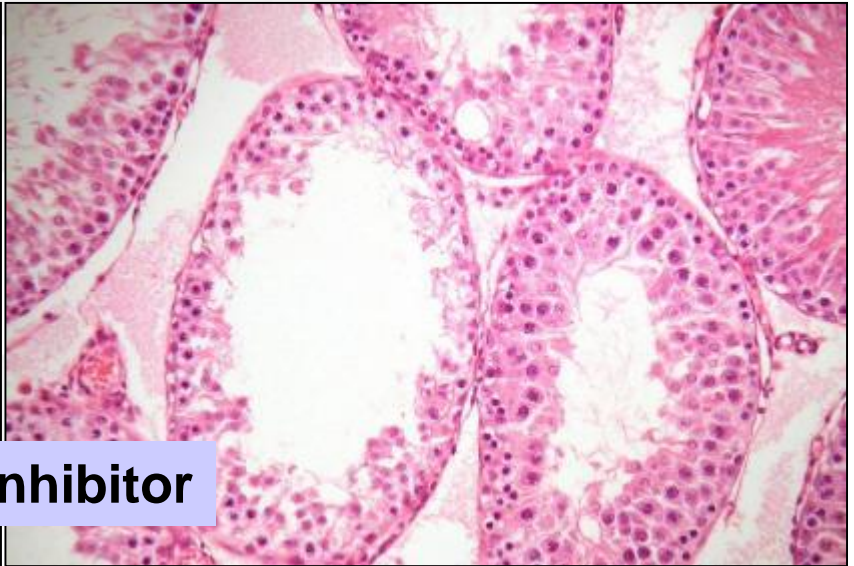
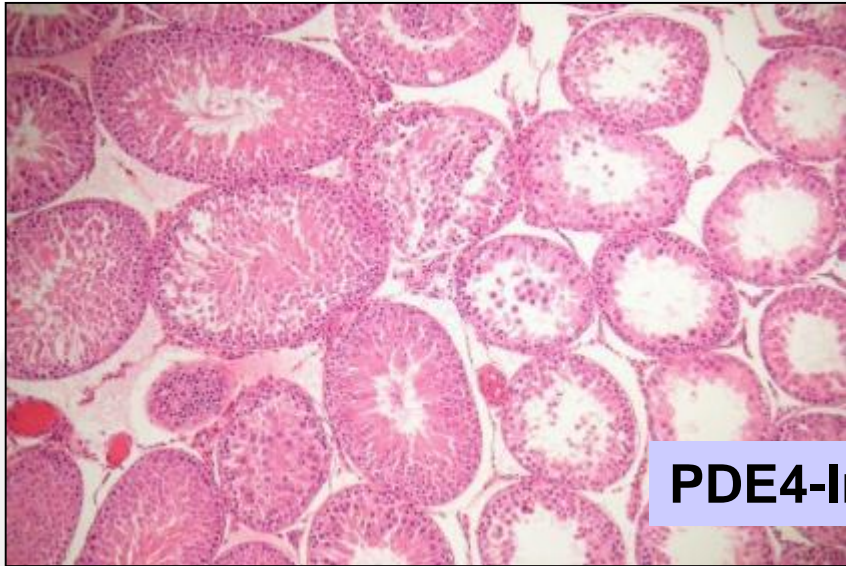
Leydig hyperplasia



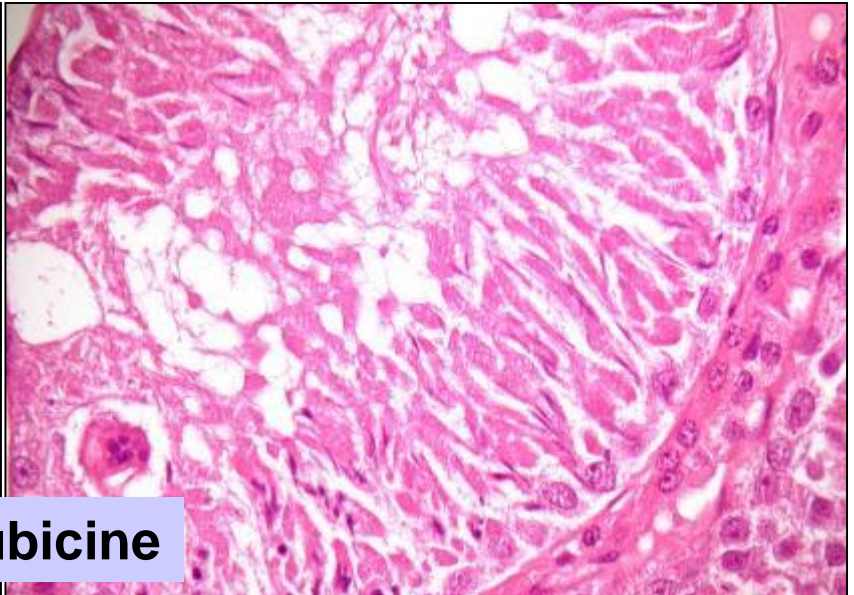
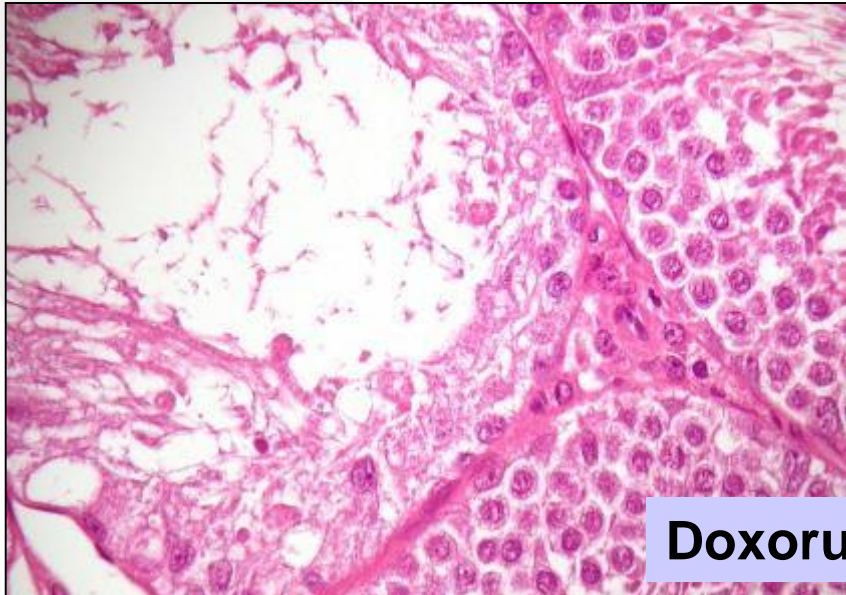
Induced Lesions - Testes: RccHan™:WIST



Induced Lesions - Testes: RccHan™:WIST

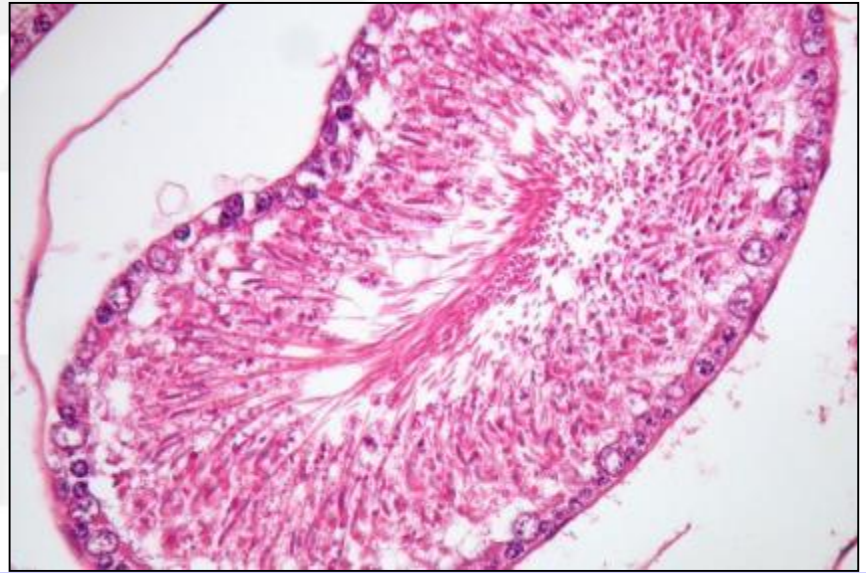
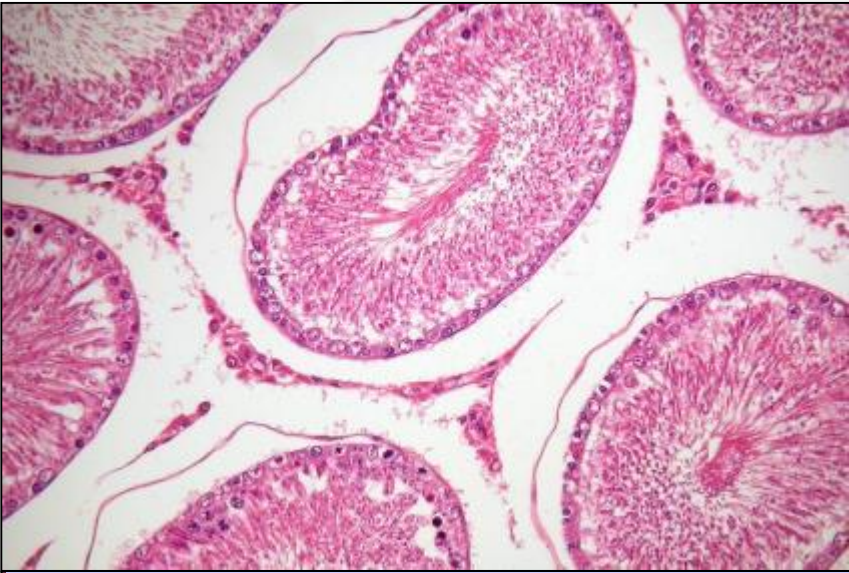


PDE4-Inhibitor

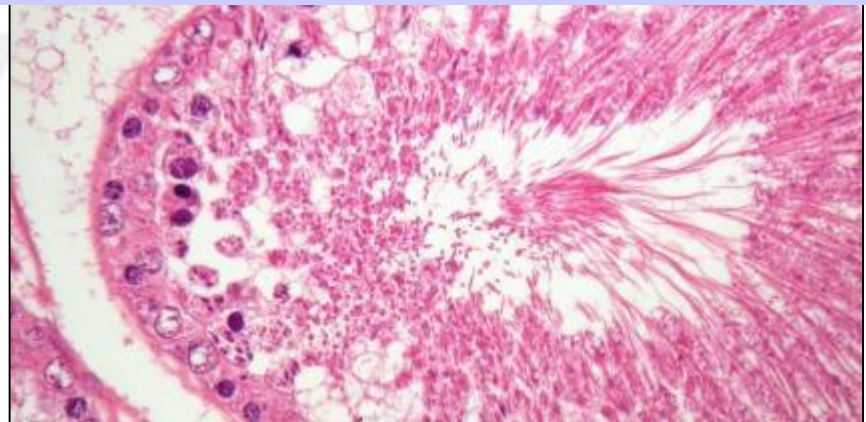
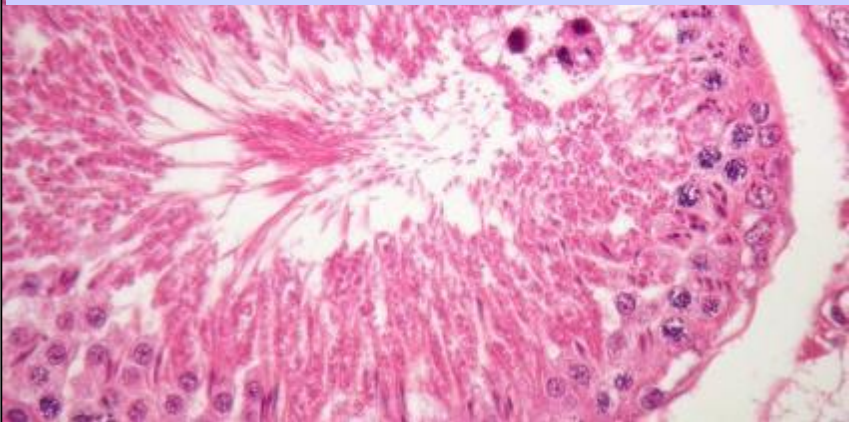


Doxorubicine

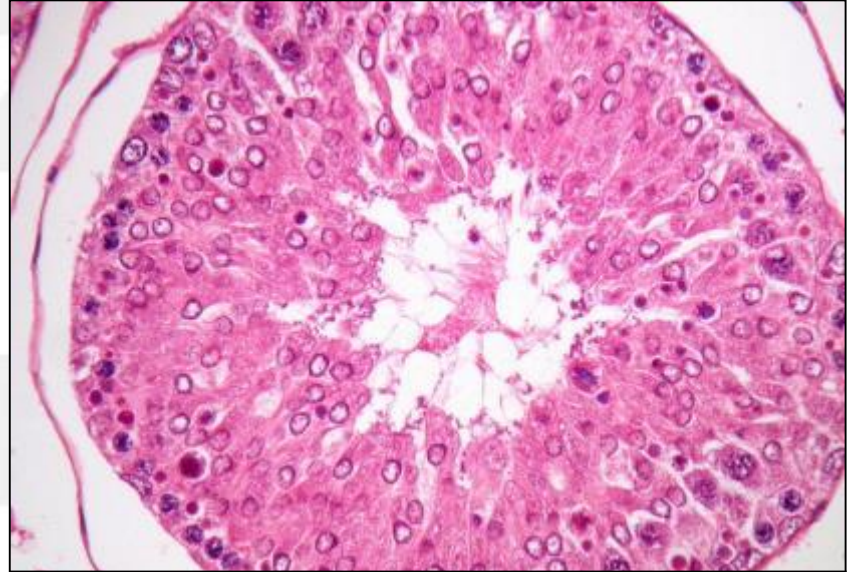
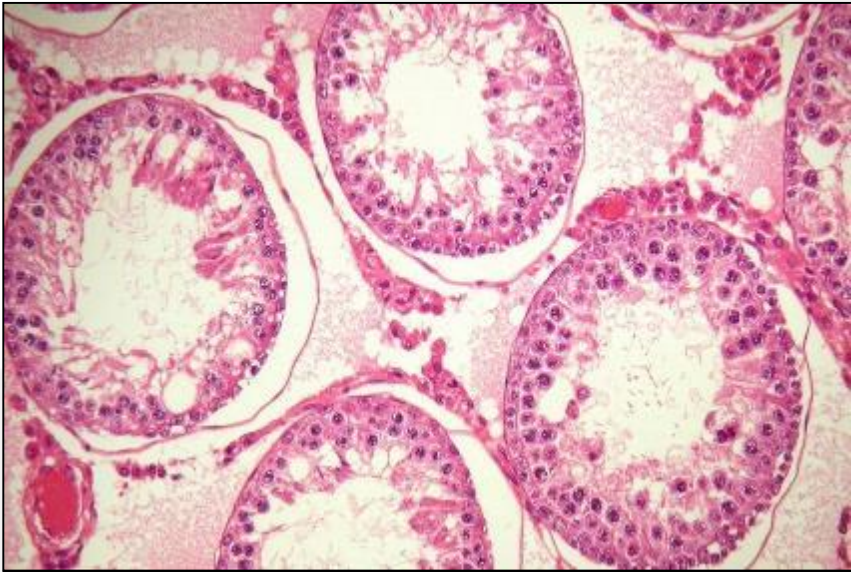
Induced Lesions - Testes: RccHan™:WIST



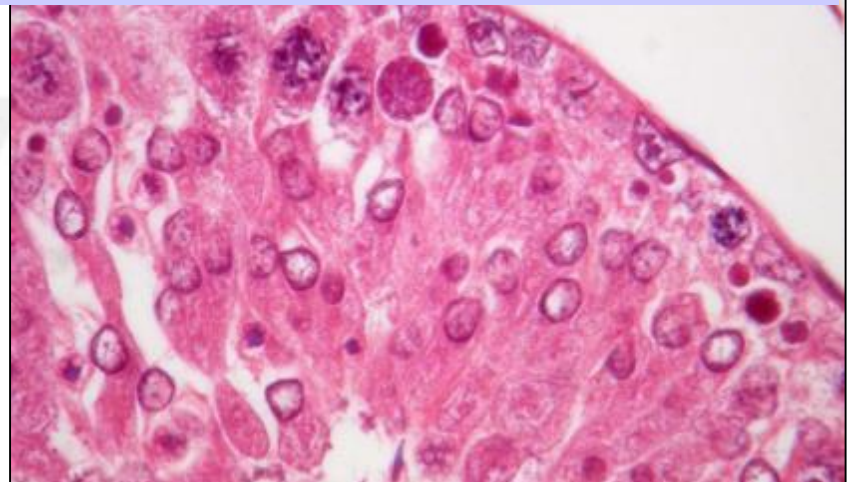
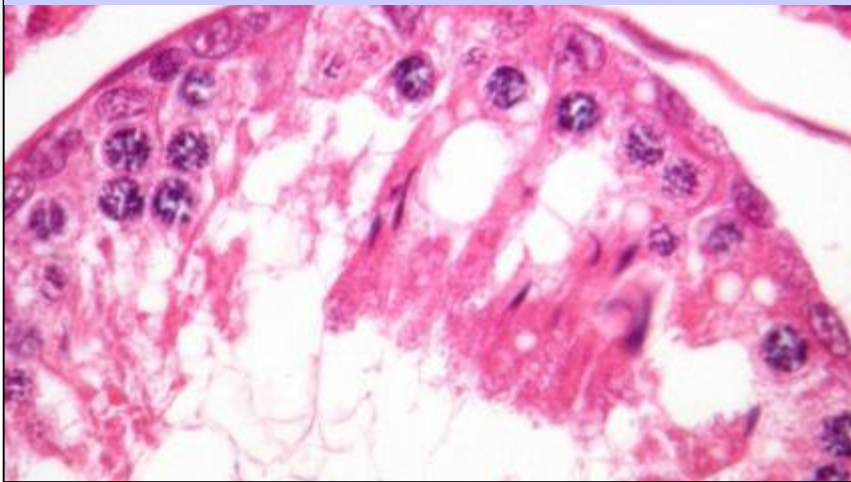
**Degeneration of spermatogonia and spermatides
(Antipsychotic) - Main Test.**



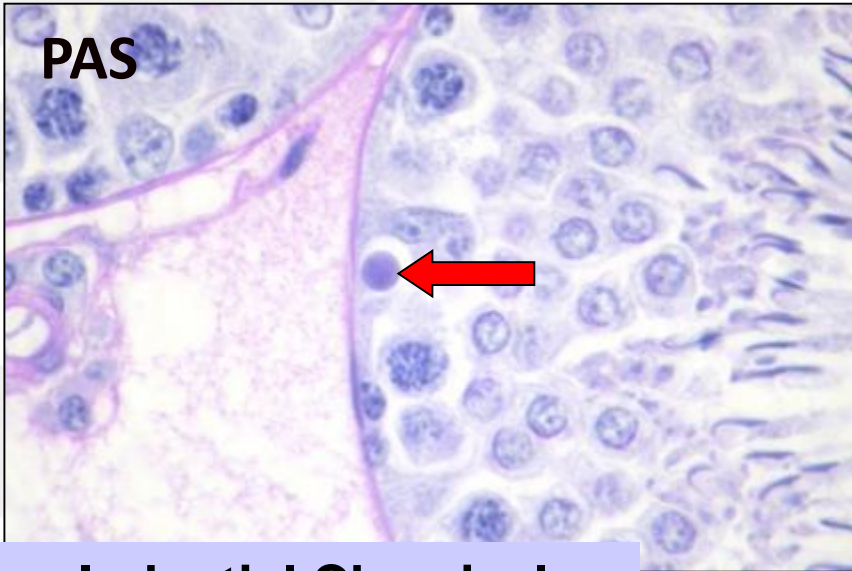
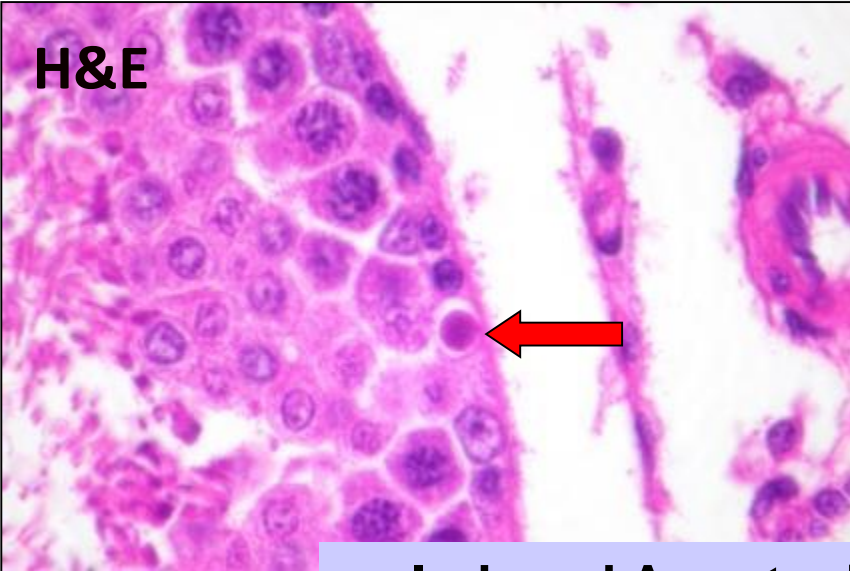
Induced Lesions - Testes: RccHan™:WIST



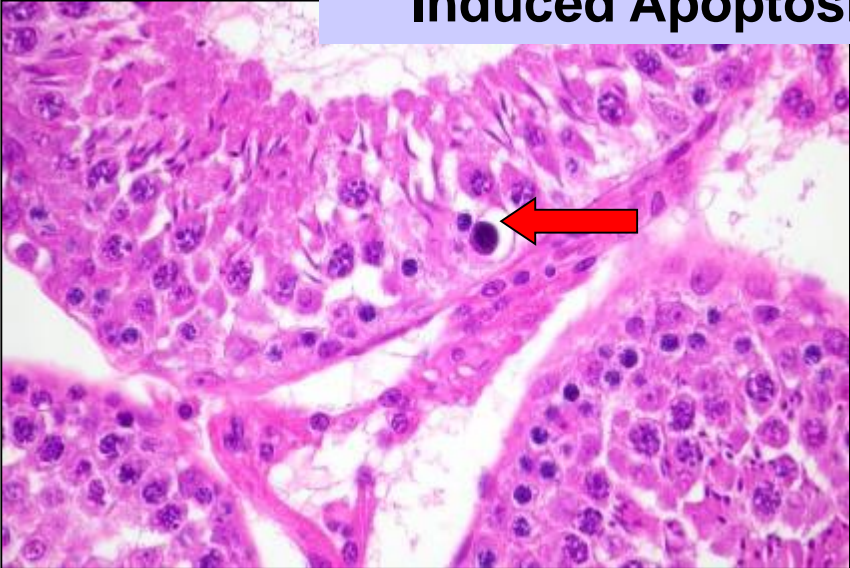
Partial Recovery (Antipsychotic, Neuroleptic). Recovery.



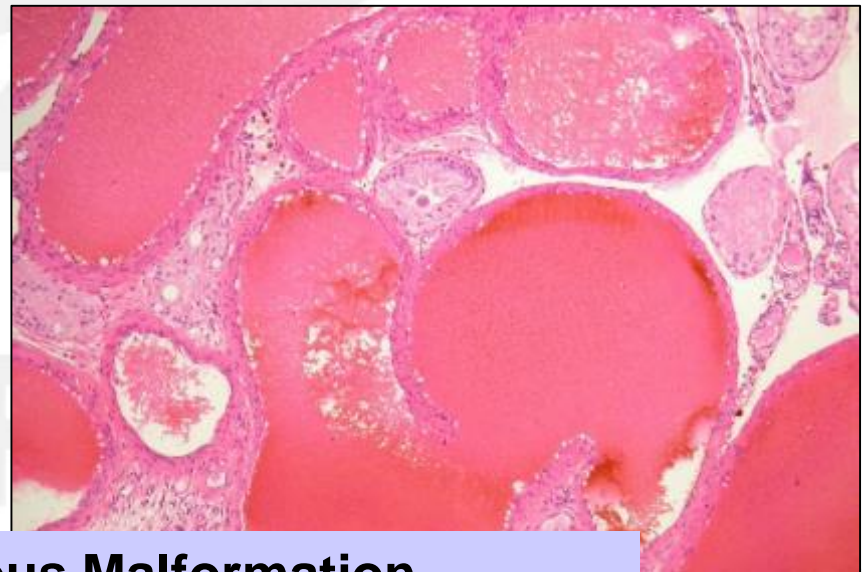
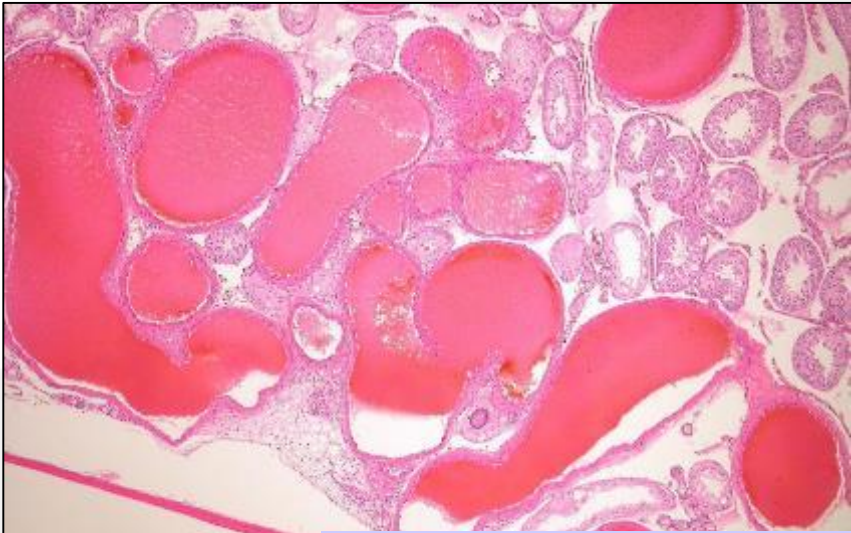
Induced Lesions - Testes: RccHan™:WIST



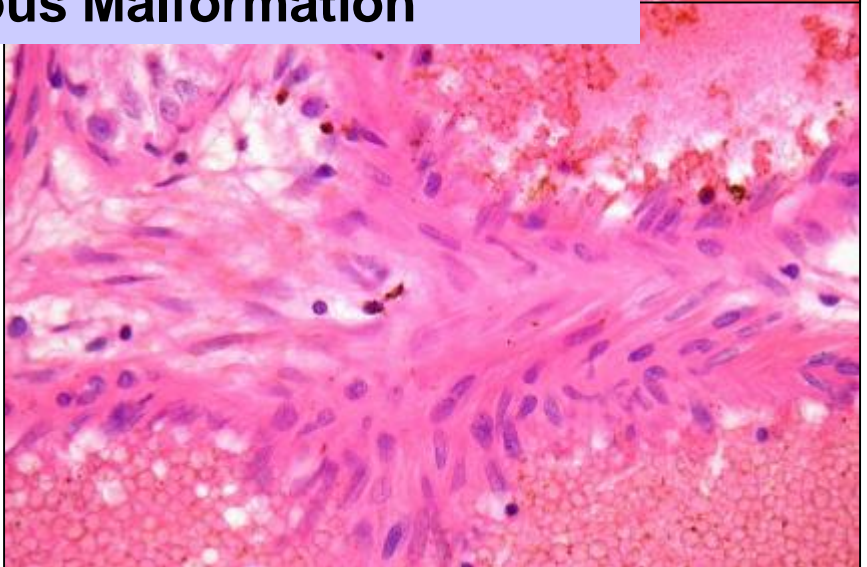
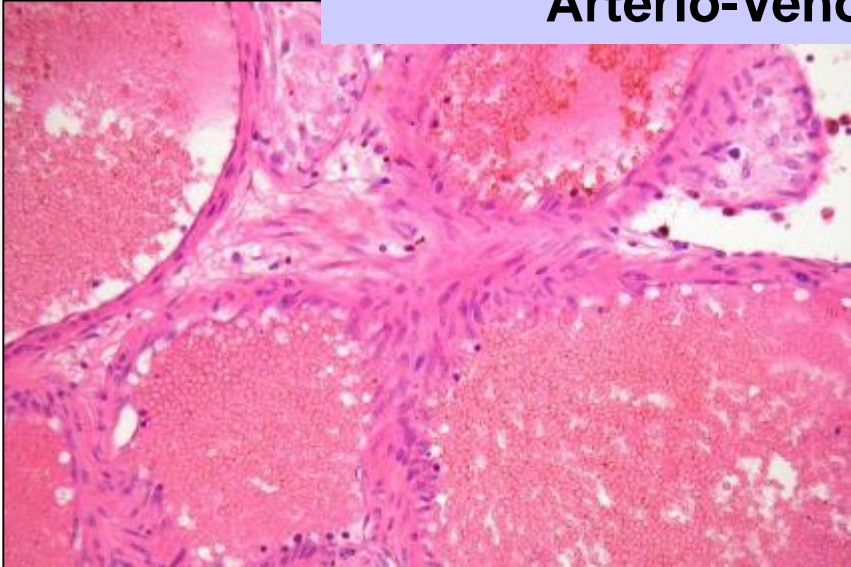
Induced Apoptosis - Industrial Chemical



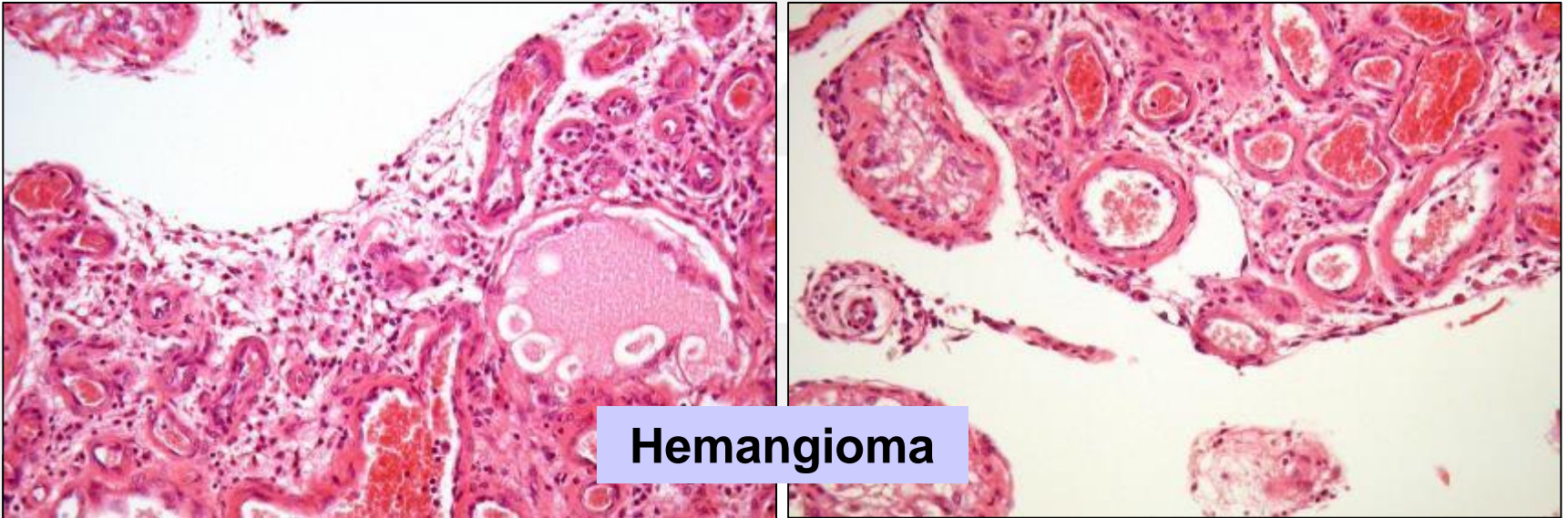
Testes - RccHan™:WIST: Pseudotumor, spontaneous, rare



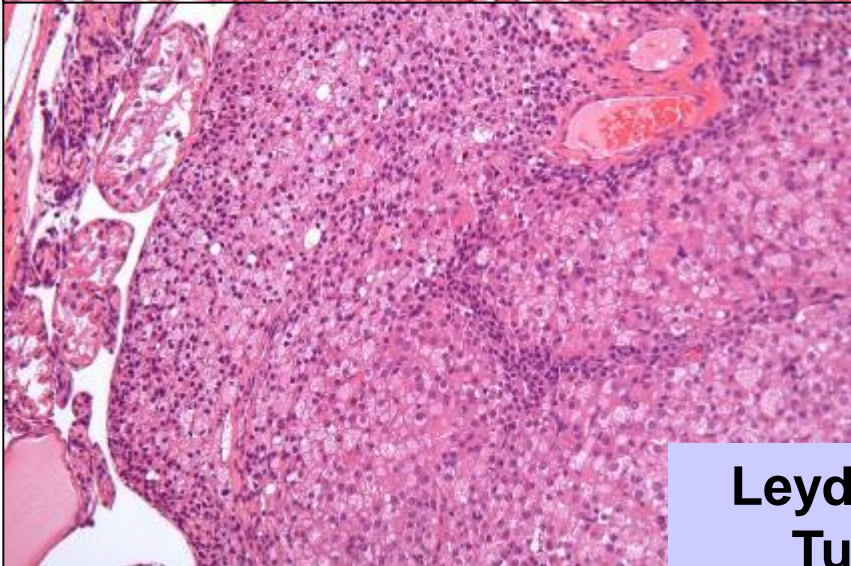
Arterio-Venous Malformation



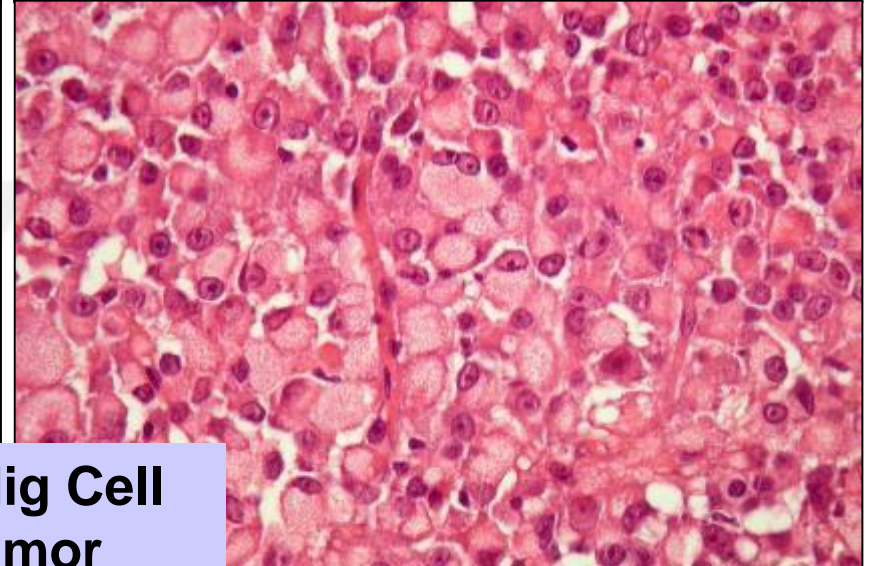
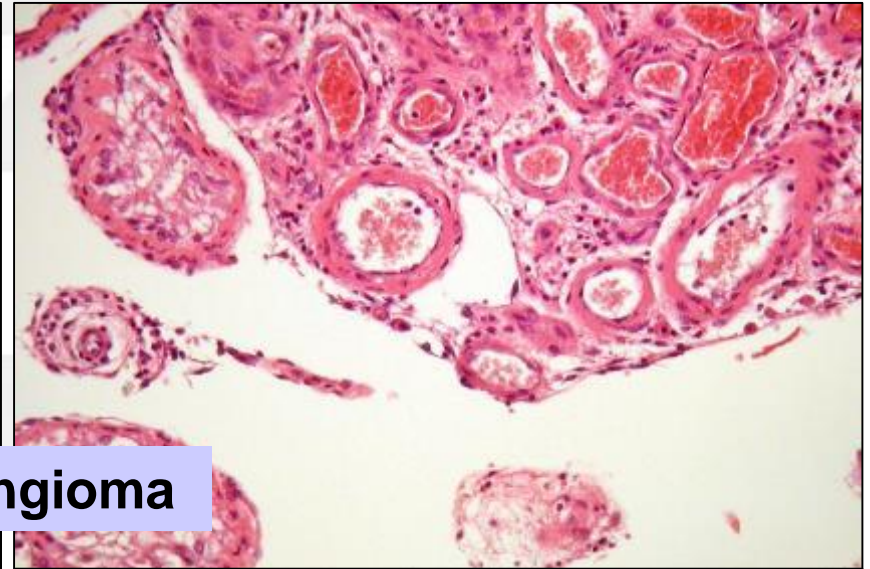
Testes - RccHan™:WIST: Neoplasms



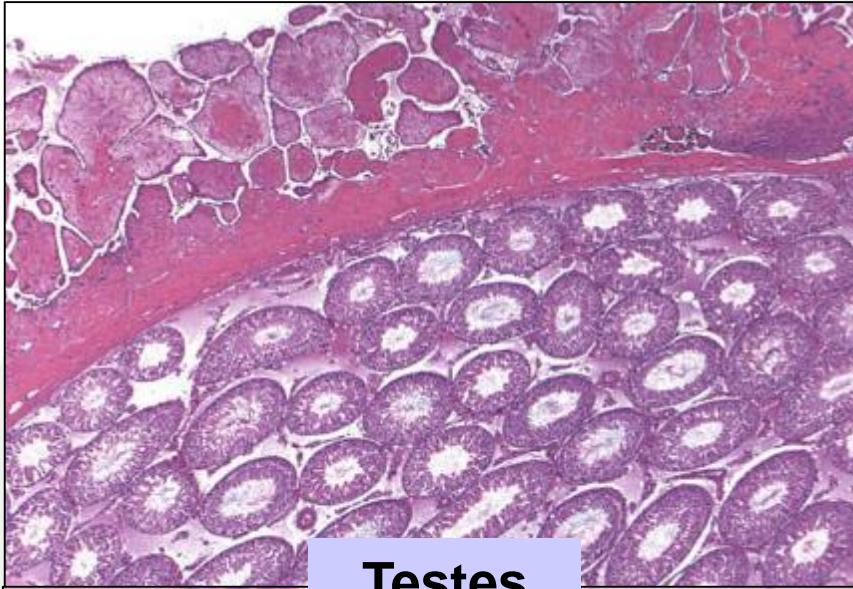
Hemangioma



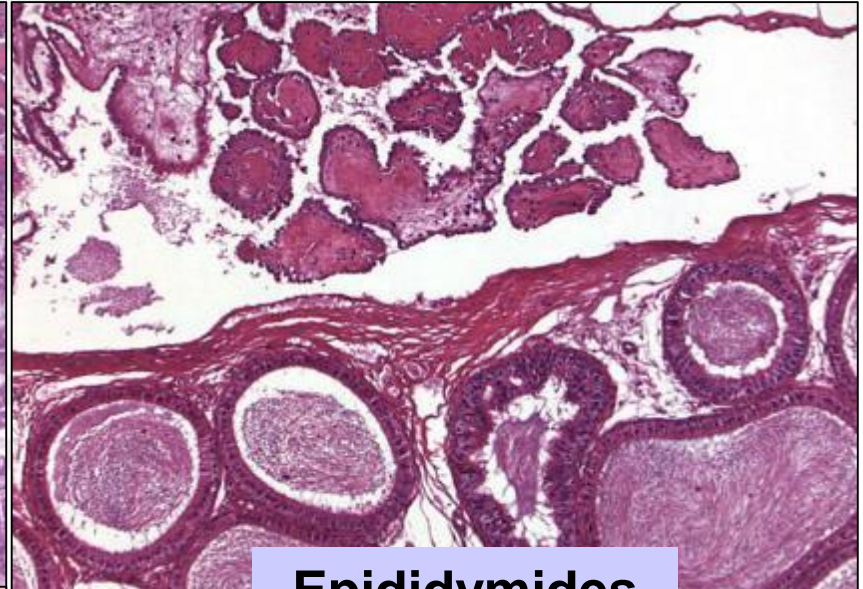
**Leydig Cell
Tumor**



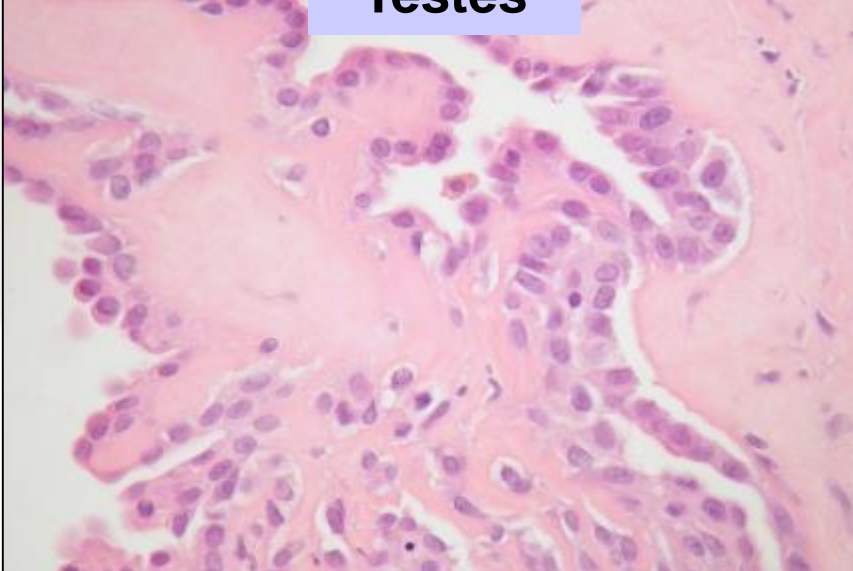
Testes/Epididymides - RccHan™:WIST: Neoplasms



Testes



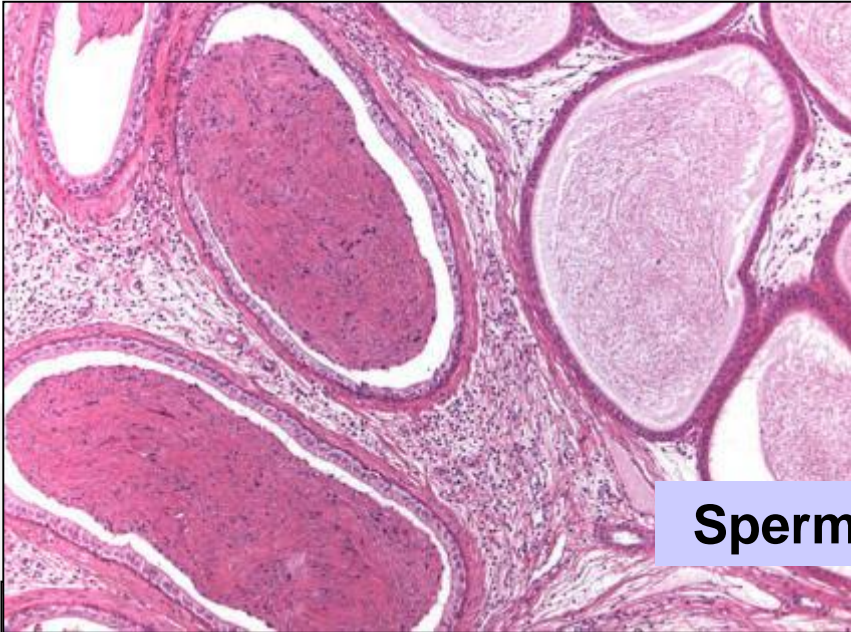
Epididymides



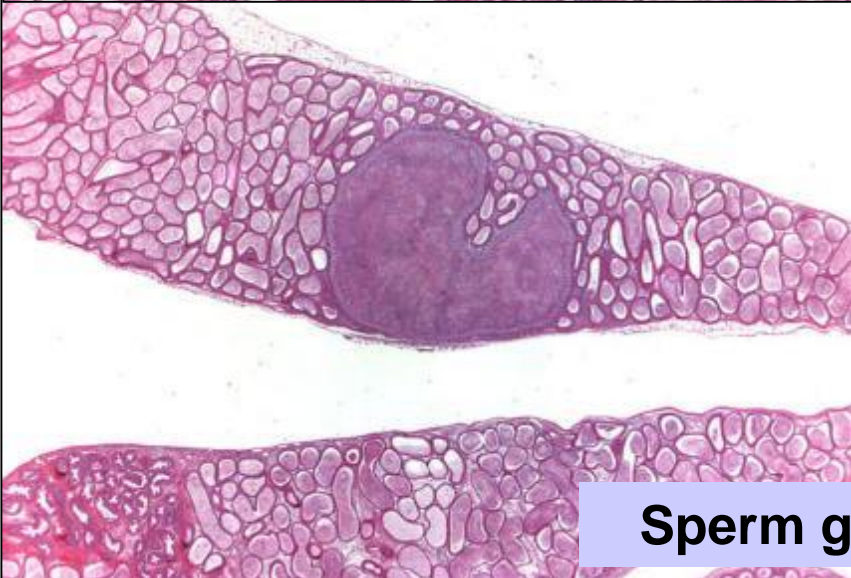
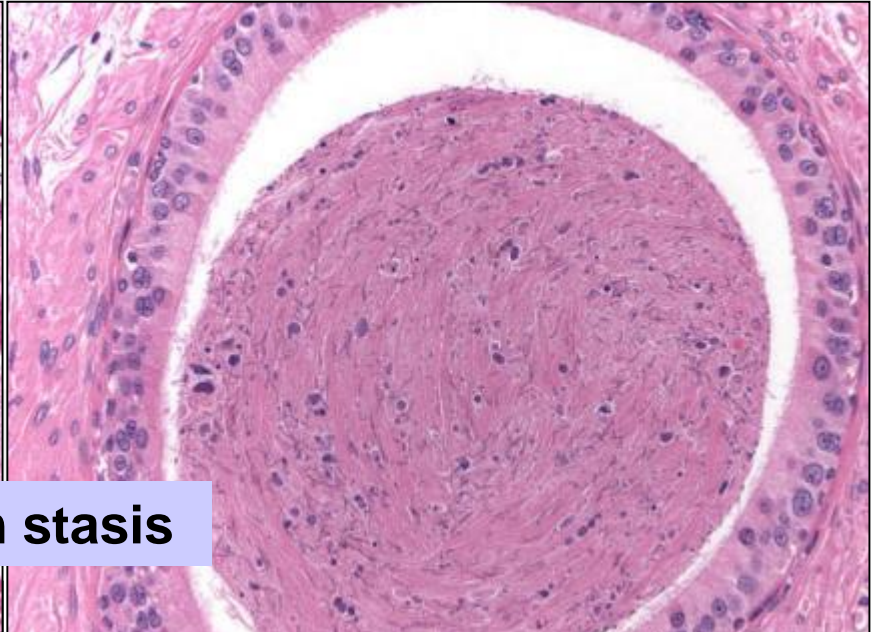
Epididymides - Spontaneous lesions: RccHan™ : WIST (4-Week)

Male	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Numbers of rats examined	1933					
Sperm stasis	1	0.05	0.03	0.57	0.00	10.00
Oligospermia	6	0.31	0.36	2.59	0.00	20.00
Aspermia	5	0.26	0.23	1.88	0.00	20.00
Cellular debris	6	0.31	0.32	2.40	0.00	20.00
Epithelial vacuolation	21	1.09	1.36	7.53	0.00	60.00
Interstitial edema	1	0.05	0.03	0.57	0.00	10.00
Mononuclear cell foci	150	7.76	7.67	19.59	0.00	100.00
Mixed cell infiltration	4	0.21	0.13	2.28	0.00	40.00
Sperm granuloma	6	0.31	0.88	8.28	0.00	100.00
Peritonitis	1	0.05	0.05	0.95	0.00	16.67
Inflammation	2	0.10	0.39	5.81	0.00	100.00
Capsular inflammation	2	0.10	0.11	1.90	0.00	33.33
Atrophy	2	0.10	0.10	1.27	0.00	20.00
Epithelial hypertrophy	1	0.05	0.06	1.14	0.00	20.00
Intratubular cell debris	1	0.05	0.06	1.14	0.00	20.00

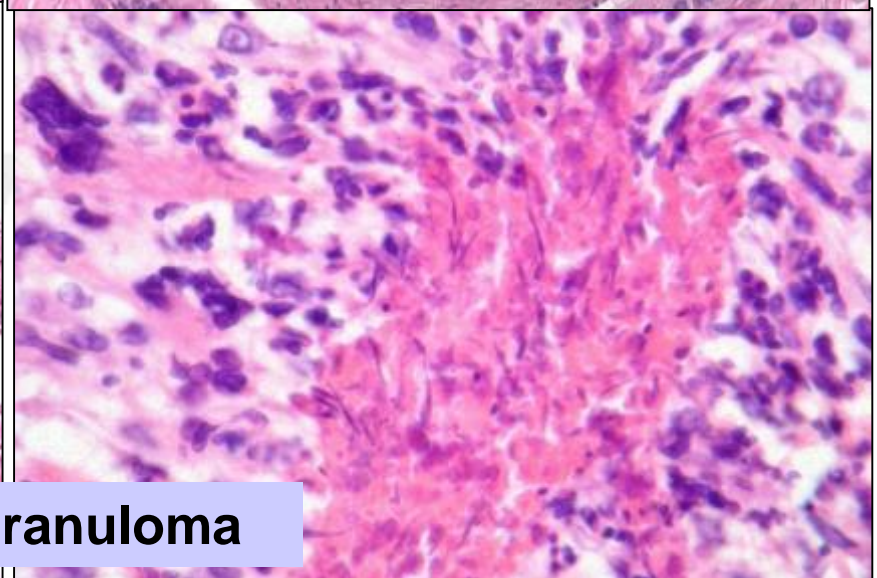
Spontaneous/Induced Lesions – Epididymides, Rat



Sperm stasis



Sperm granuloma

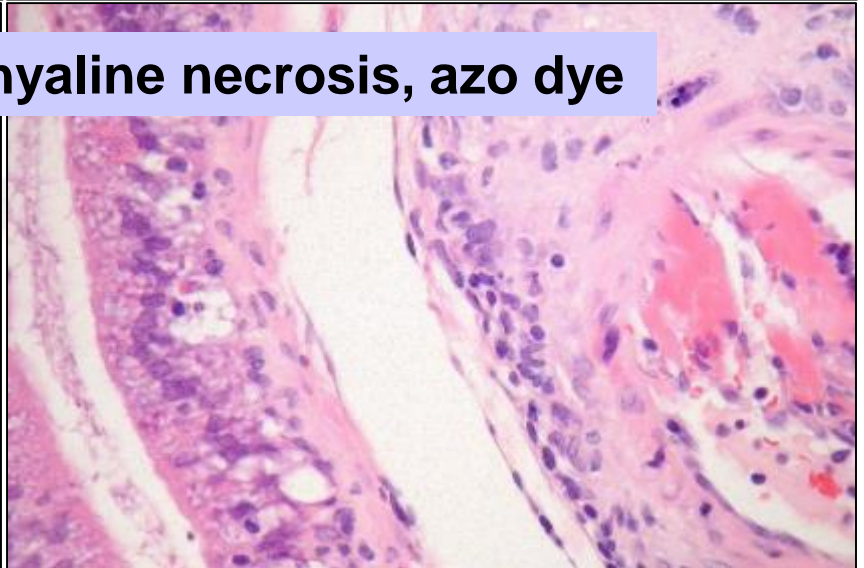


Induced Lesions – Epididymides, Rat

**Aspermia,
PDE4 inhibitor**

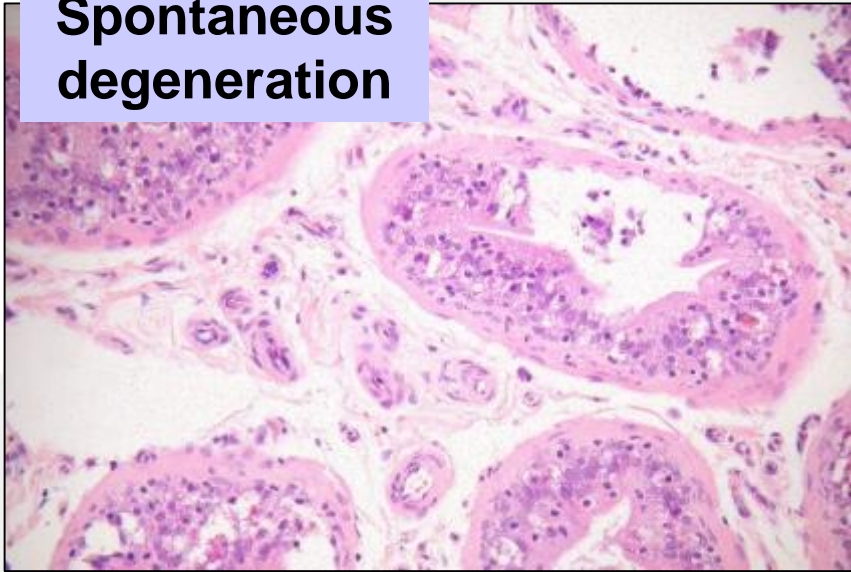
**Detritus,
azo dye**

Epithelial degeneration, hyaline necrosis, azo dye

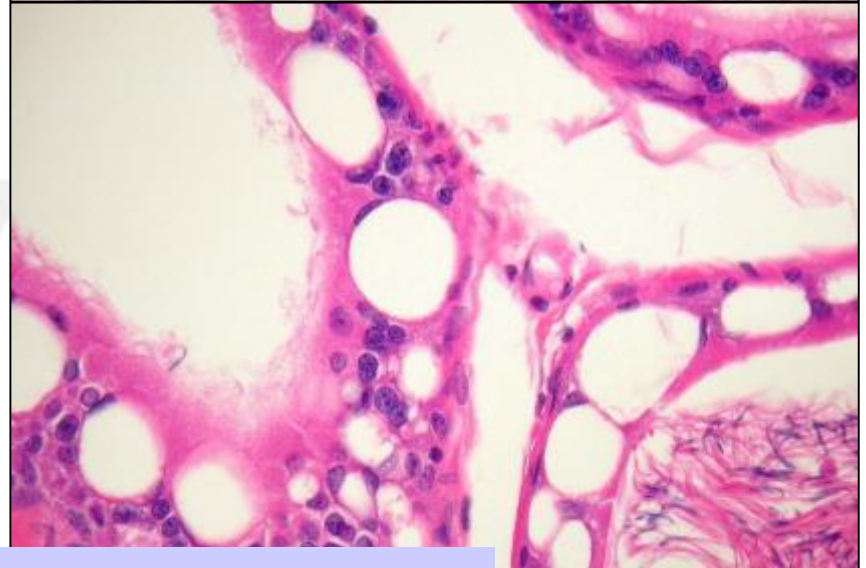
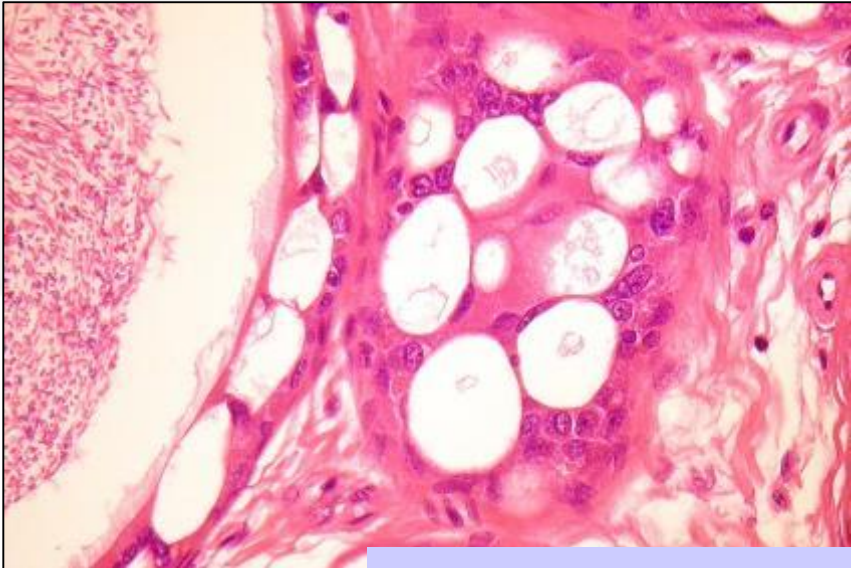
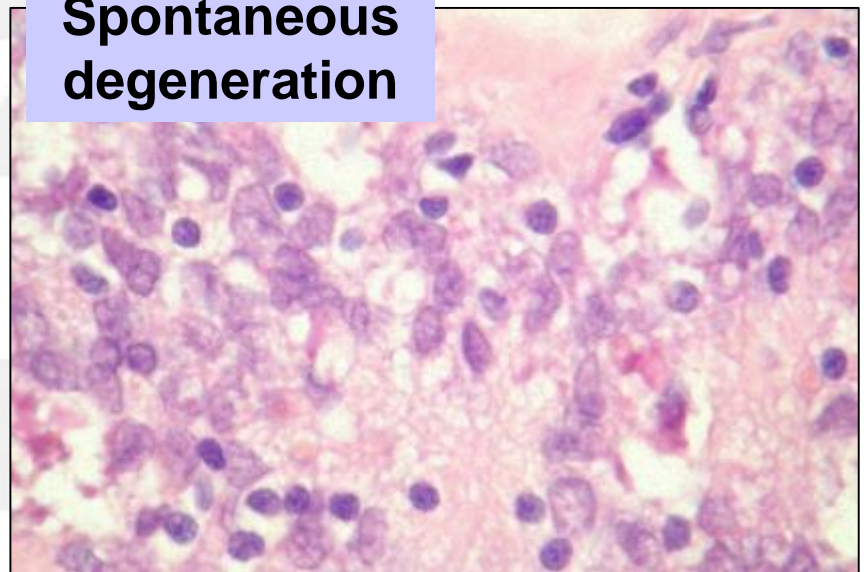


Spontaneous/Induced Lesions – Epididymides, Rat

Spontaneous degeneration



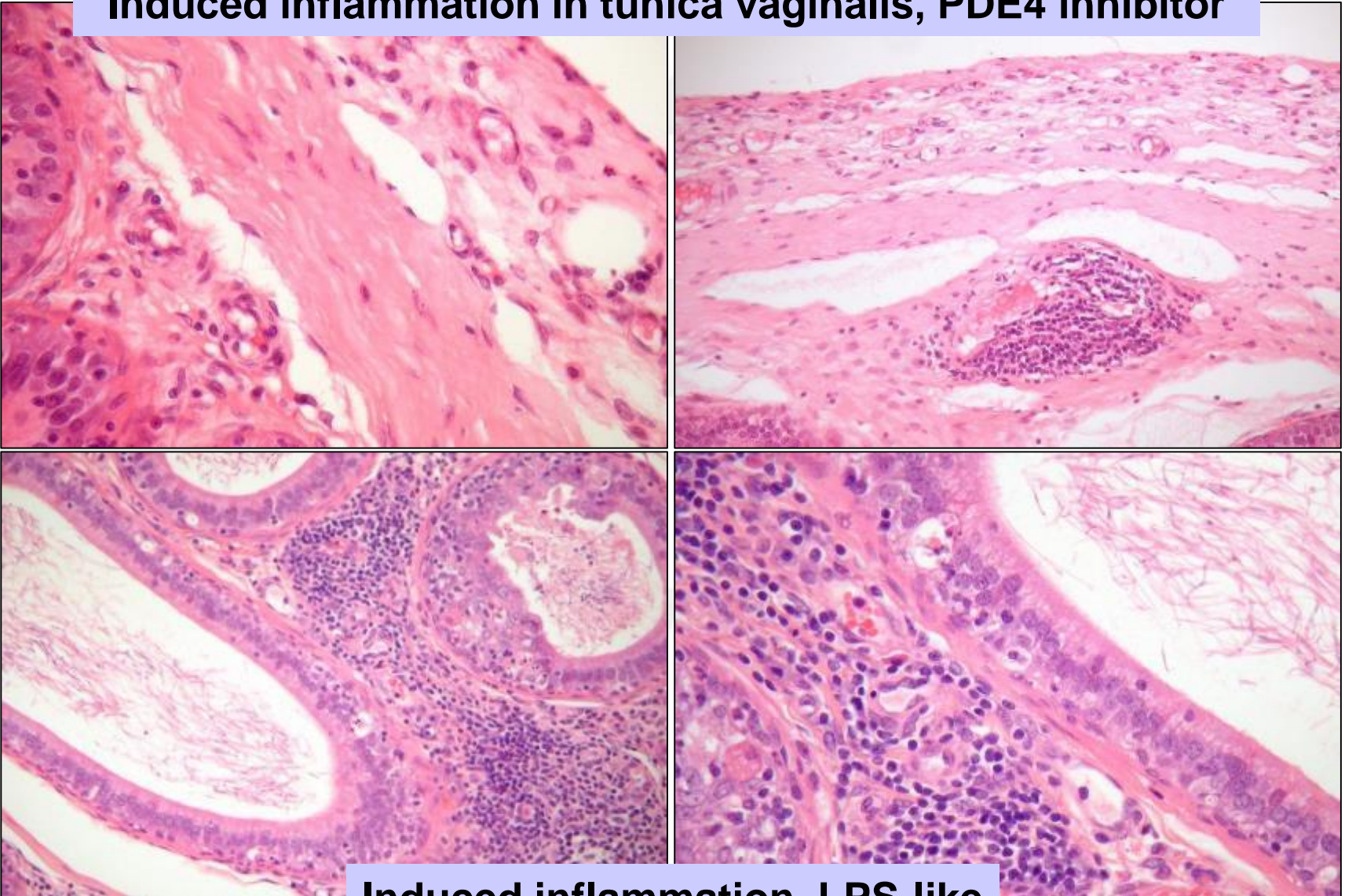
Spontaneous degeneration



Induced vacuolation, phytoestrogen

Induced Lesions – Epididymides, Rat

Induced inflammation in tunica vaginalis, PDE4 inhibitor



Induced inflammation, LPS-like

Prostate - Spontaneous lesions: RcchHan™ : WIST (4-Week)

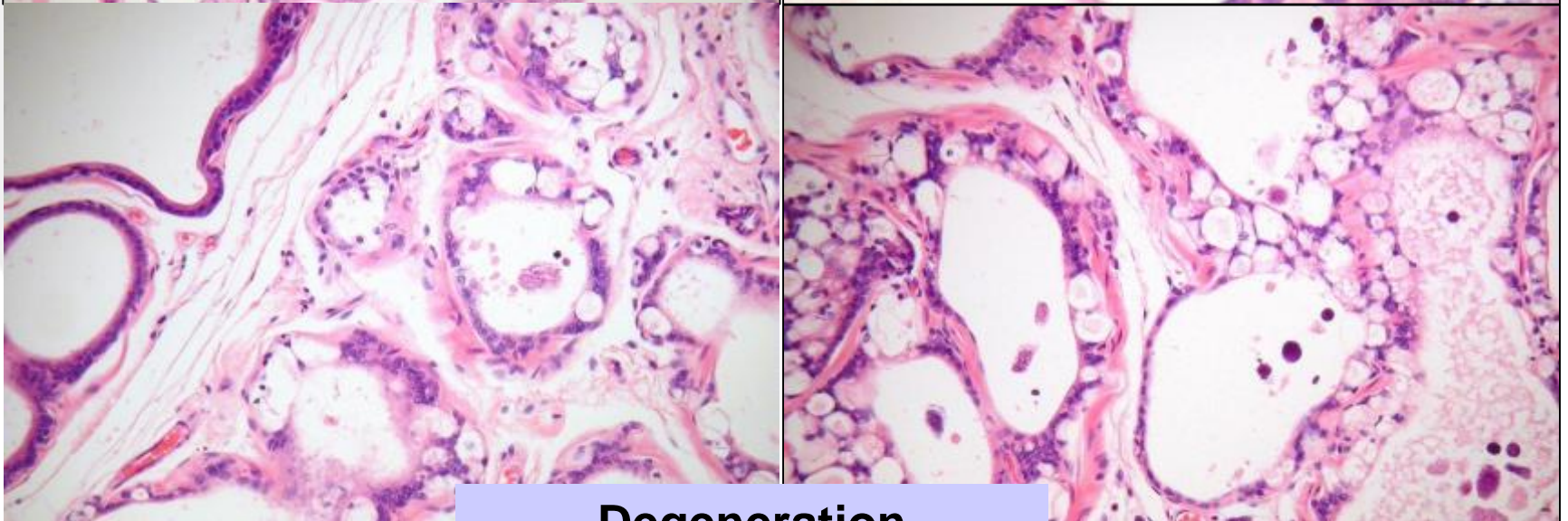
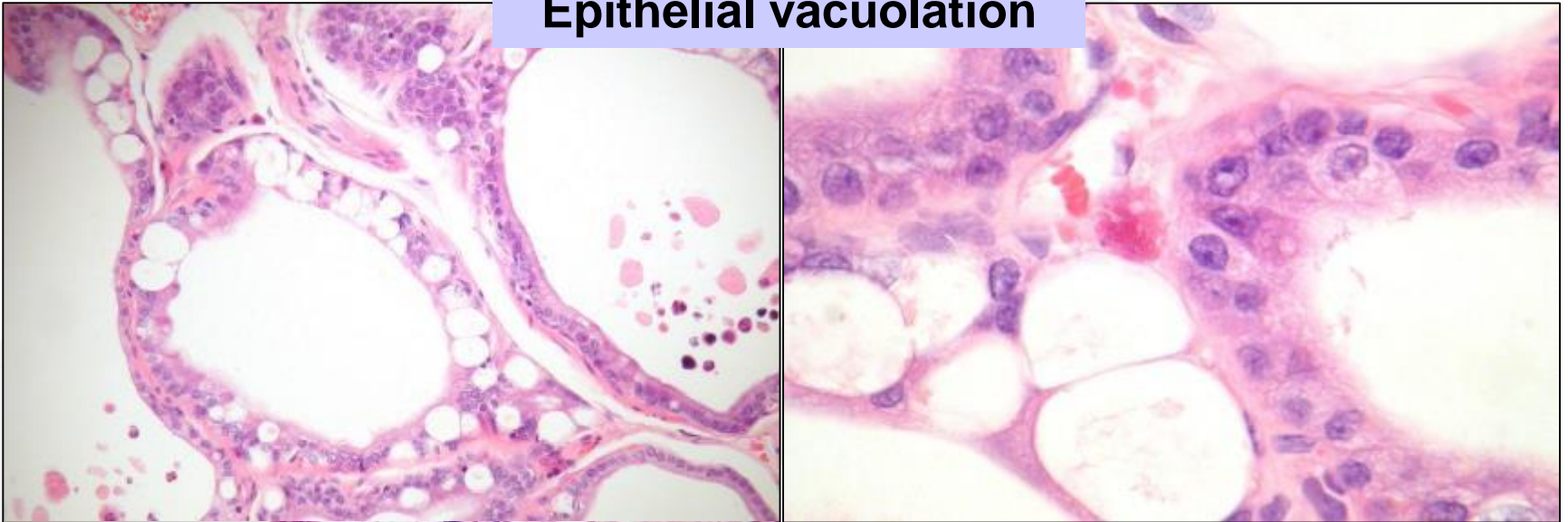
Male	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Numbers of rats examined	1888					
Glandular dilation	5	0.26	0.34	5.83	0.00	100.00
Changed colloid	1	0.05	0.07	1.17	0.00	20.00
Concrements	4	0.21	0.24	2.67	0.00	40.00
Hemorrhage	2	0.11	0.14	1.65	0.00	20.00
Mononuclear cell foci	46	2.44	2.41	8.38	0.00	60.00
Inflammatory cell foci	16	0.85	0.68	3.63	0.00	30.00
Inflammation	53	2.81	2.30	6.65	0.00	40.00
Glandular atrophy	8	0.42	0.31	2.08	0.00	20.00
Focal hyperplasia	3	0.16	0.14	1.65	0.00	20.00

Seminals - Spontaneous lesions: RcchHan™ : WIST (4-Week)

Male	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Numbers of rats examined	1782					
Immature	1	0.06	0.07	1.17	0.00	20.00
Reduced colloid	10	0.56	1.43	10.82	0.00	100.00
Congestion	33	1.85	5.03	20.08	0.00	100.00
Hemorrhage	4	0.22	0.24	2.10	0.00	20.00
Mononuclear cell foci	2	0.11	0.14	1.65	0.00	20.00
Inflammatory cell foci	1	0.06	0.07	1.17	0.00	20.00
Atrophy	6	0.34	0.20	1.64	0.00	20.00

Spontaneous Lesions – Prostate, Rat

Epithelial vacuolation

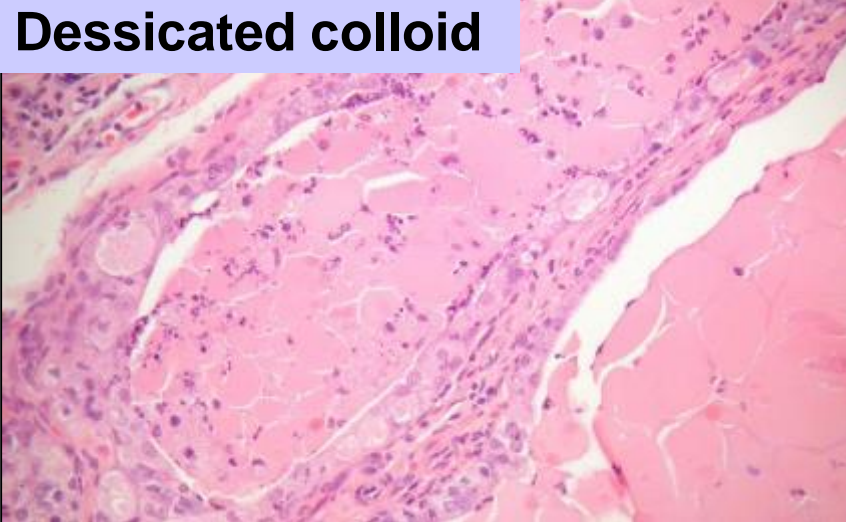


Degeneration

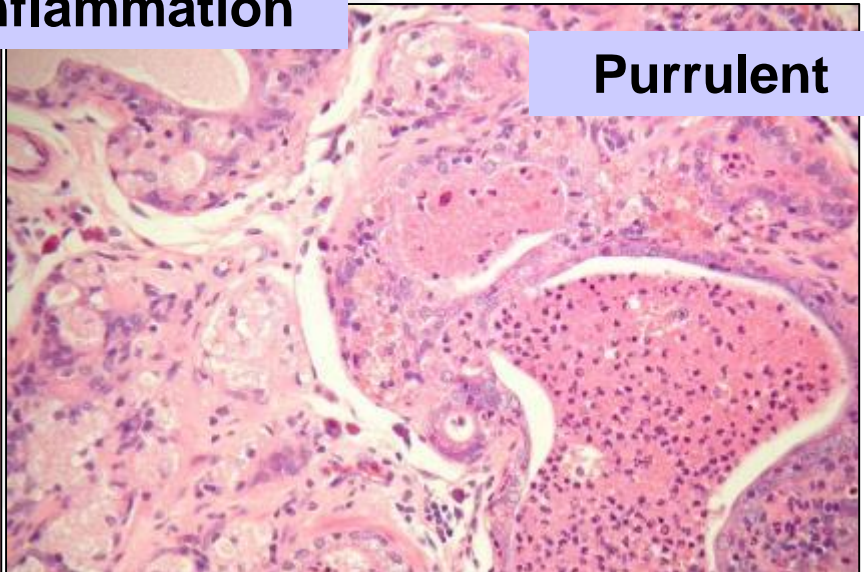
Spontaneous Lesions – Prostate, Rat

Chronic inflammation

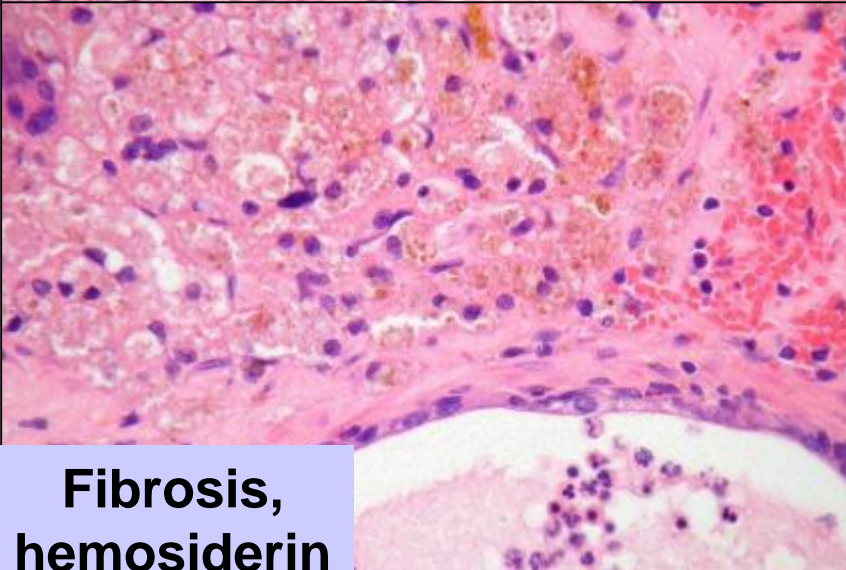
Dessicated colloid



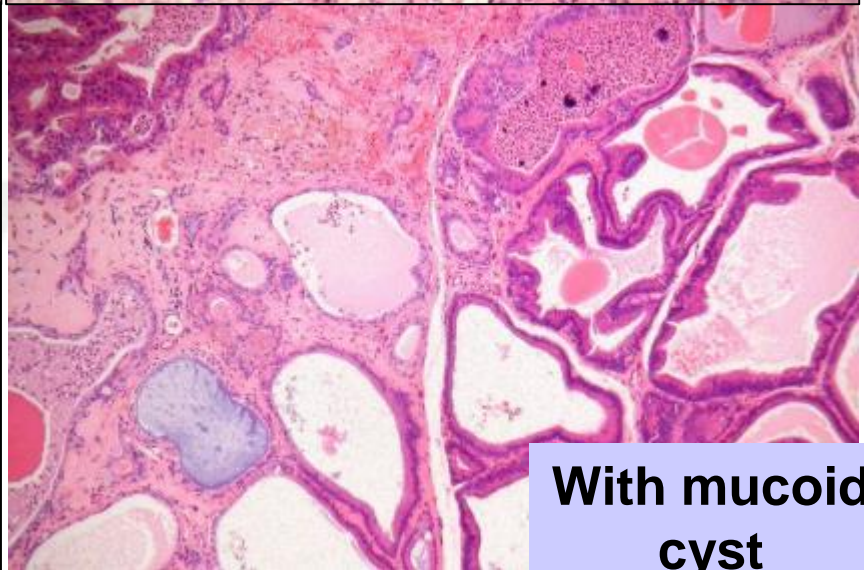
Purulent



Fibrosis,
hemosiderin

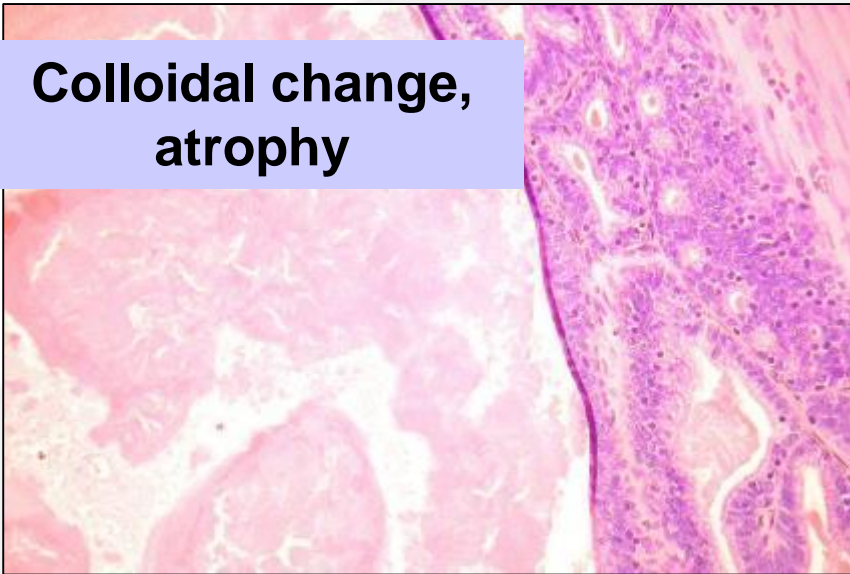


With mucoid
cyst

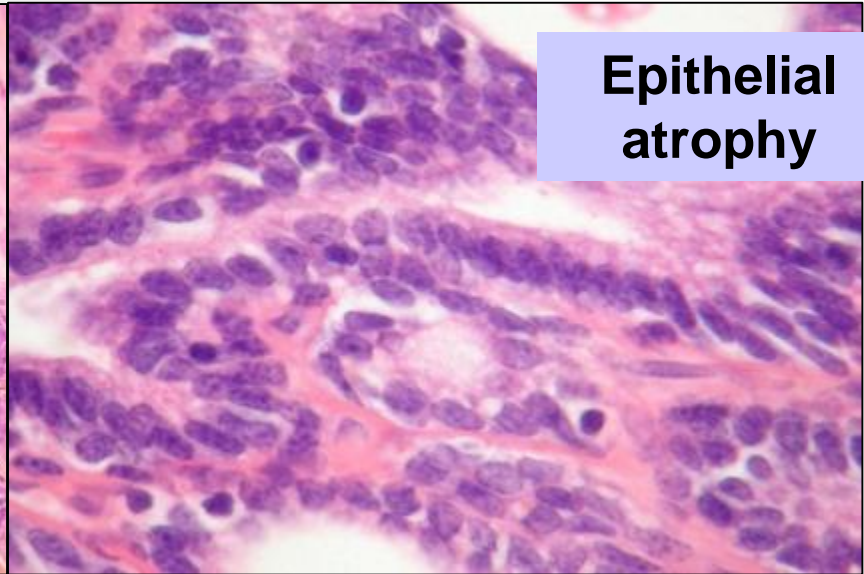


Spontaneous Lesions – Seminal Vesicles, Rat

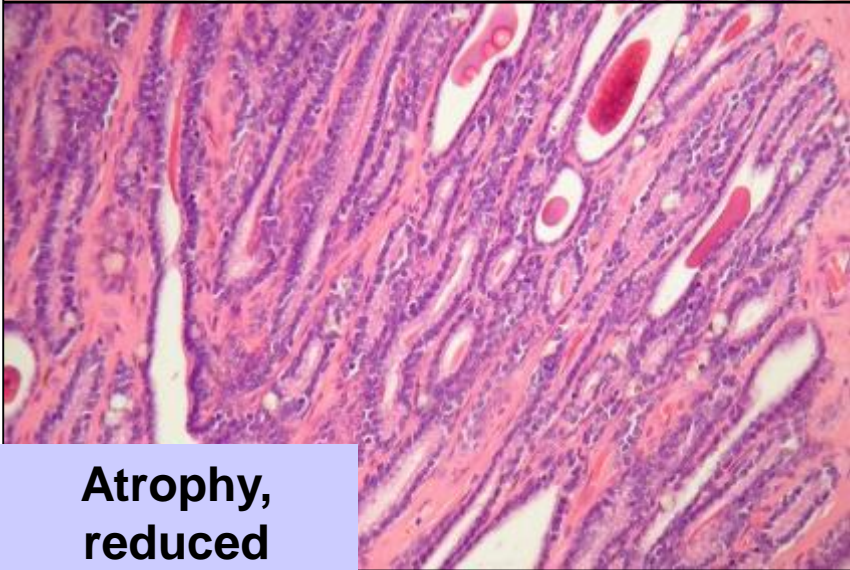
**Colloidal change,
atrophy**



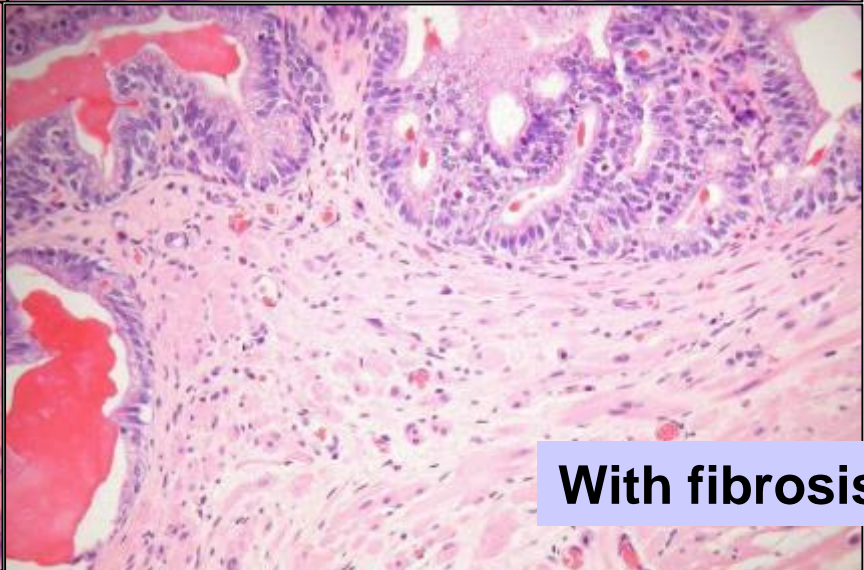
**Epithelial
atrophy**



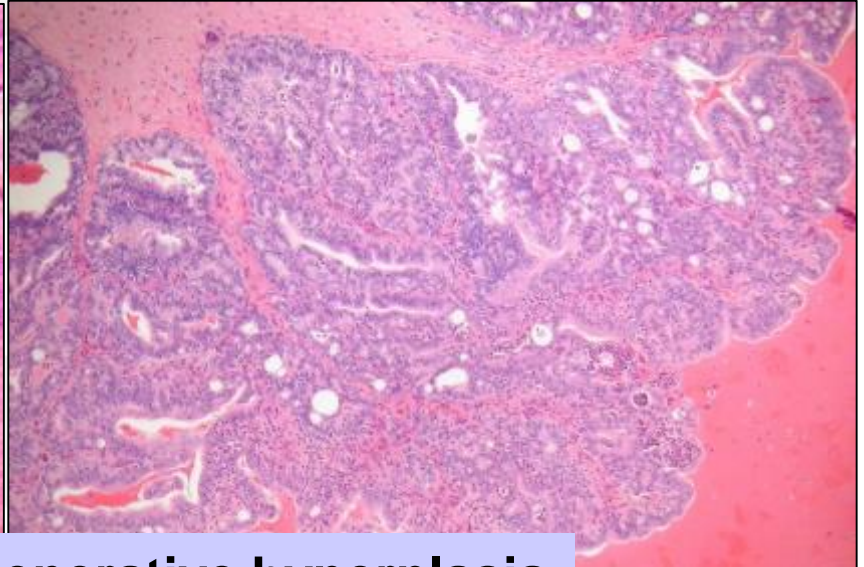
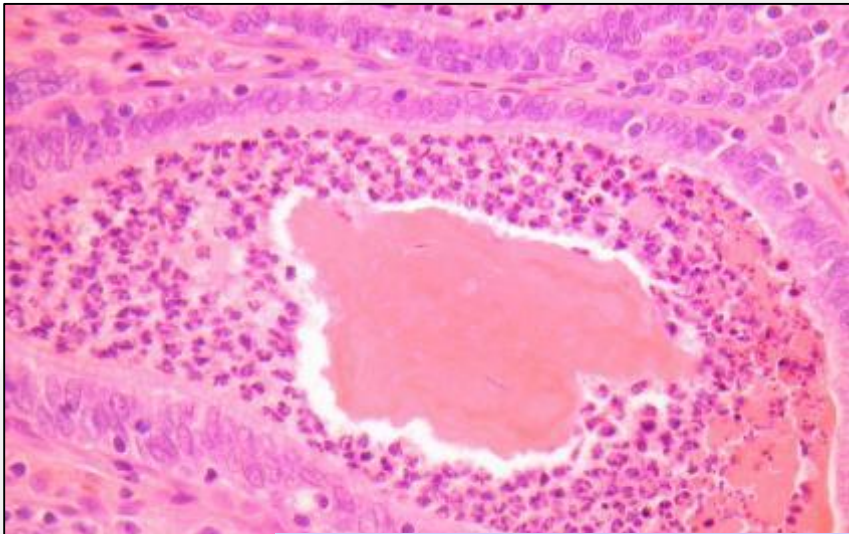
**Atrophy,
reduced
colloid**



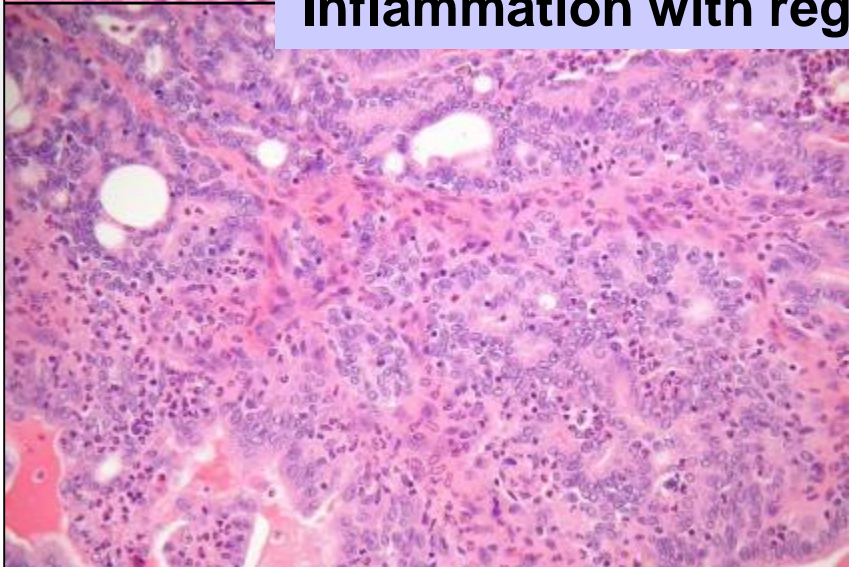
With fibrosis



Spontaneous Lesions – Seminal Vesicles, Rat

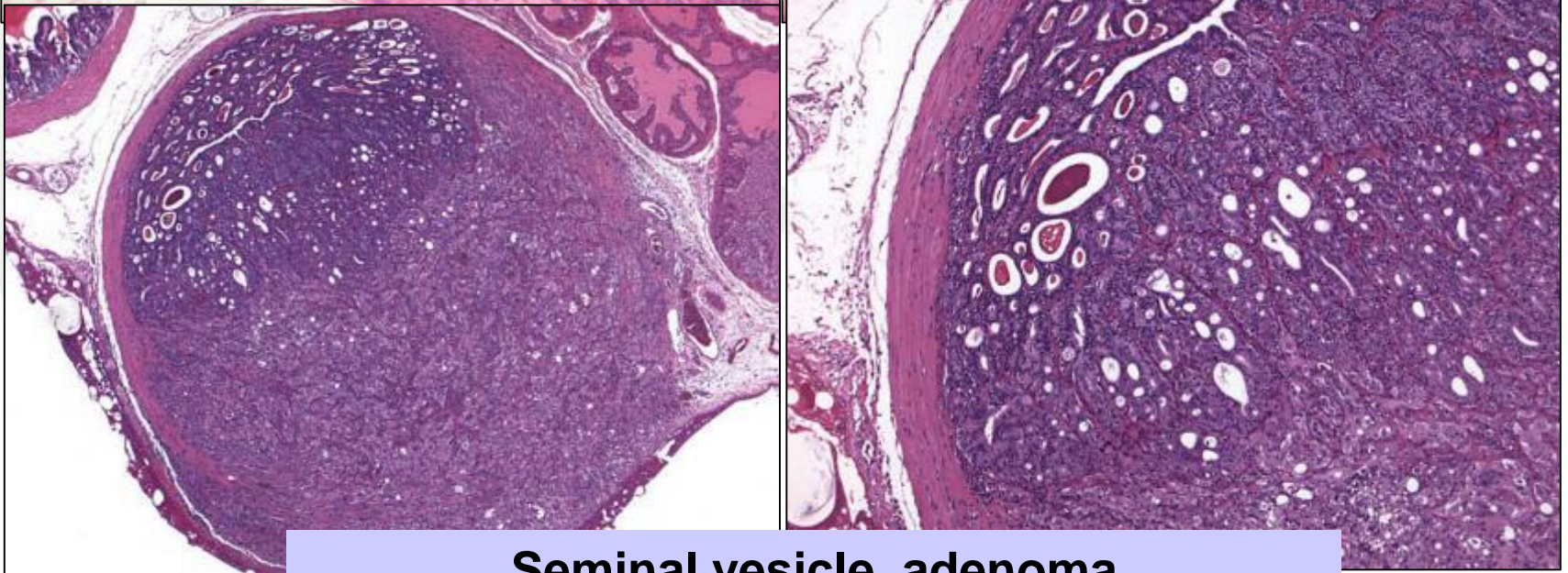
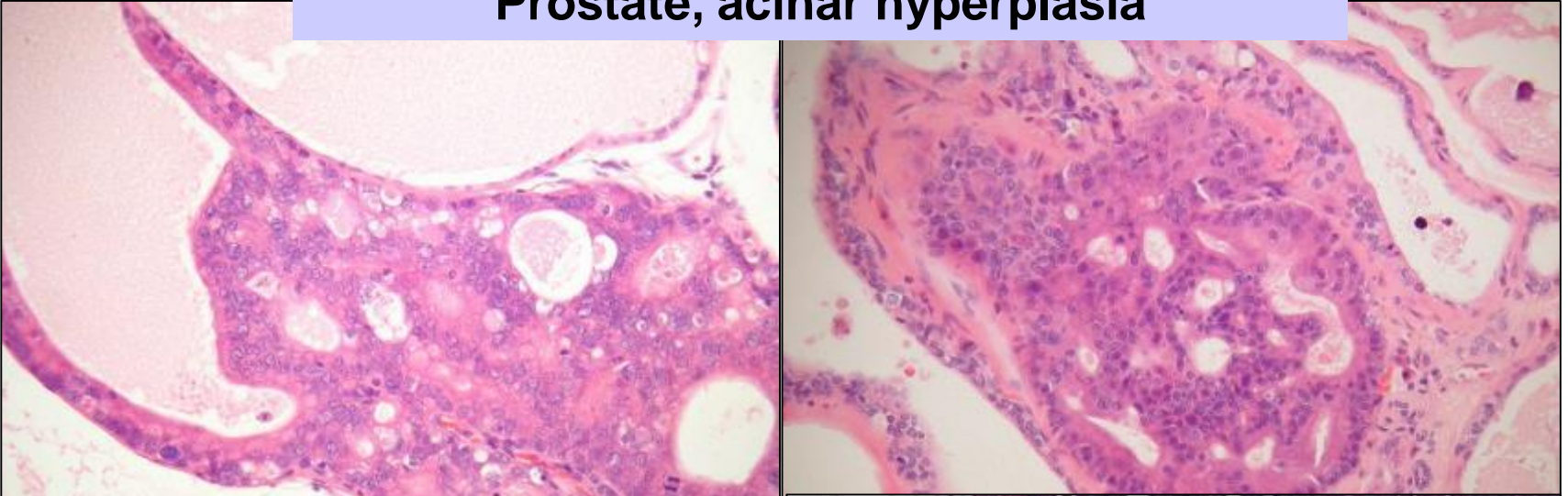


Inflammation with regenerative hyperplasia



Prostate/Seminal Vesicles: Hyperplasia/Adenoma, Rat

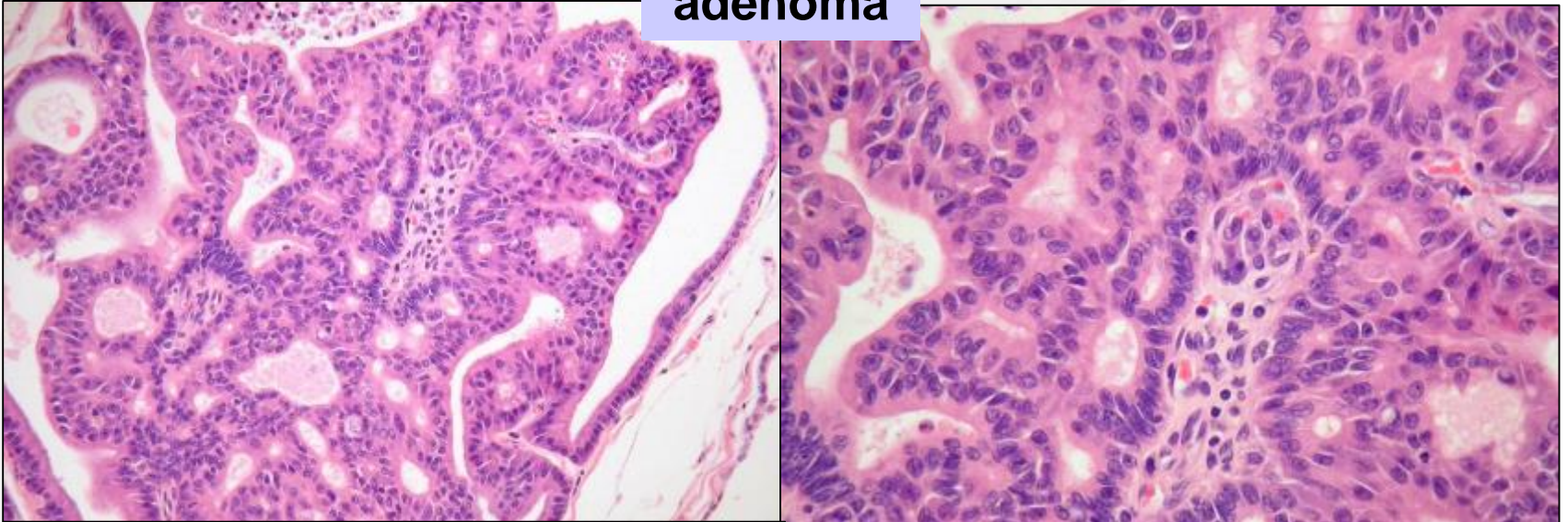
Prostate, acinar hyperplasia



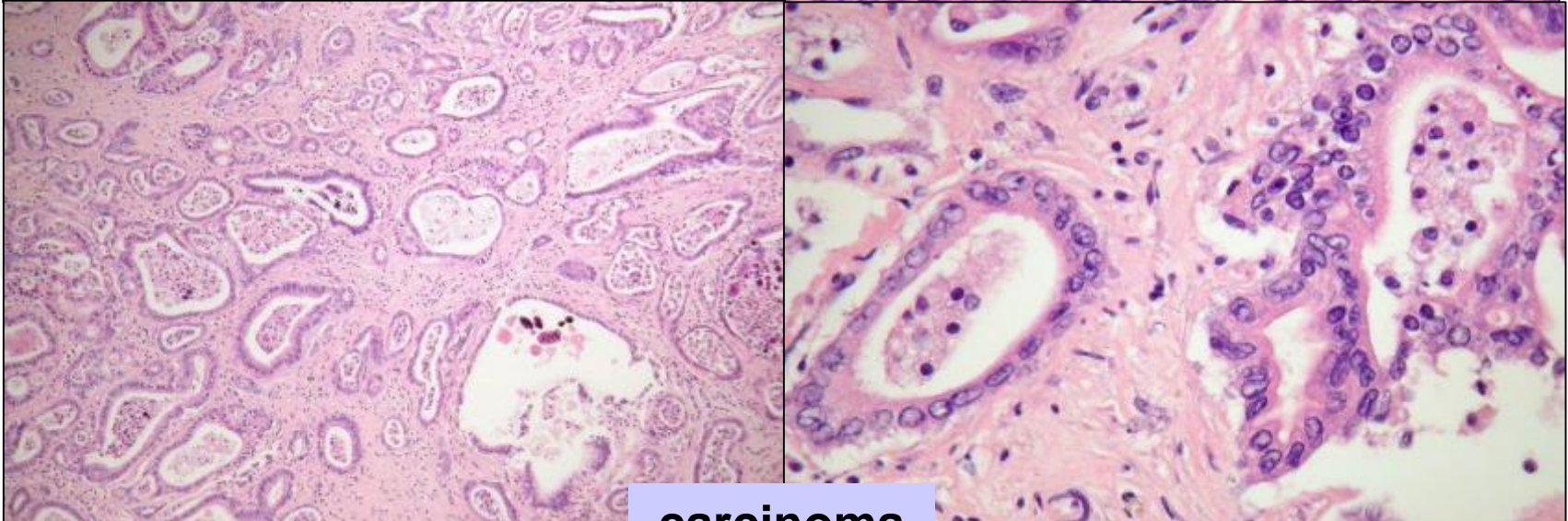
Seminal vesicle, adenoma

Prostate: Adenoma/Carcinoma, Rat

adenoma



carcinoma



Spontaneous lesions: Beagle

Rehm S. Spontaneous testicular lesions in purpose-bred beagle dogs. Toxicol Pathol. 2000, 28:782-787.

... **most significant** finding was bilateral **segmental hypospermatogenesis in (30%)** of the dogs. .. occasionally **associated with giant cells, with cellular debris, and in 40% with atrophic tubules** devoid of germ cells... Focal subcapsular tubular atrophy or **hypoplasia** (tubules lined by Sertoli cells only) found in **26%** of dogs without hypospermatogenesis. Inhibited spermiation with retention of mature sperm in tubules was seen in In conclusion, about 30% of control beagle dogs show segmental hypospermatogenesis, which may be associated with degenerative changes...

Major Differences in Strains: Maturity Males

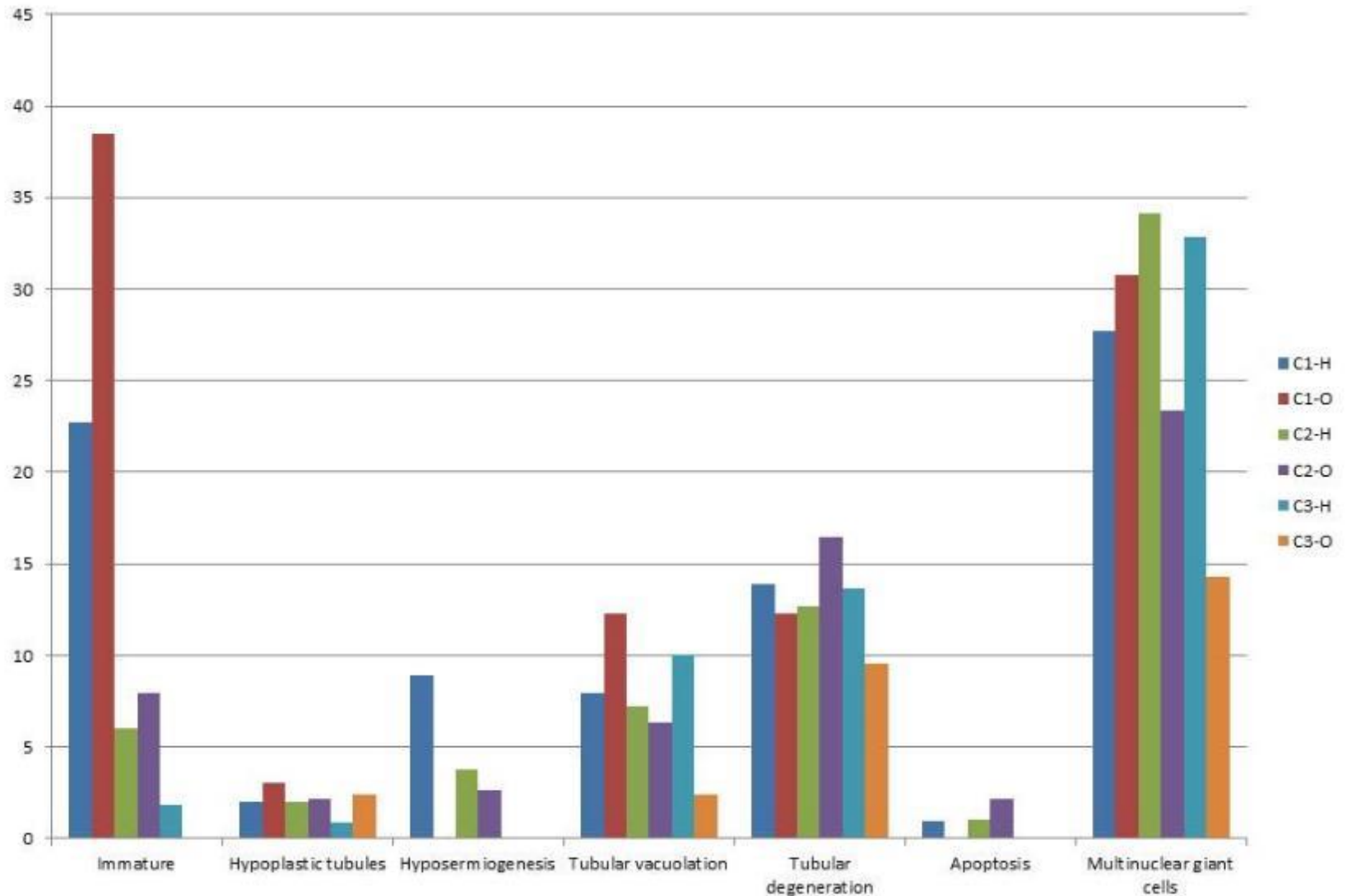
Historical Control Data in 4 Weeks Studies																
	Category ALL				Category 1				Category 2				Category 3			
	COBE		Others		COBE		Others		COBE		Others		COBE		Others	
No. of Studies	81		50		46		31		29		13		4		2	
No. of Pathologists	18		16		14		12		11		6		4		2	
Organ/Finding	COBE		Others		COBE		Others		COBE		Others		COBE		Others	
Testes	315 M	-	189 M	-	179 M	-	122 M	-	118 M	-	44 M	-	12 M	-	8 M	-
Immature	9.56		19.75		15.04		24.66		2.62		15.28	-	0	-	0	-

Historical Control Data in 13 Weeks Studies																
	Category ALL				Category 1				Category 2				Category 3			
	COBE		Others		COBE		Others		COBE		Others		COBE		Others	
No. of Studies	35		20		11		3		18		12		3		3	
No. of Pathologists	14		10		9		3		11		7		3		2	
Organ/Finding	COBE		Others		COBE		Others		COBE		Others		COBE		Others	
Testes	187 M	-	93 M	-	63 M	-	13 M	-	95 M	-	66 M	-	17 M	-	7 M	-
Immature	1.72		7.46		0		25		2.45		5.56	-	0	-	0	-

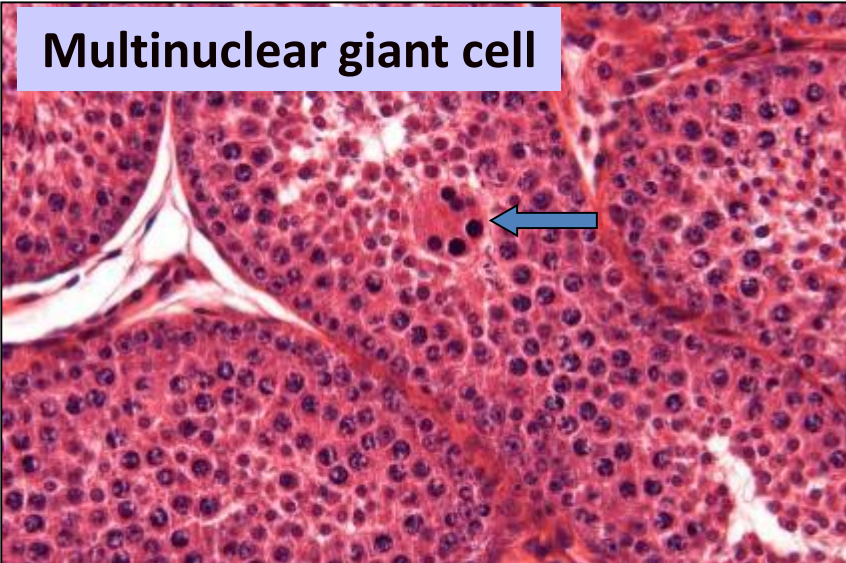
C1=6-8 months, C2=9-11 months, C3=12-14 months, H=Harlan, O=Others

Testes

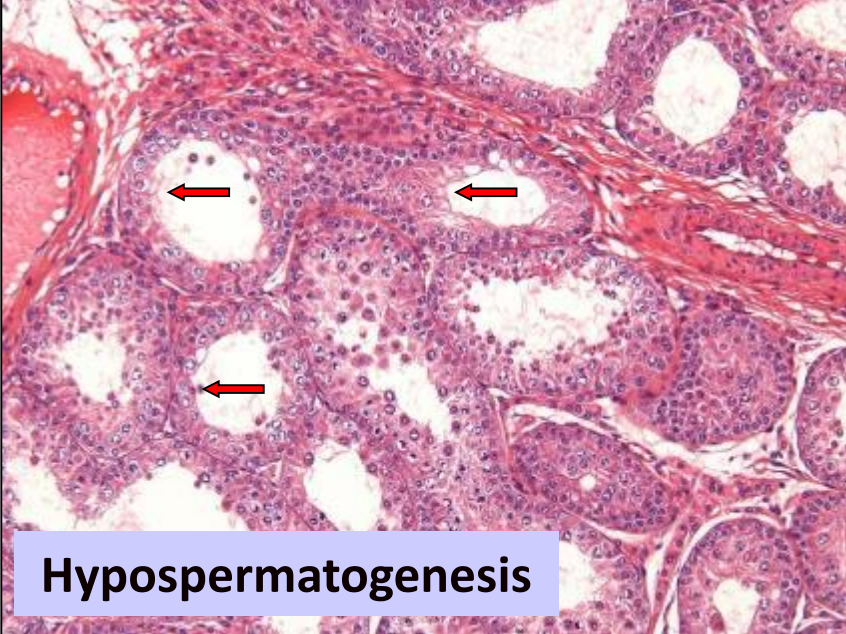
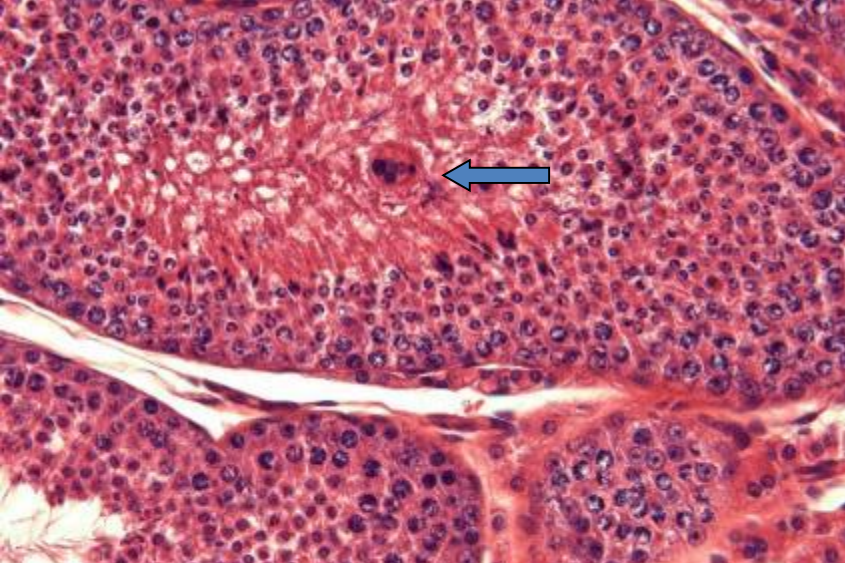
C1=6-8 months, C2=9-11 months, C3=12-14 months, H=Harlan, O=Others



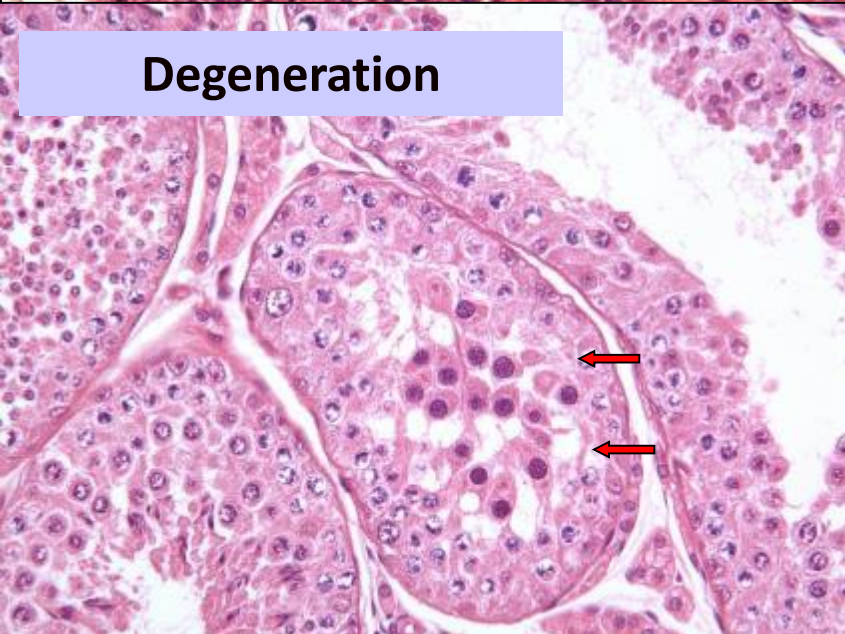
Spontaneous Lesions: DOBE



Multinuclear giant cell



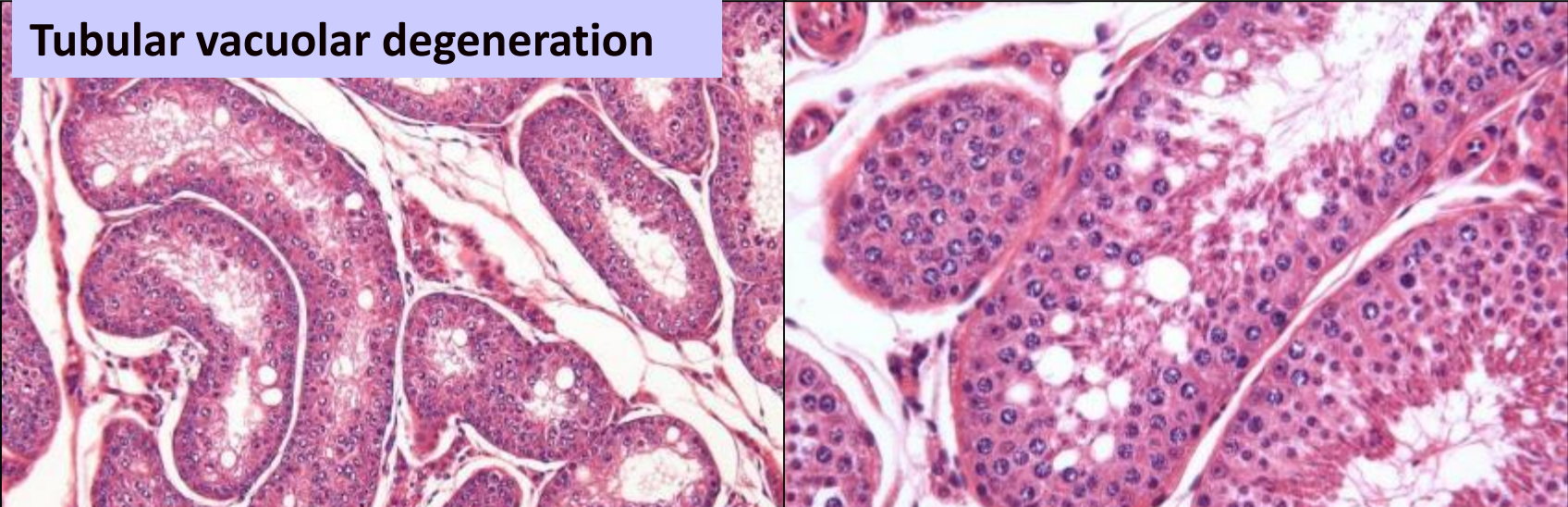
Hypospermatogenesis



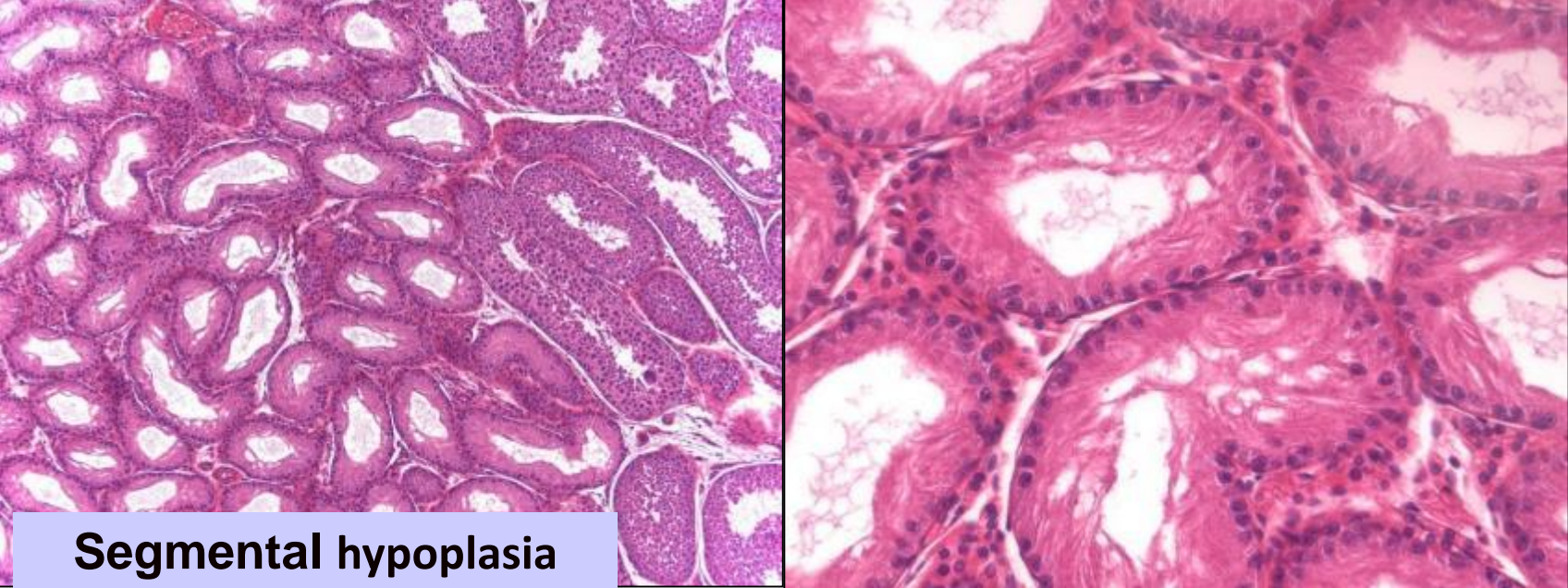
Degeneration

Spontaneous lesions: DOBE

Tubular vacuolar degeneration

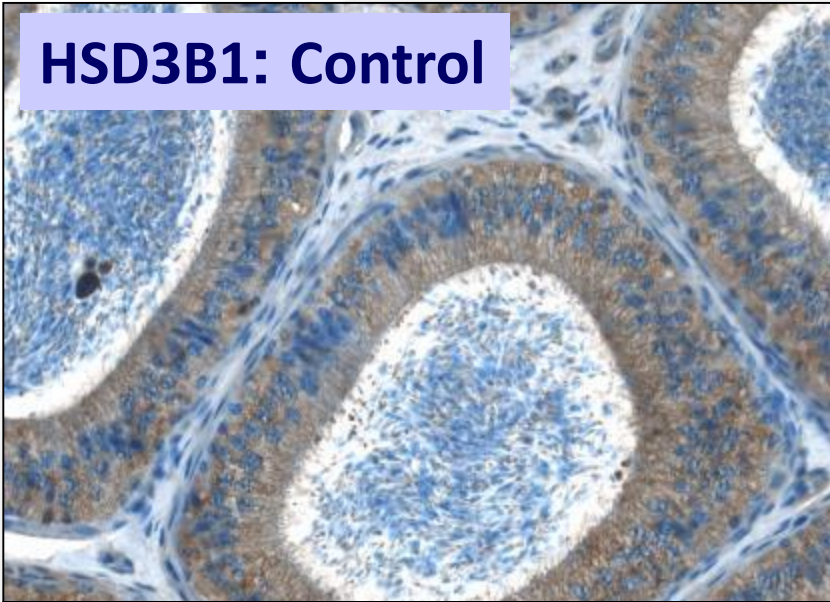


Segmental hypoplasia

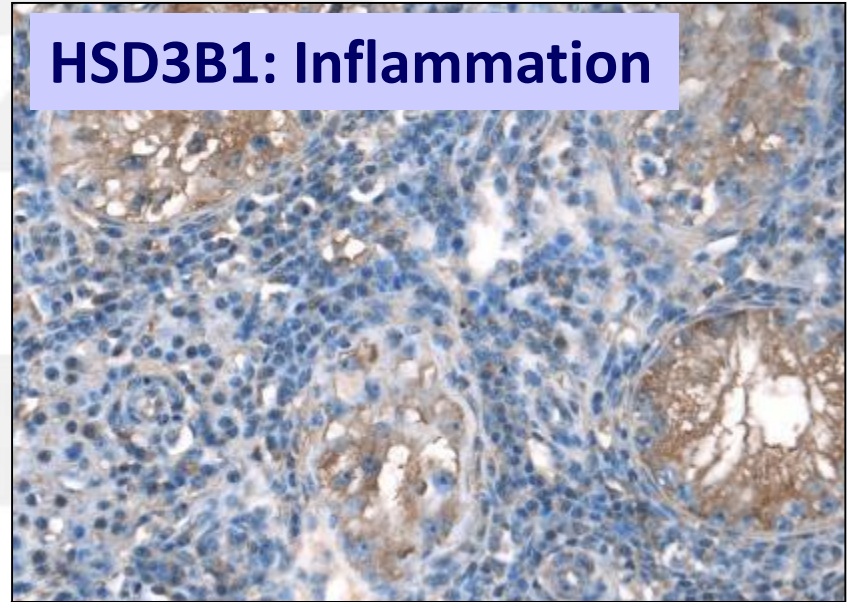


Spontaneous lesions: Marshall (USA)

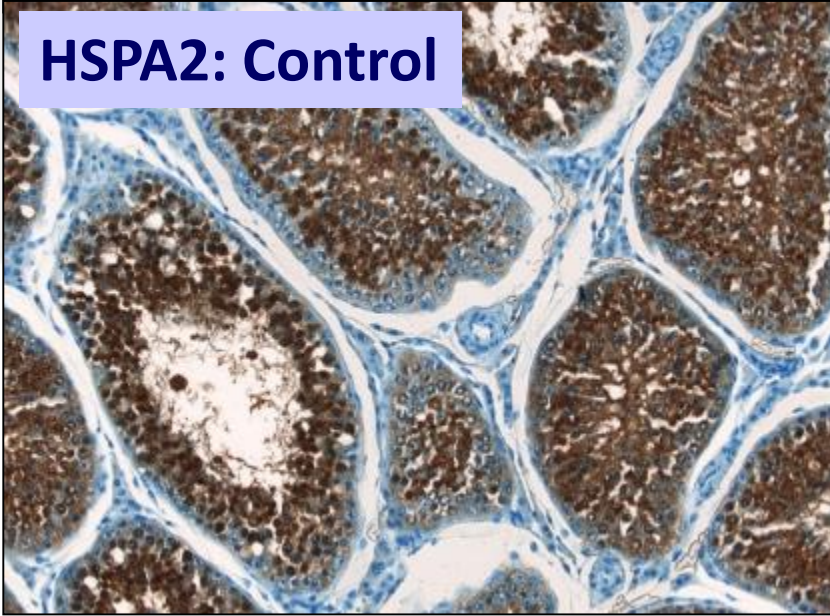
HSD3B1: Control



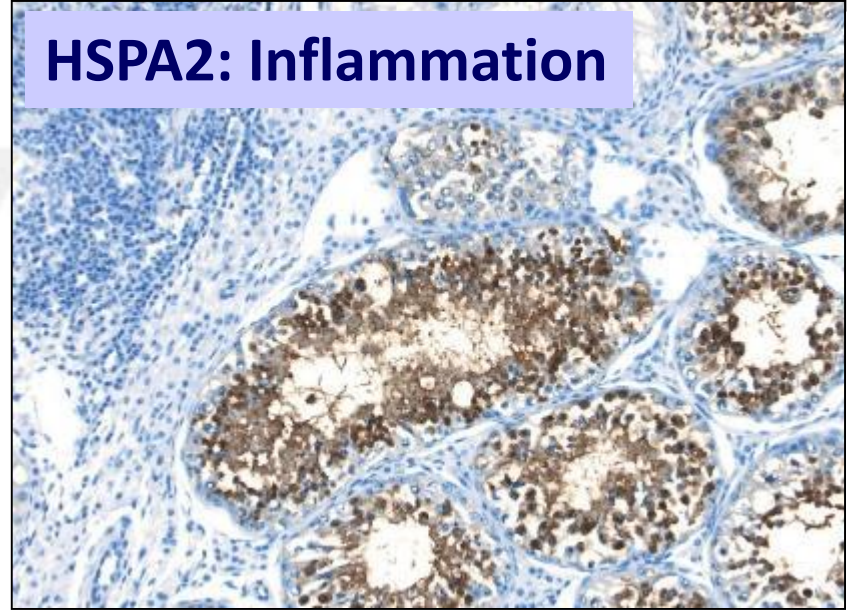
HSD3B1: Inflammation



HSPA2: Control

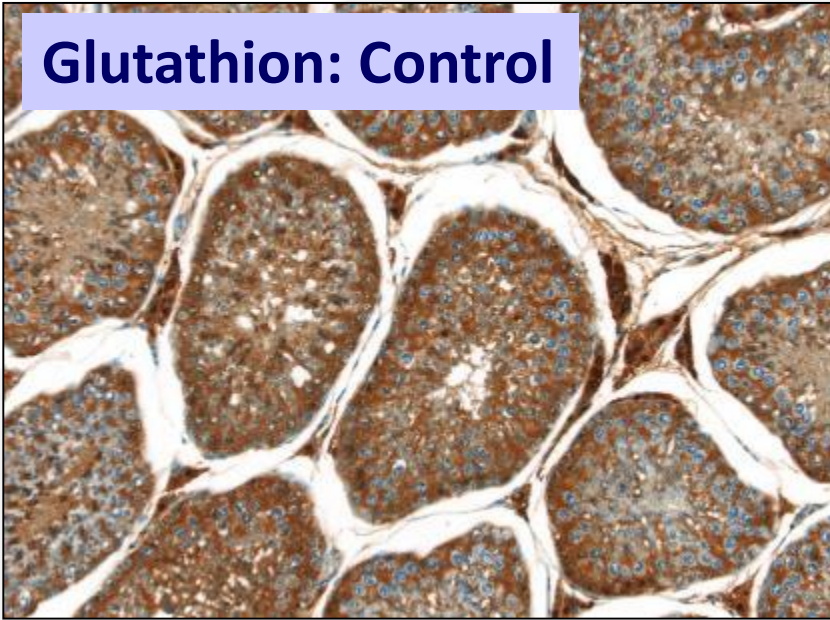


HSPA2: Inflammation

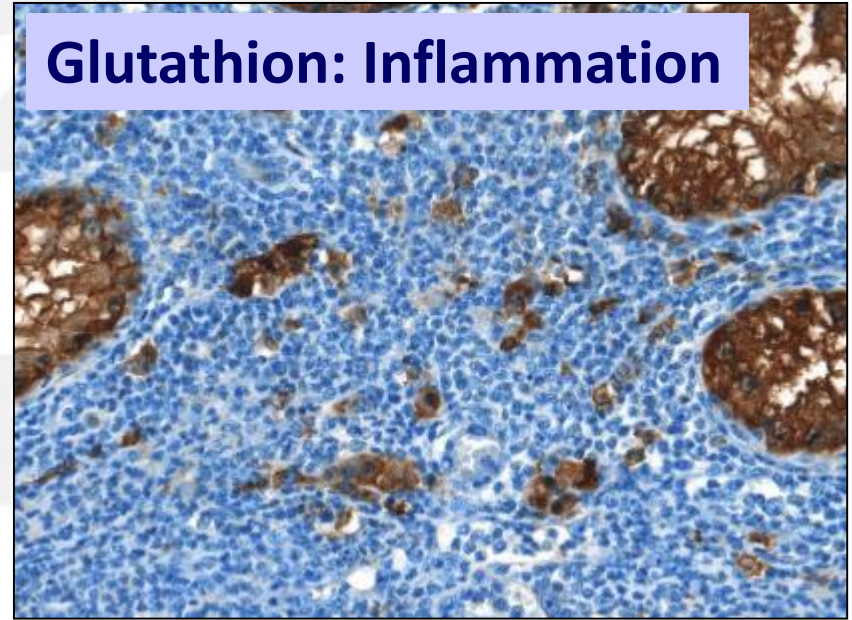


Spontaneous lesions: Beagle

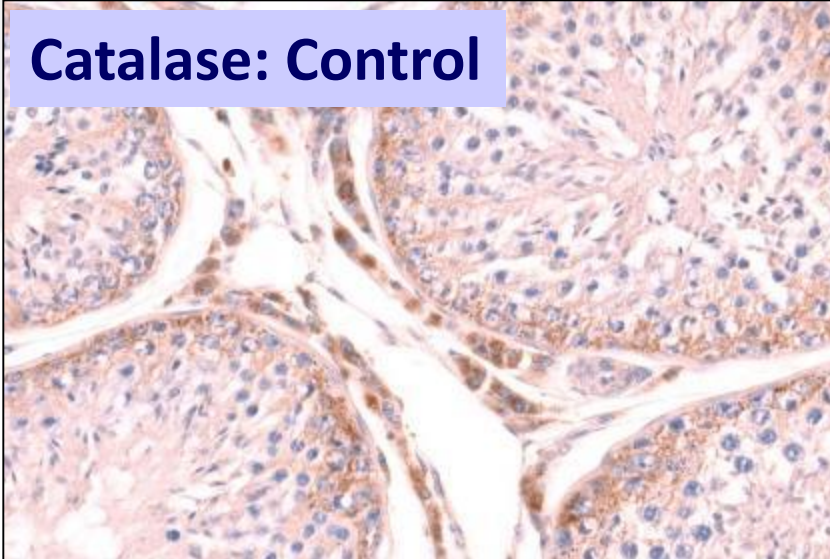
Glutathion: Control



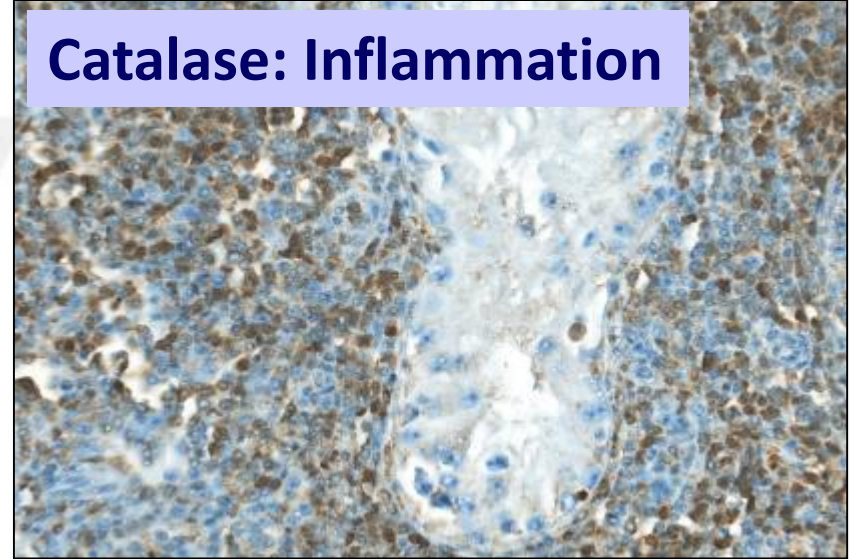
Glutathion: Inflammation



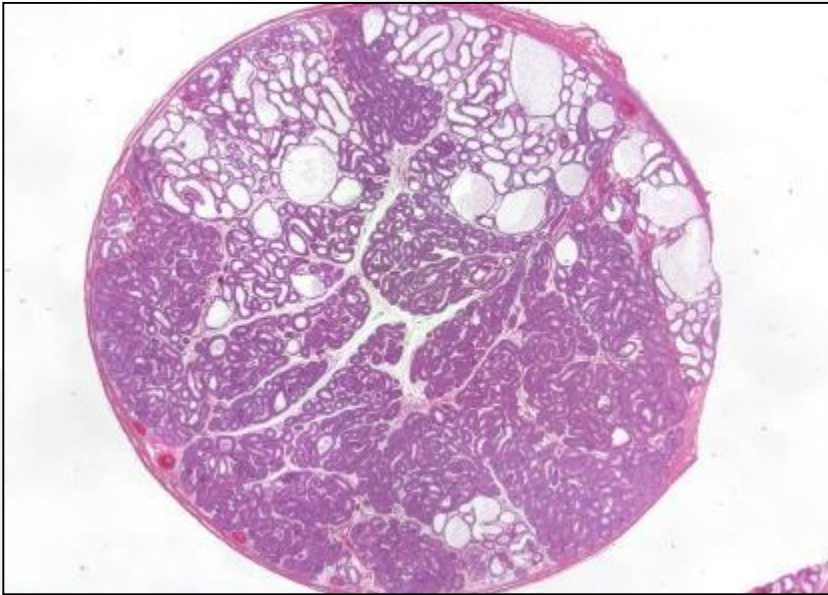
Catalase: Control



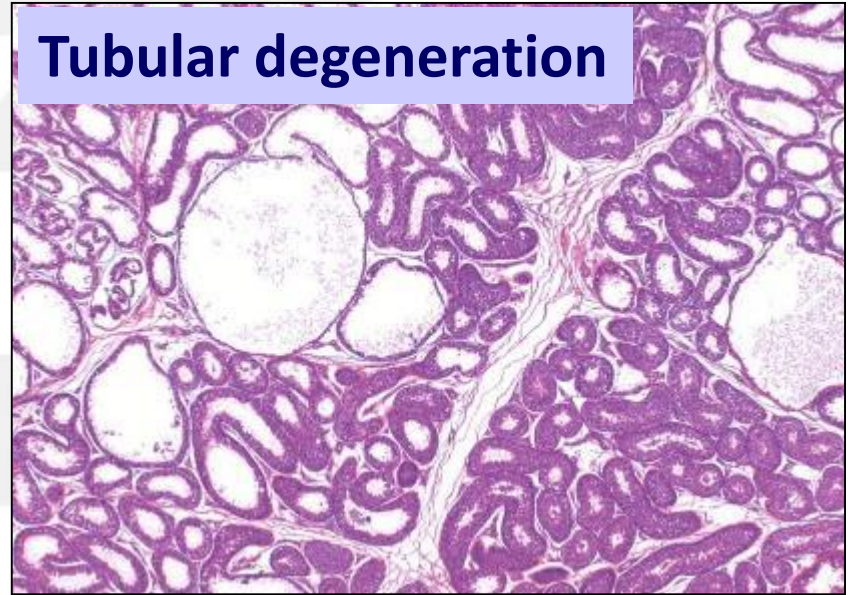
Catalase: Inflammation



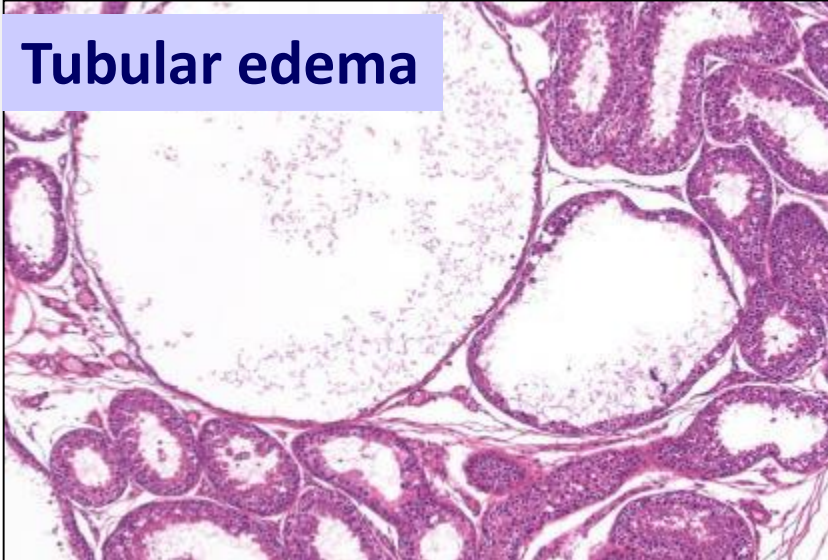
Normal situation: Rabbit



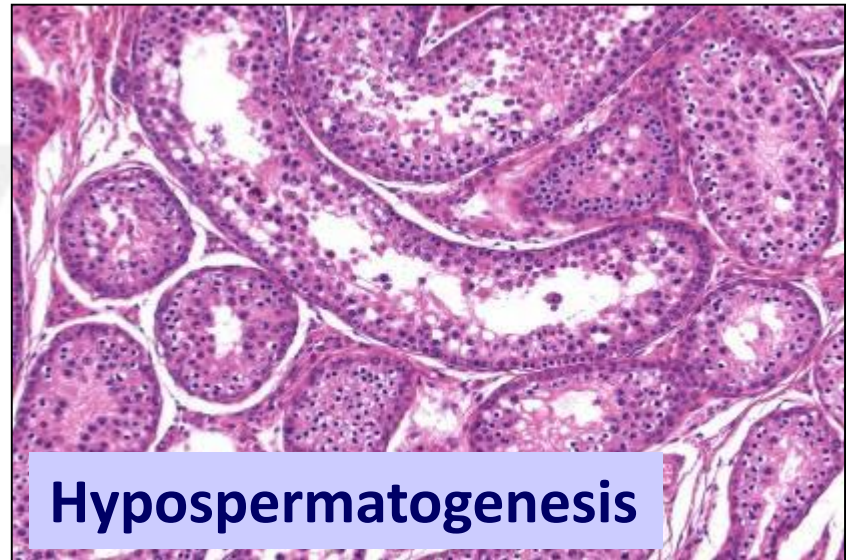
Tubular degeneration



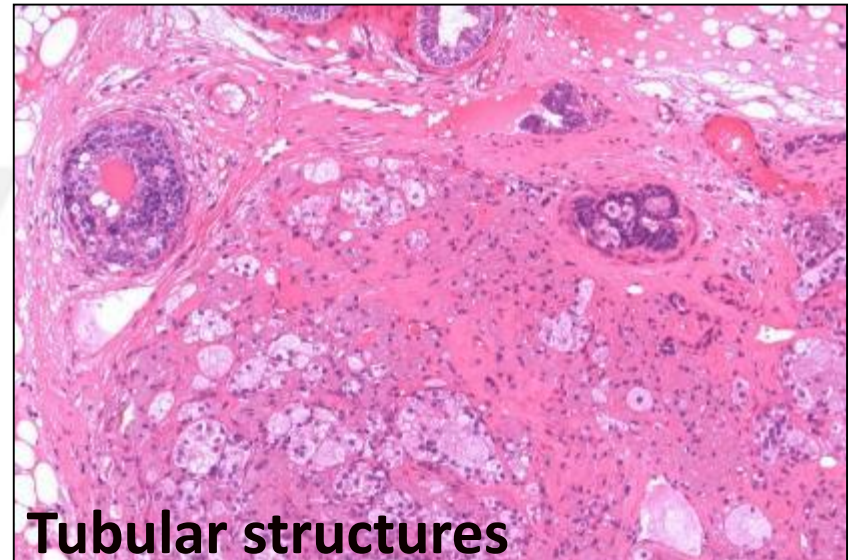
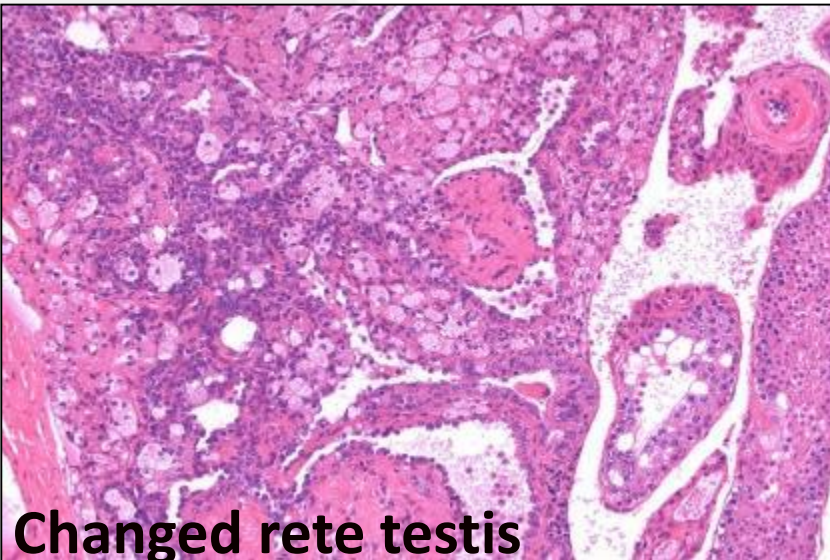
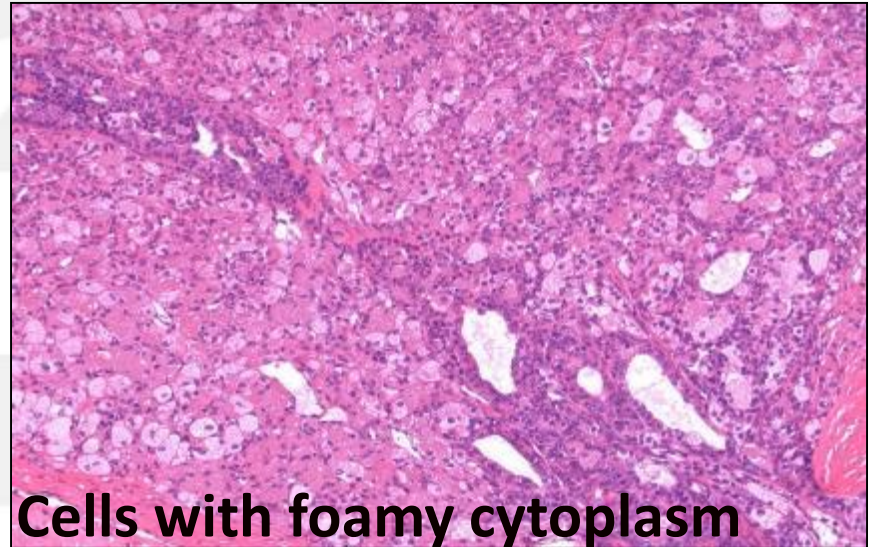
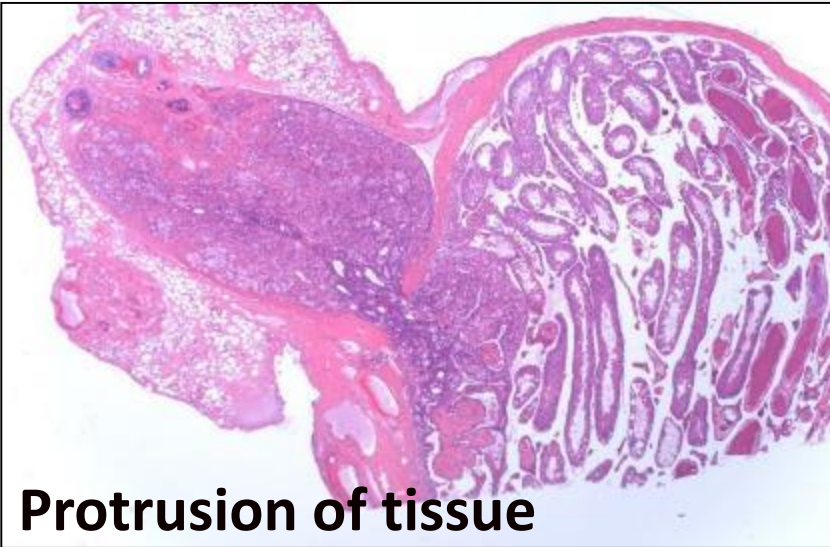
Tubular edema



Hypospermatogenesis



Unusual Lesions: CD-1 Mouse



Conclusions

Knowledge on:

- ✓ **Physiology (hibernating animals)**
- ✓ **Anatomy (rete testes, Leydig cells etc.)**
- ✓ **Consideration of study conditions**
- ✓ **Consideration of related pathology**
- ✓ **Species-related differences**
- ✓ **Proper fixation**
- ✓ **Immunohistochemistry as special tools**
- ✓ **Overview on background lesions**
- ✓ **Qualitative staging**