



CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY REPRODUCTIVE SYSTEM

Third Conference

ORGANIZED BY SOCIETY FOR TOXICOLOGIC PATHOLOGY IN INDIA (STPI)

OCTOBER 29-31, 2010

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



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Review of Histopathological Tools: Testes - Mammalian Model Species

Life Stages, Typical Alterations, Natural
Variability

Klaus Weber, PhD, DVM, MS Biol
Head of Pathology
Chief Scientific Officer

Major Differences

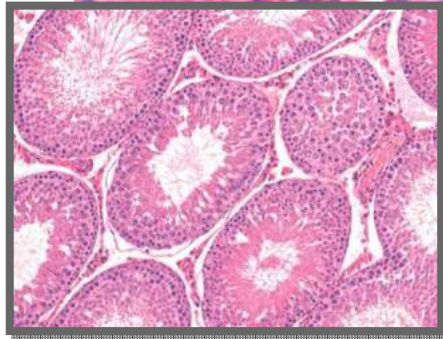
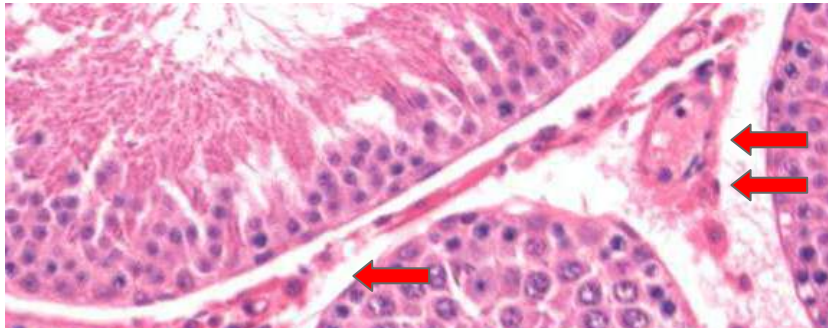
Tubular sections (transversal section):

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

Stages:

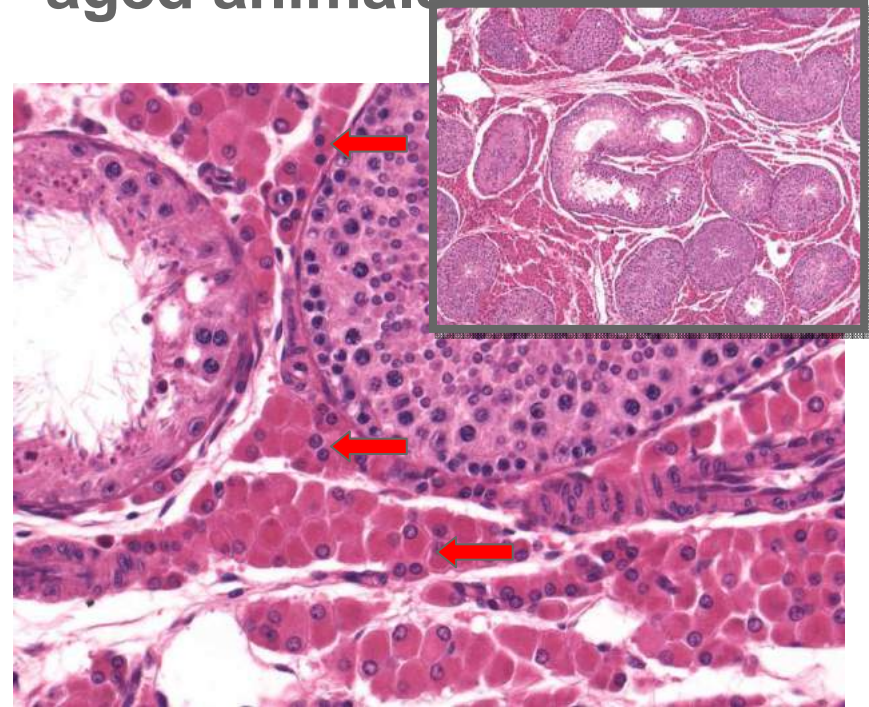
- ✓ Dog: 8
- ✓ Mouse: 12
- ✓ Rat: 14
- ✓ Cynomolgus: 12
- ✓ Human: 6

Major Differences in Testes of Laboratory Animals

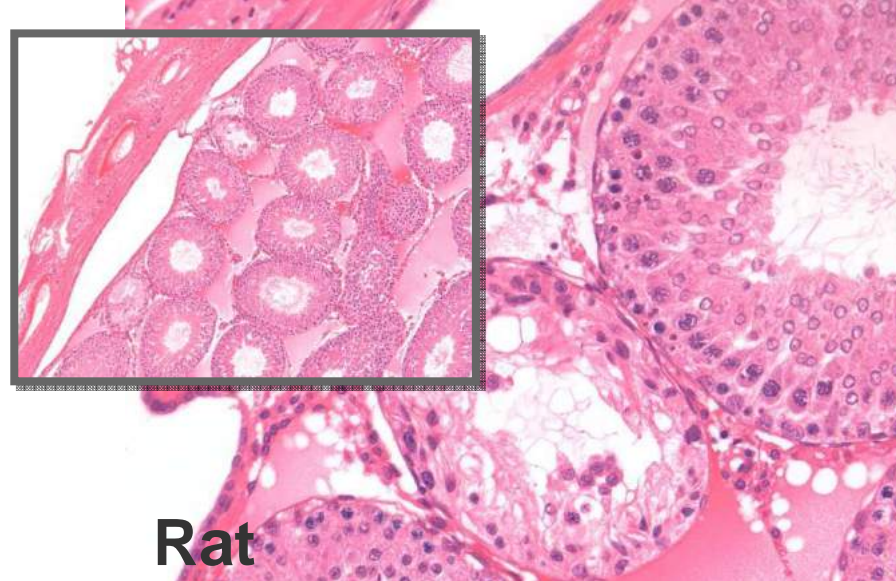
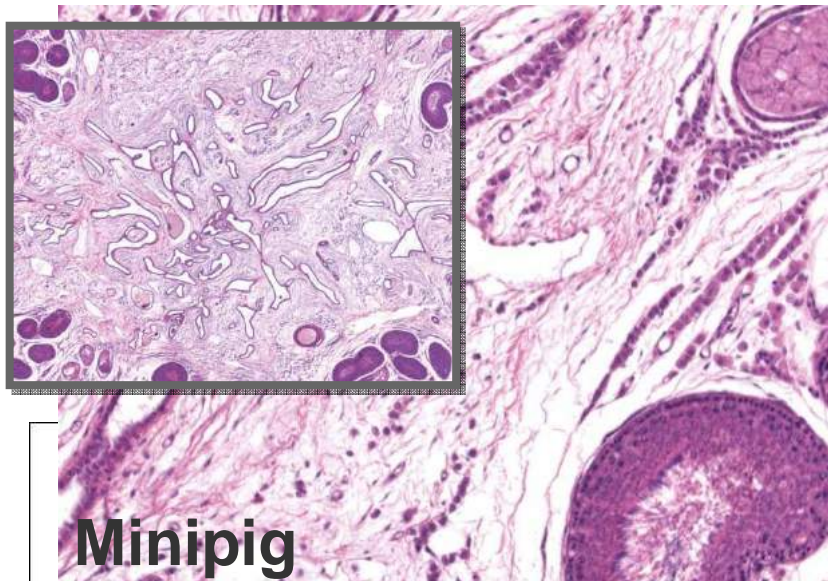
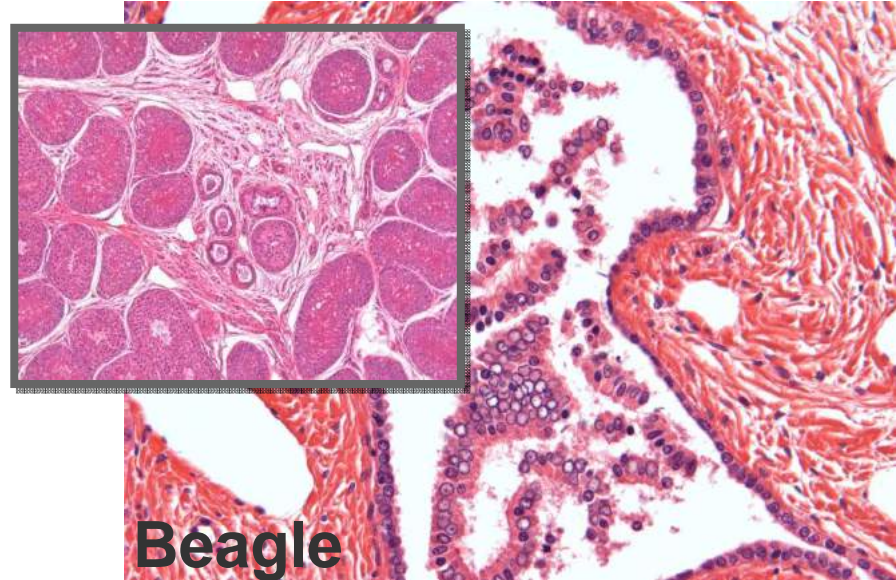
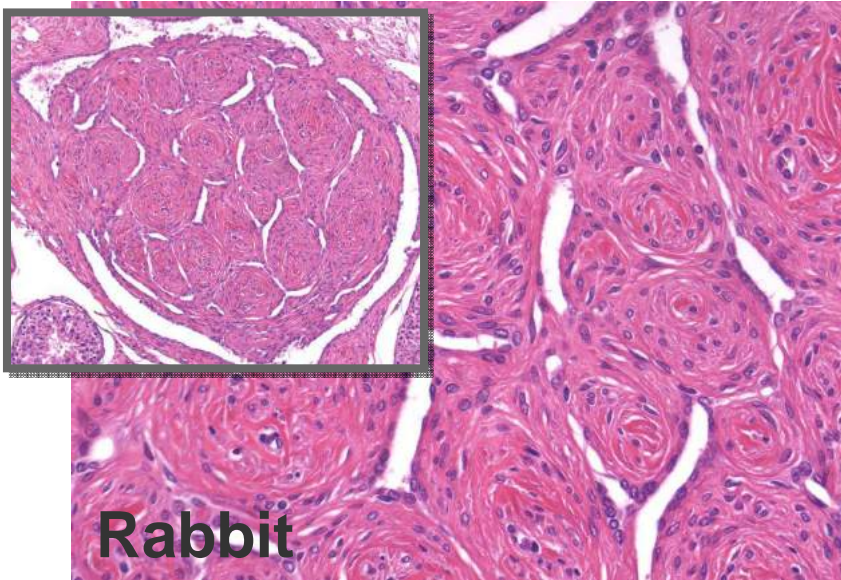


Rat:
Leydig cells,
normal amount in
young to
middle aged animals

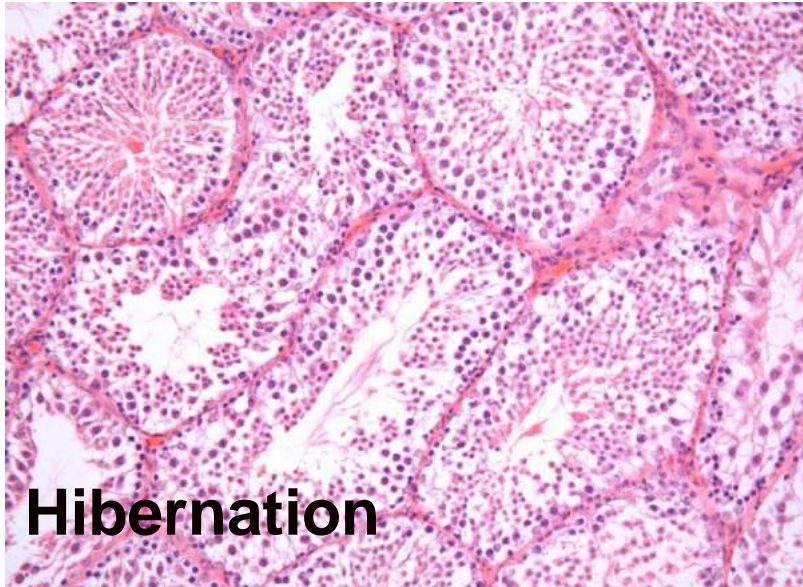
Pig:
Leydig cells,
normal amount in
young to middle
aged animals



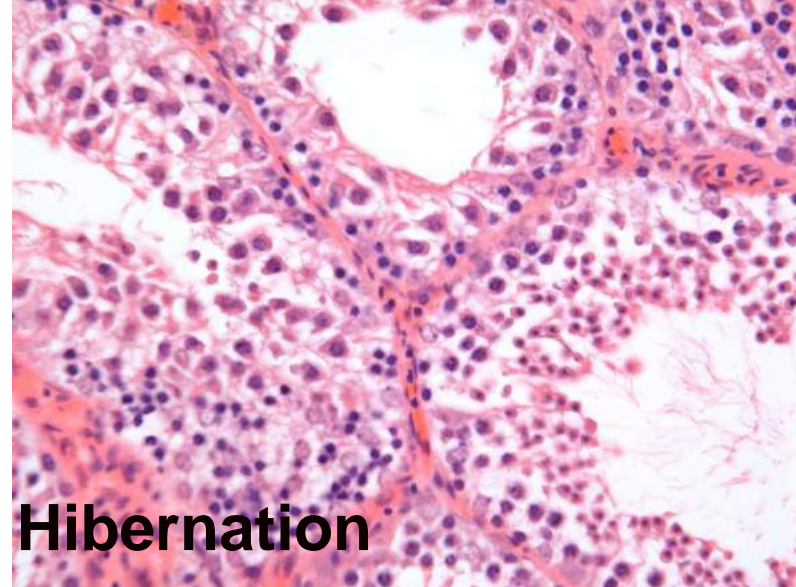
Rete Testis



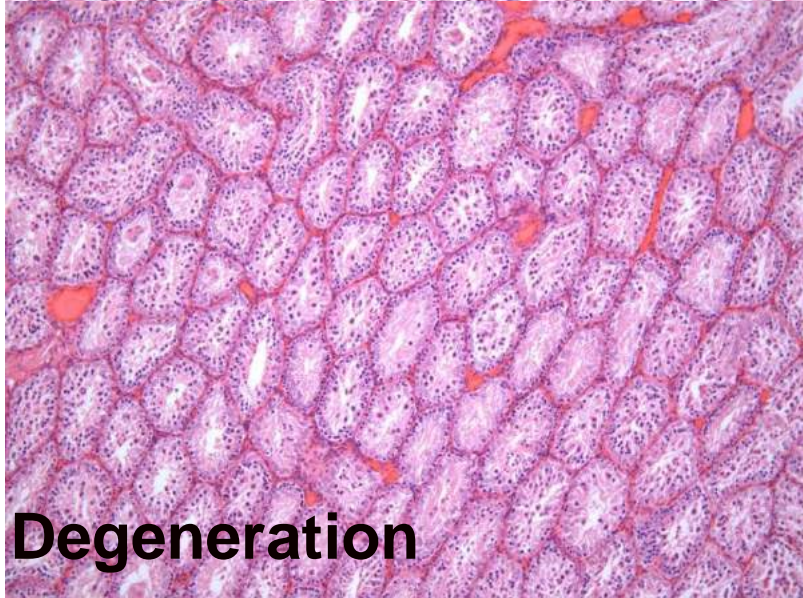
Hamster: Hibernation vs Degeneration



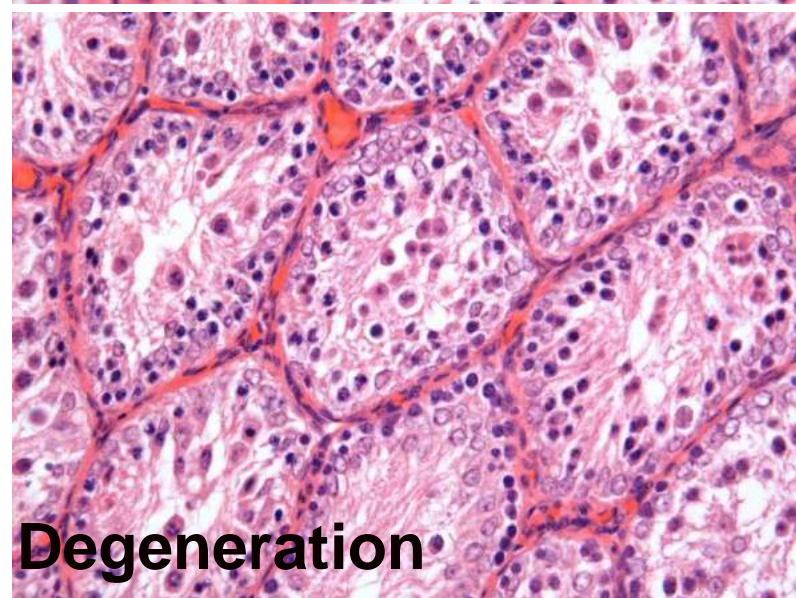
Hibernation



Hibernation



Degeneration



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.....

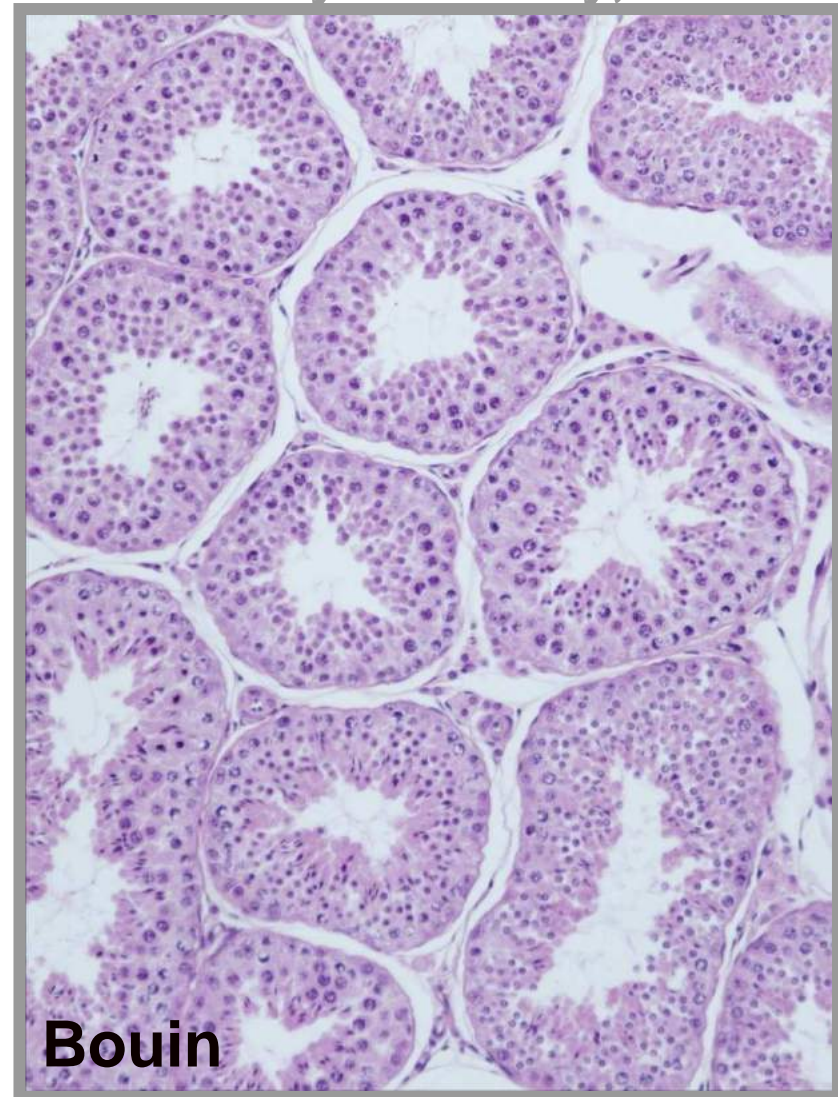
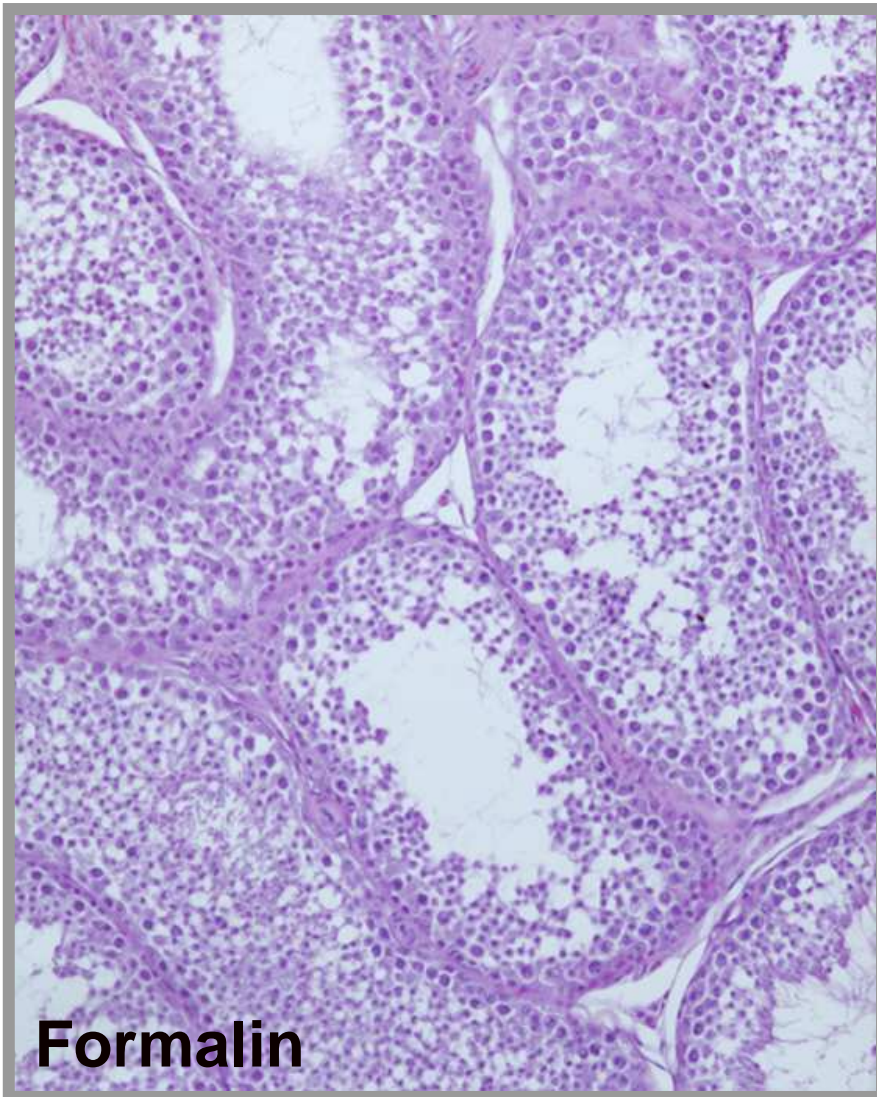
Histotechnique: Fixation

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

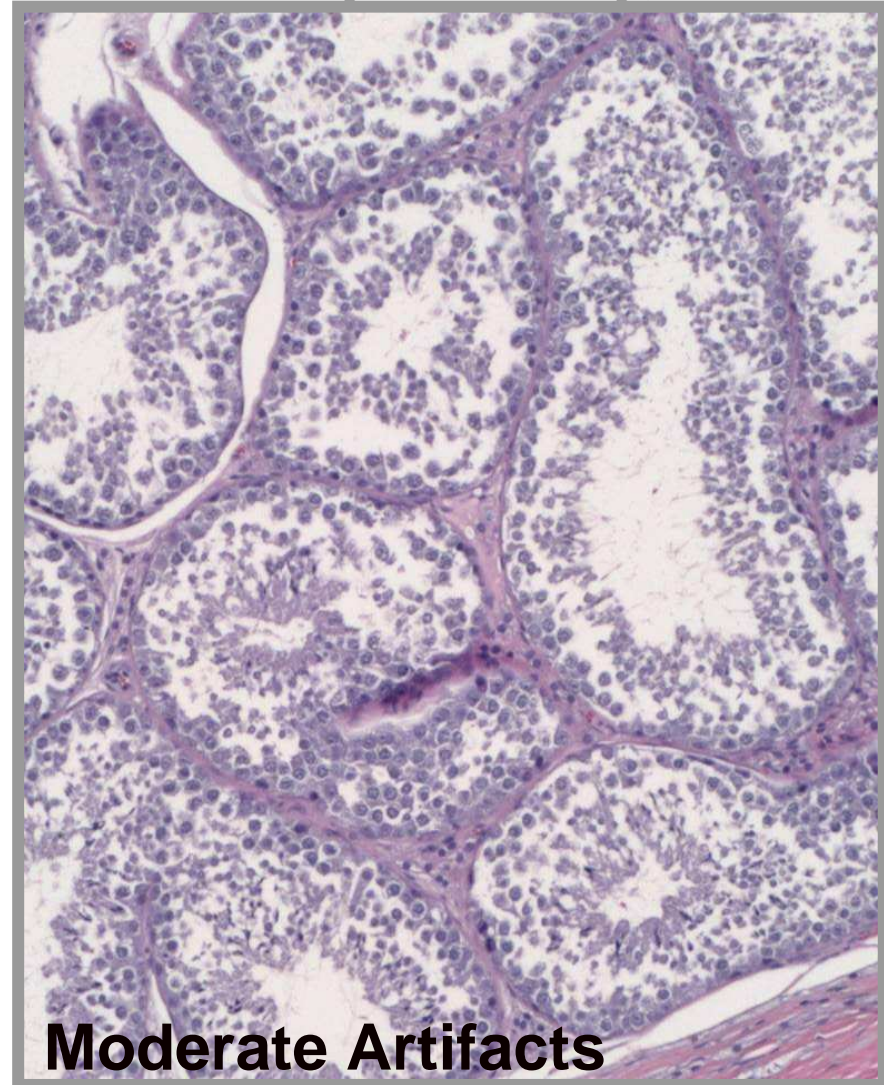
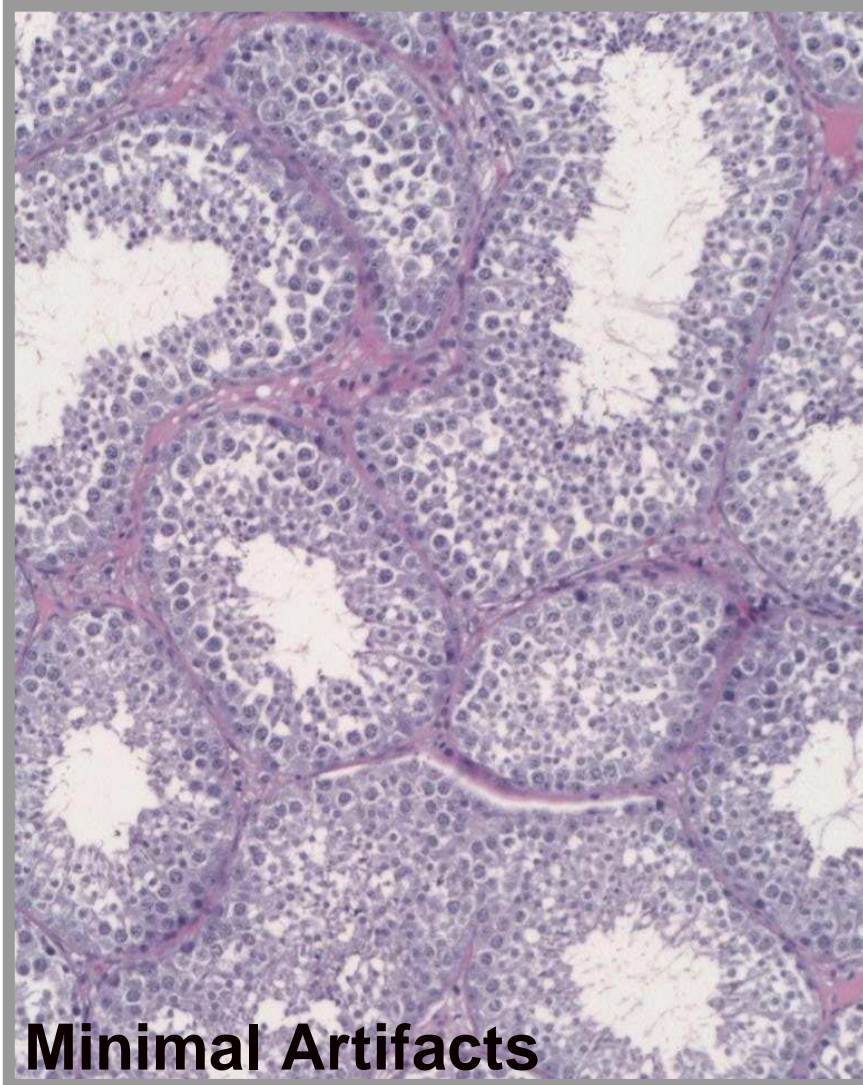
Fixation Issues

By J.Hardisty, EPL Inc



Fixation Issues

By J.Hardisty, EPL Inc



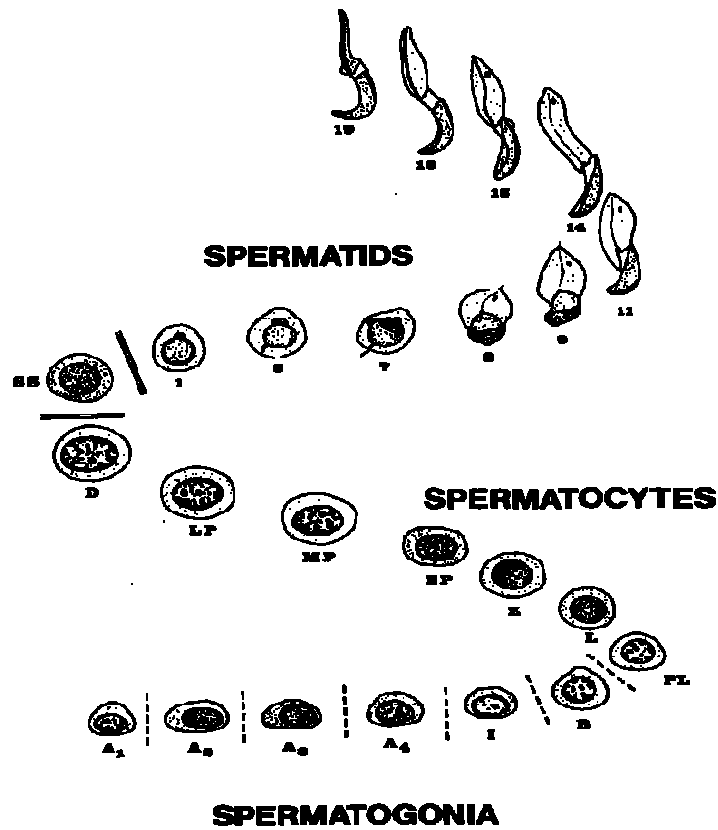
Autolysis

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

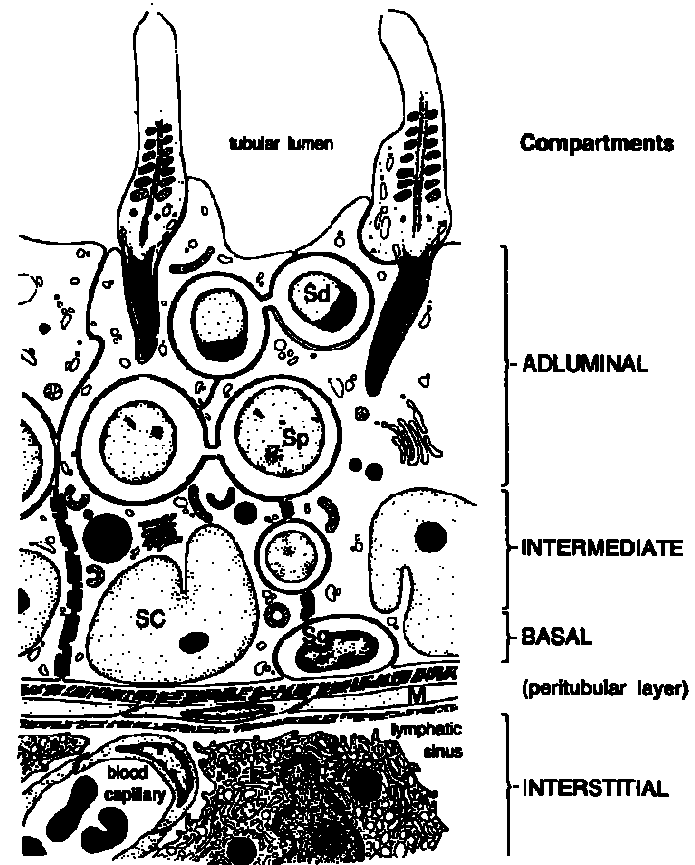
To **clarify** the contribution of spontaneous or autolytic post-mortem changes to testis histopathology...A **progressive decrease** in testis **weight** and **seminiferous tubule diameter** was observed, as well as **detachment of the seminiferous epithelium** from the basement membrane. As early as **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:

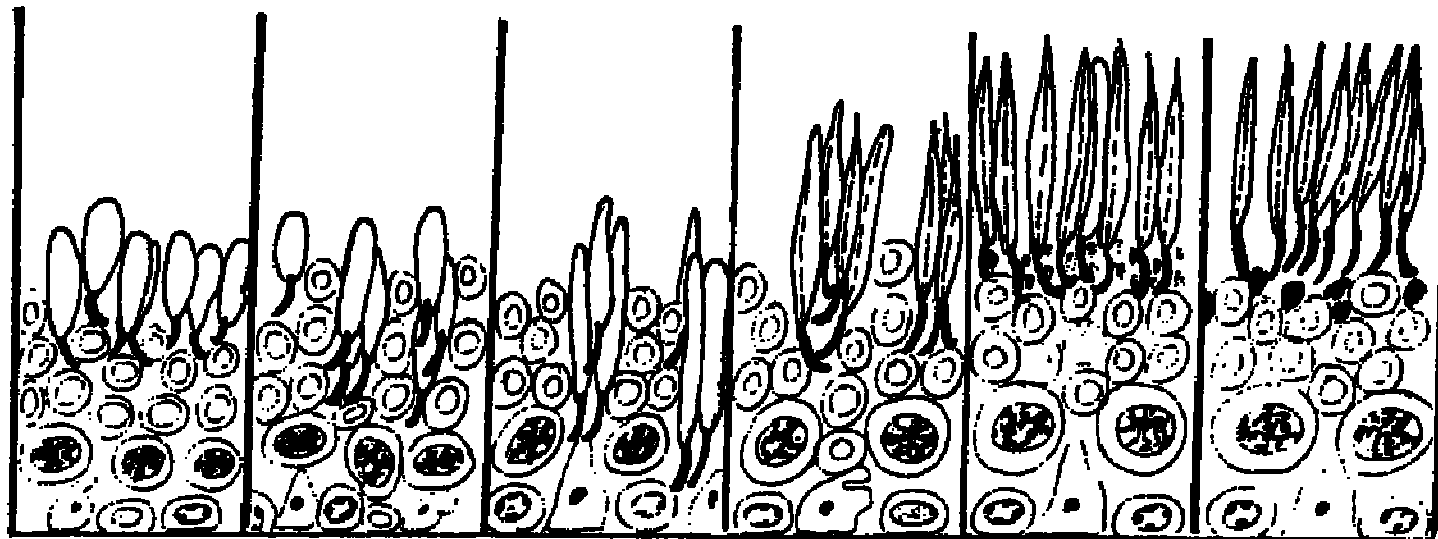


Stages: Example - Rats



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 S/ids. 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

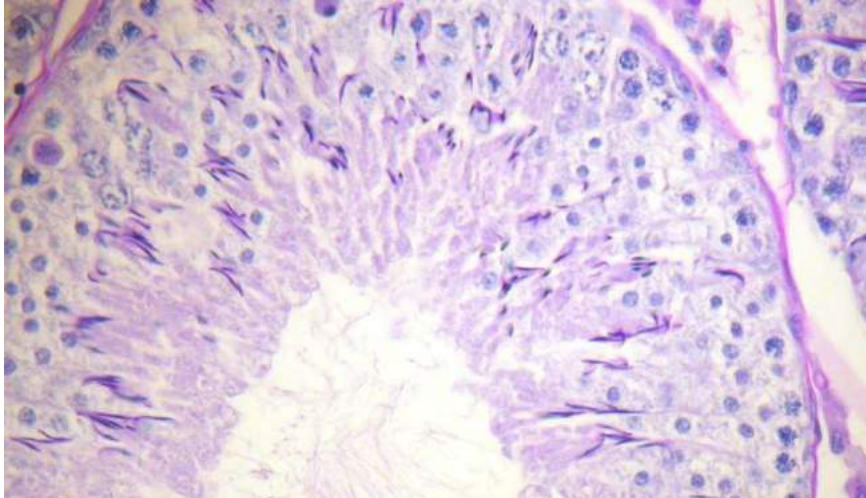
Qualitative staging:

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

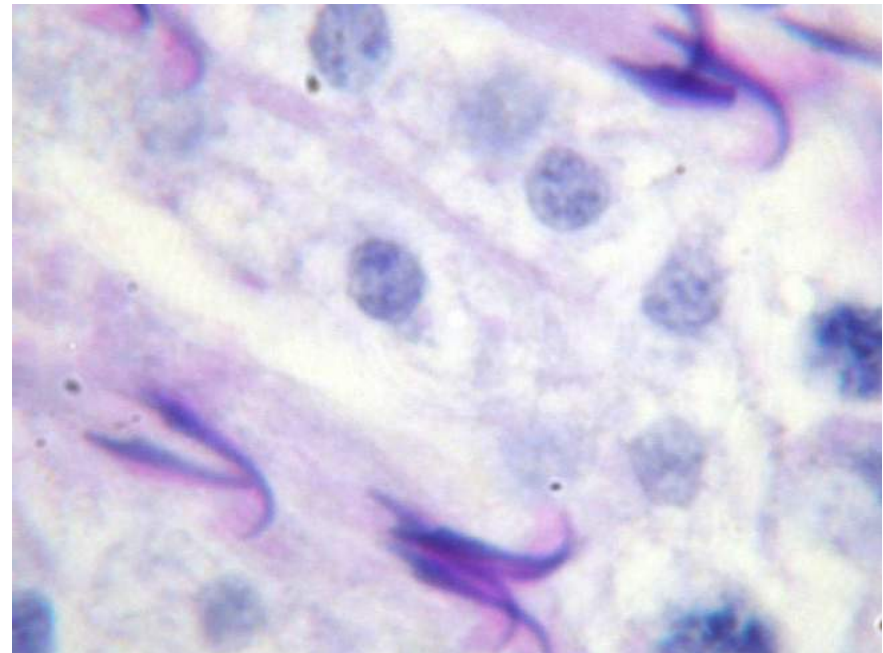
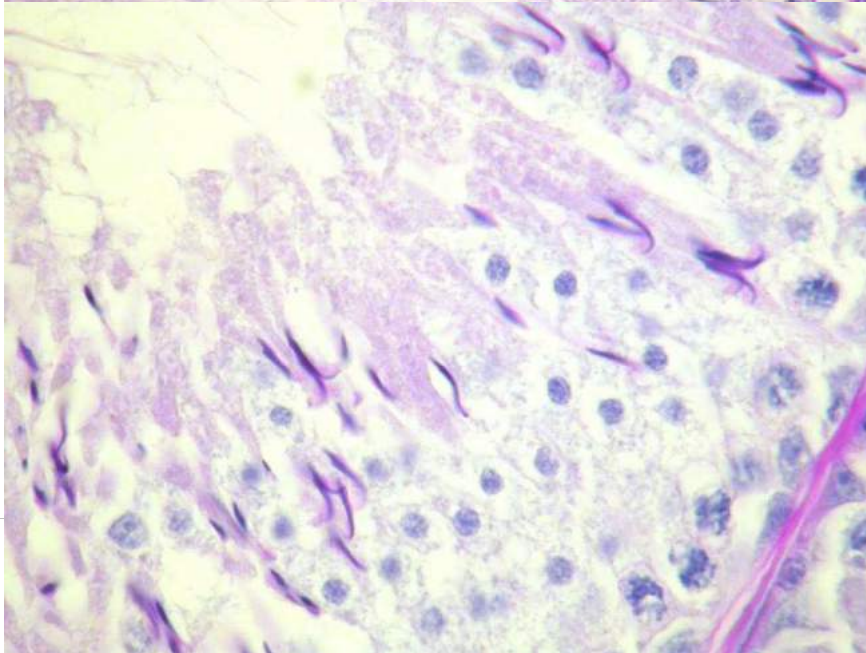
Quantitative staging:

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

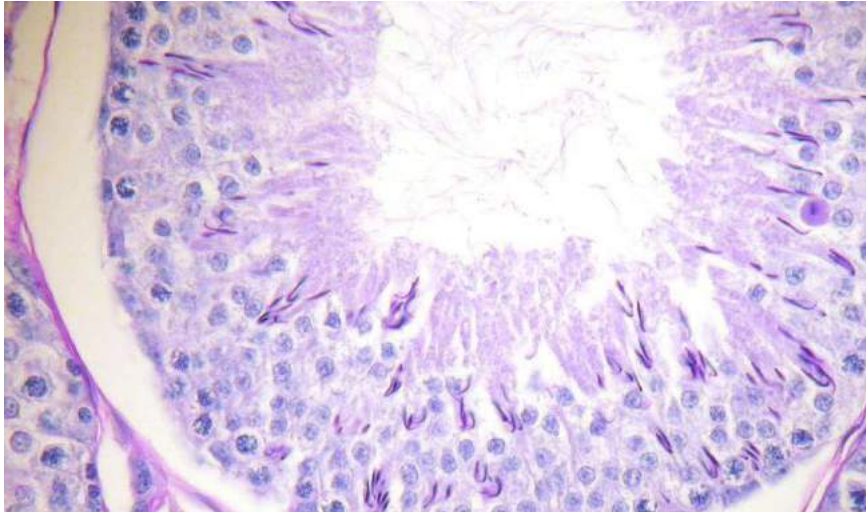
Stage I



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

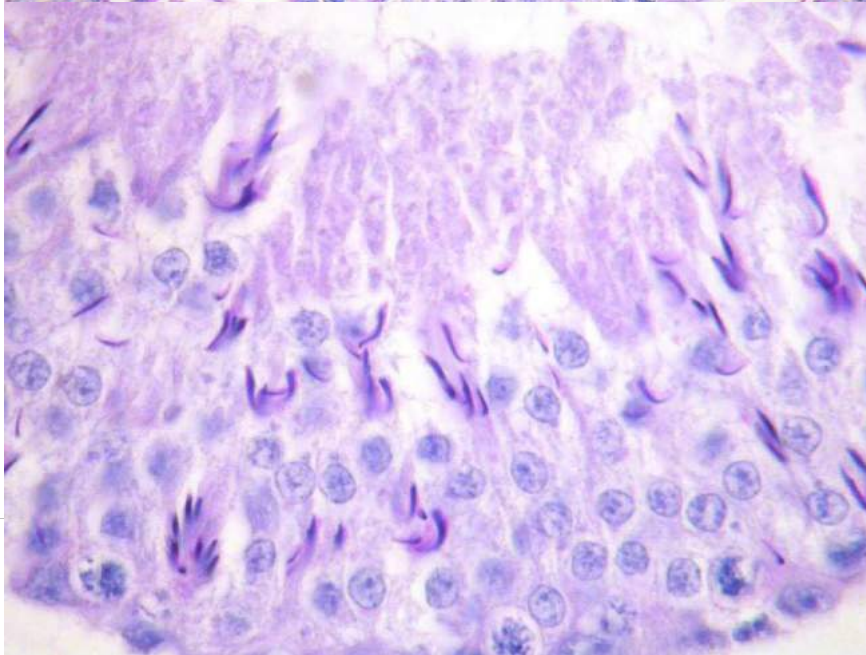


Stage II-III

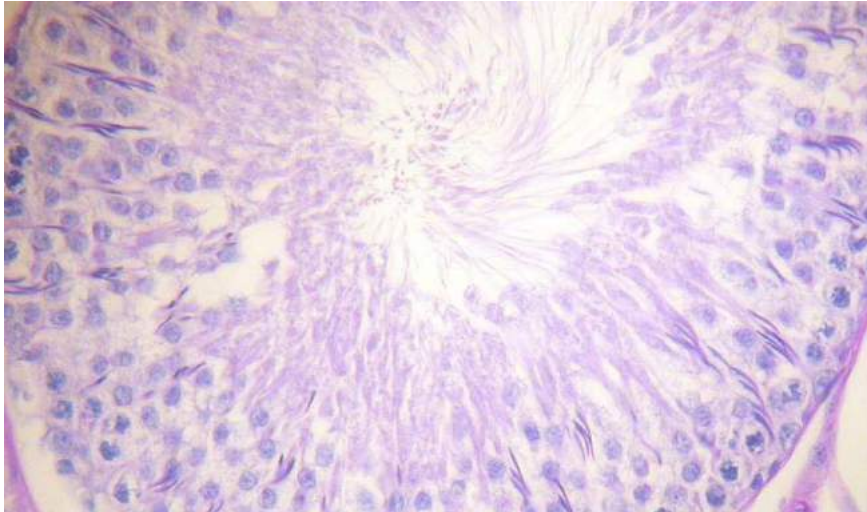


✓ Nucleus of elongated spermatids extending into deeper layers (between round spermatids)

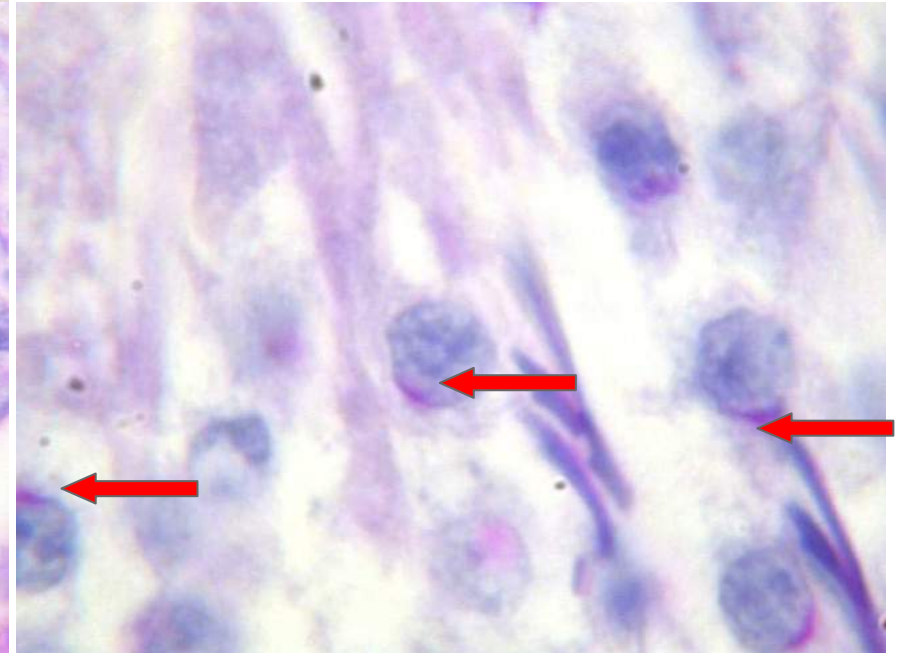
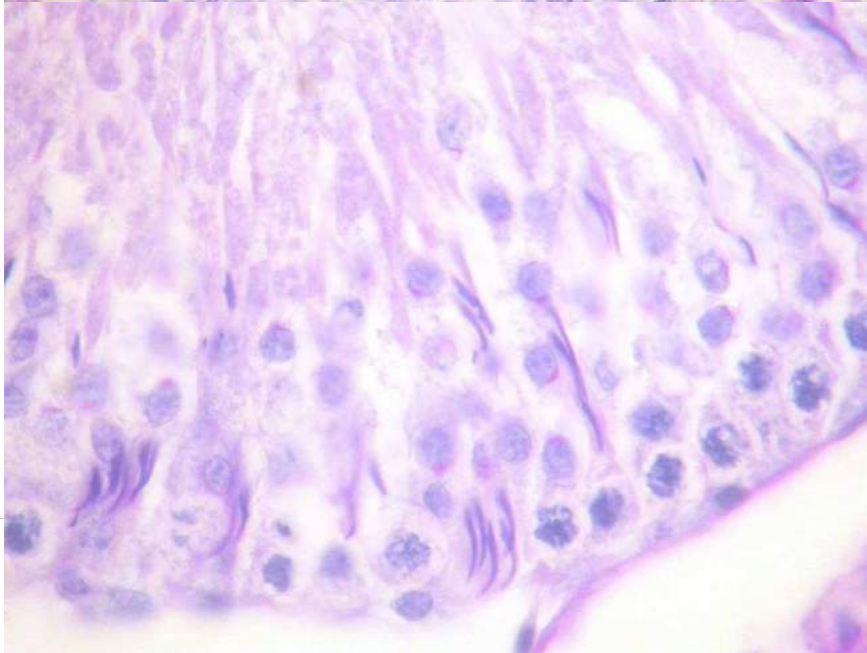
✓ In round spermatids no clear acrosome but 1 or 2 small granula



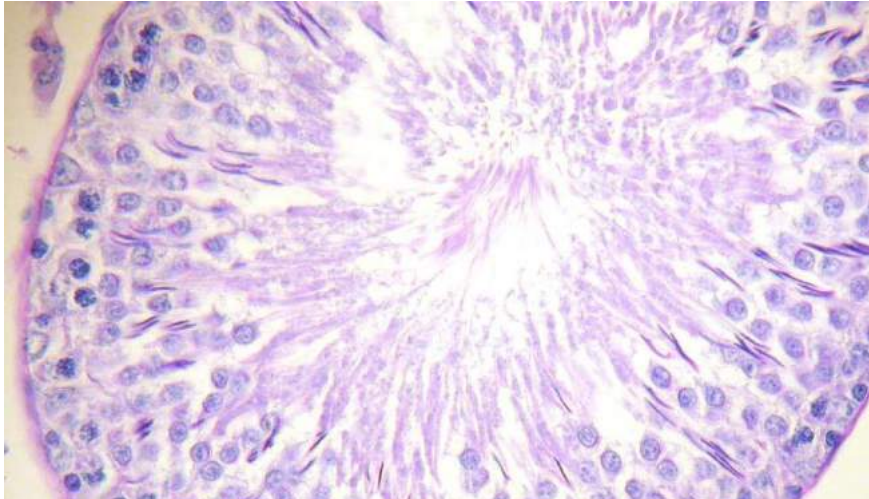
Stage IV



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

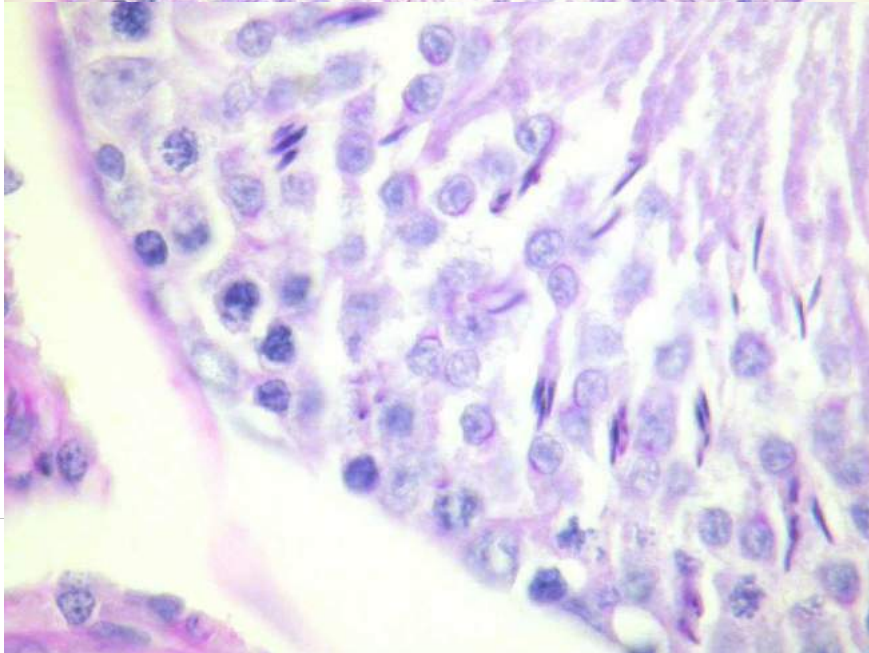


Stage V

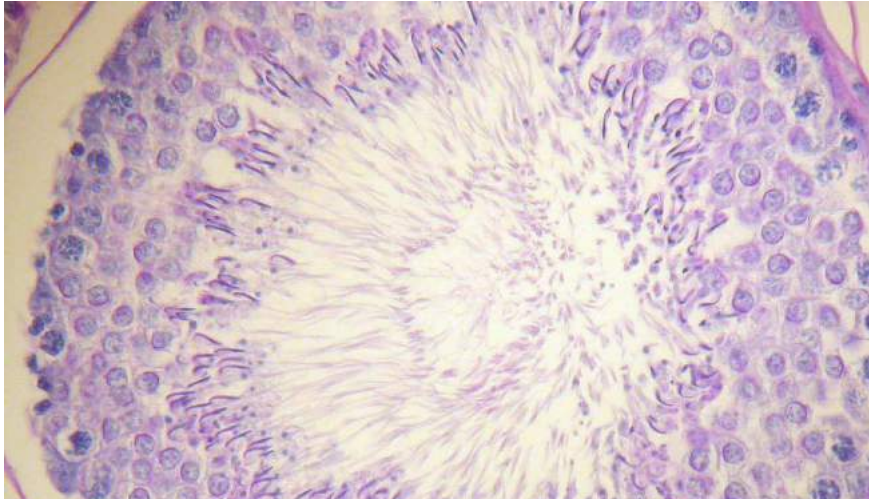


✓ Nucleus of elongated spermatids deep, but tending another one into luminal direction

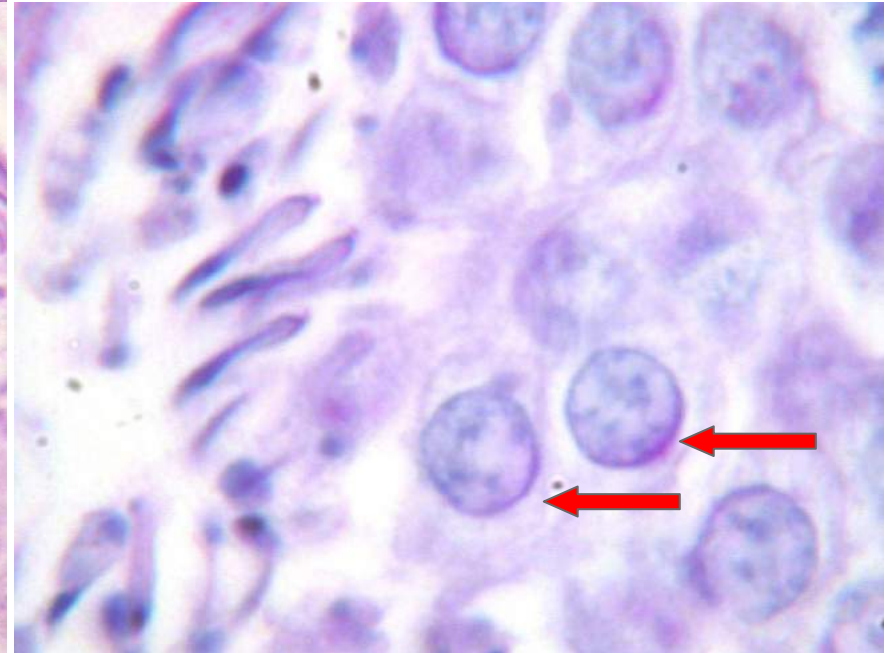
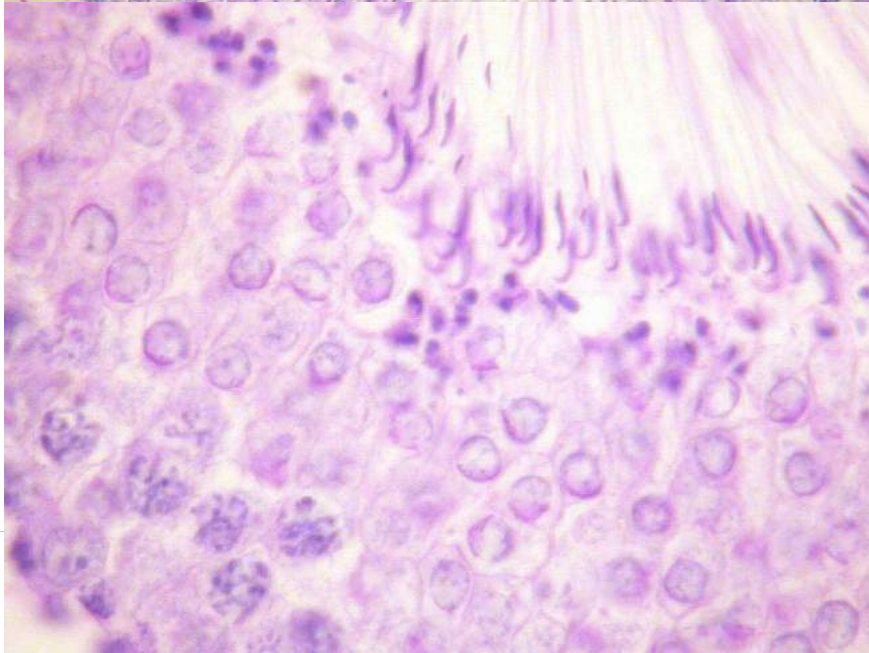
✓ Acrosome on round spermatids covering approximately 45°



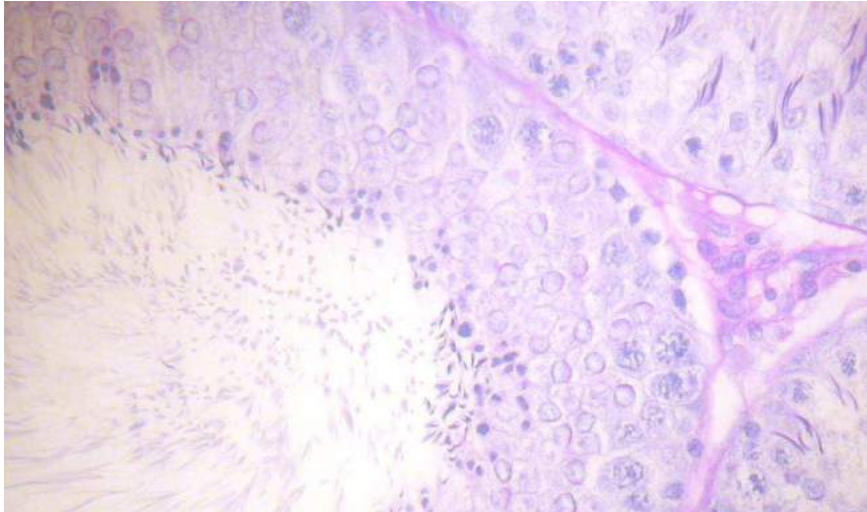
Stage VI



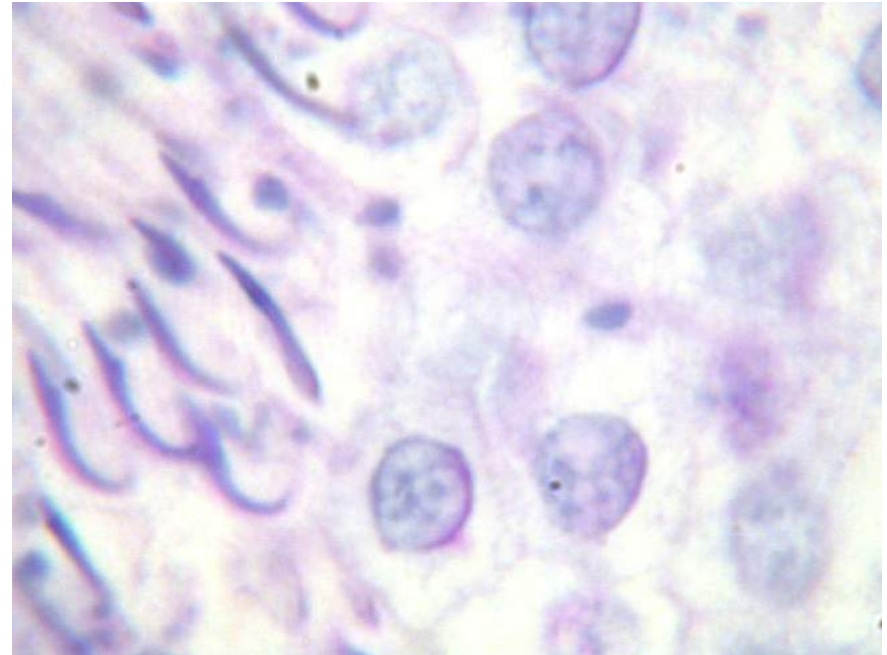
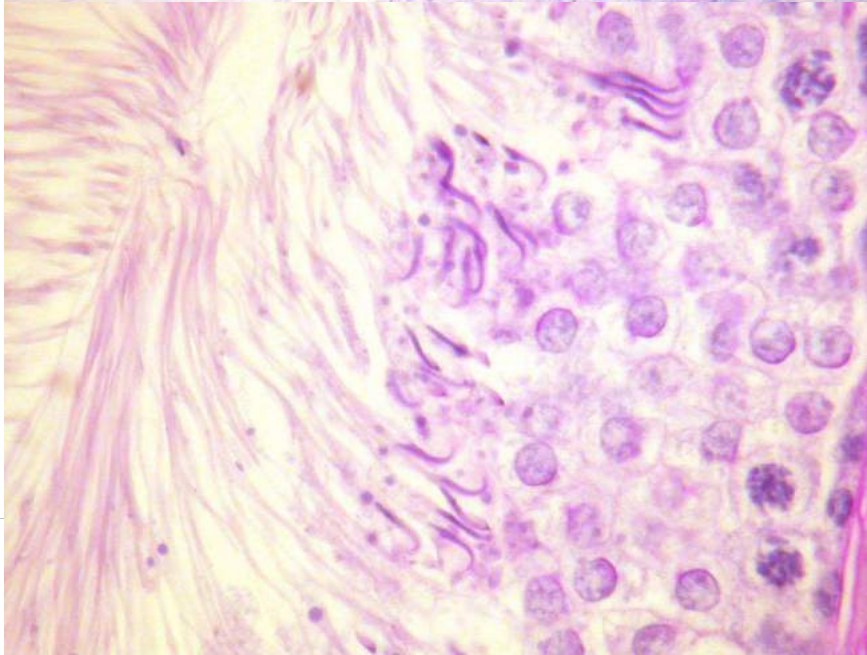
- ✓ Elongated spermatids on surface,
- ✓ Acrosome on round spermatids covering approximately 80°



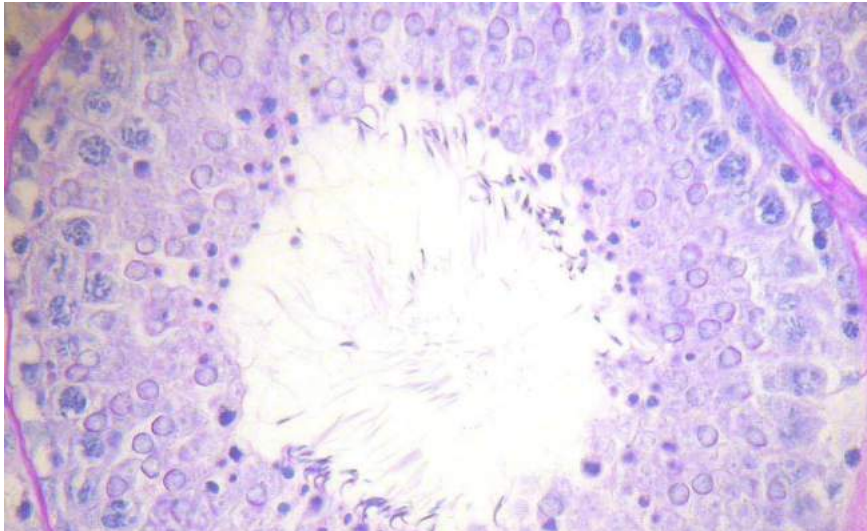
Stage VII



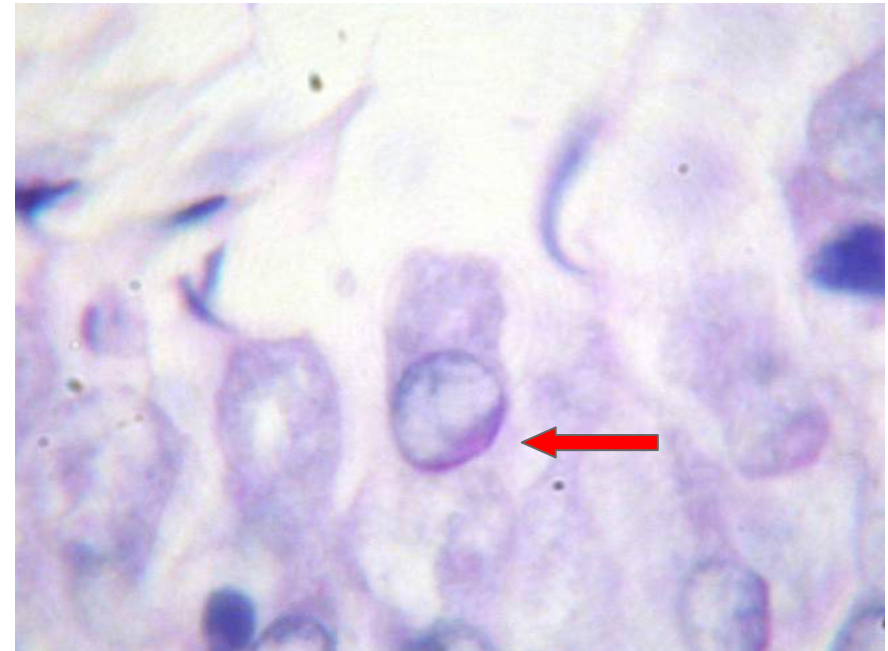
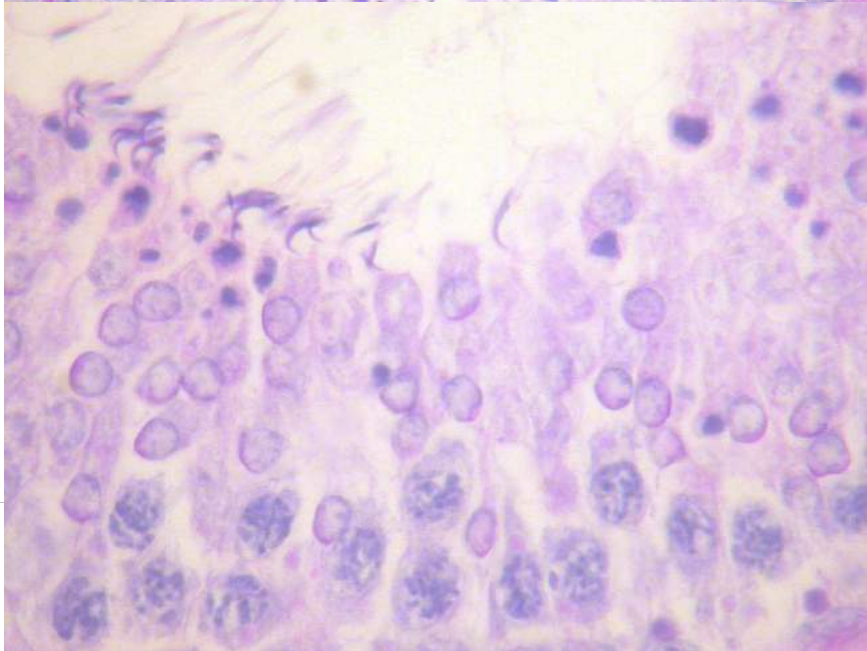
- ✓ Spermia on surface
- ✓ Residual bodies
- ✓ Acrosome/nucleus of round spermatids is round



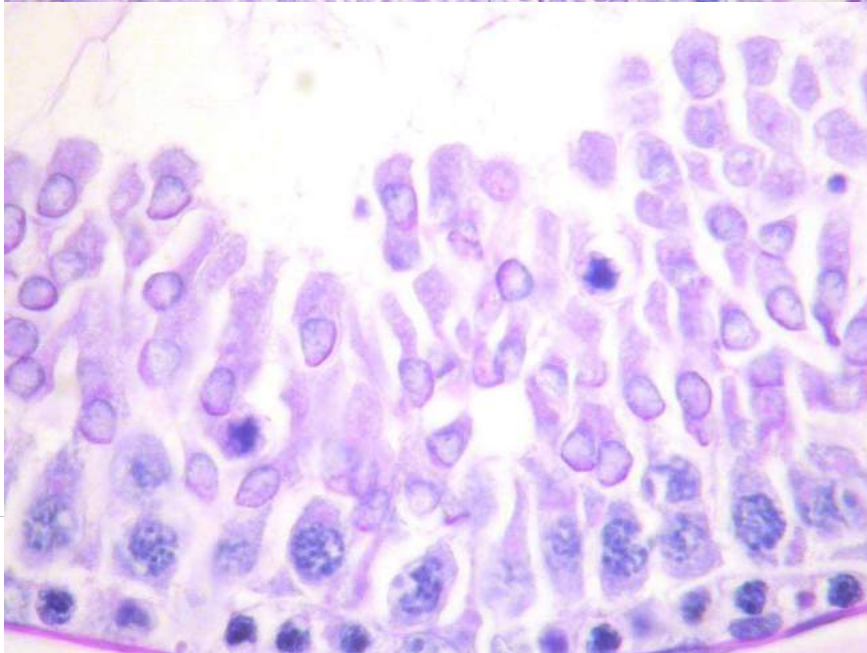
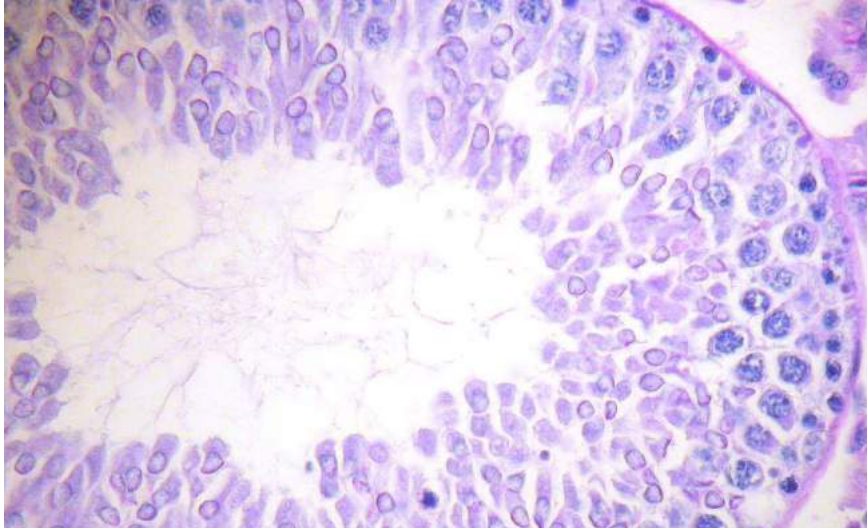
Stage VIII



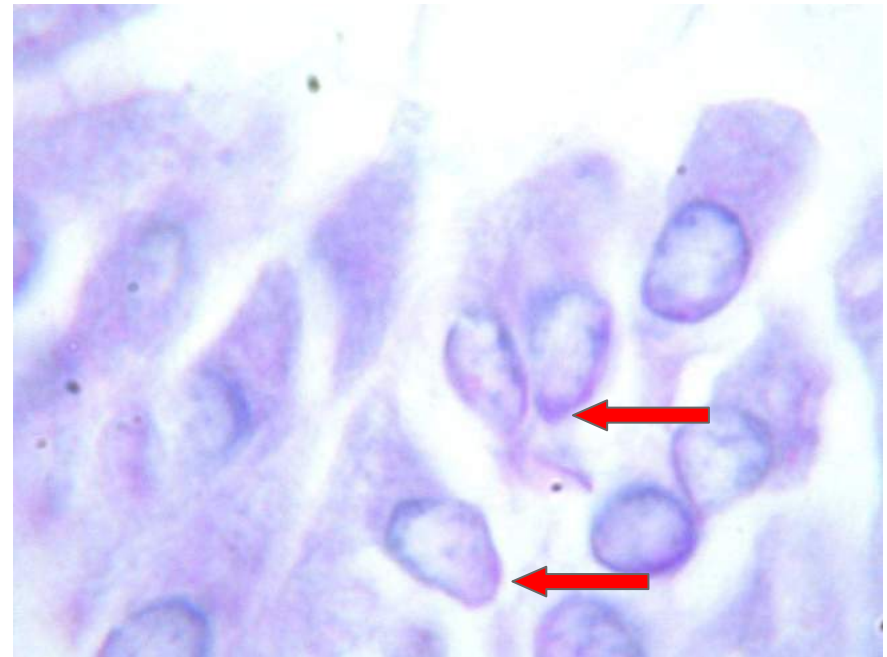
- ✓ Only a few spermia
- ✓ Residual bodies often resorbed
- ✓ Acrosome of elongated spermatids ,umbrella'-like on round nucleus



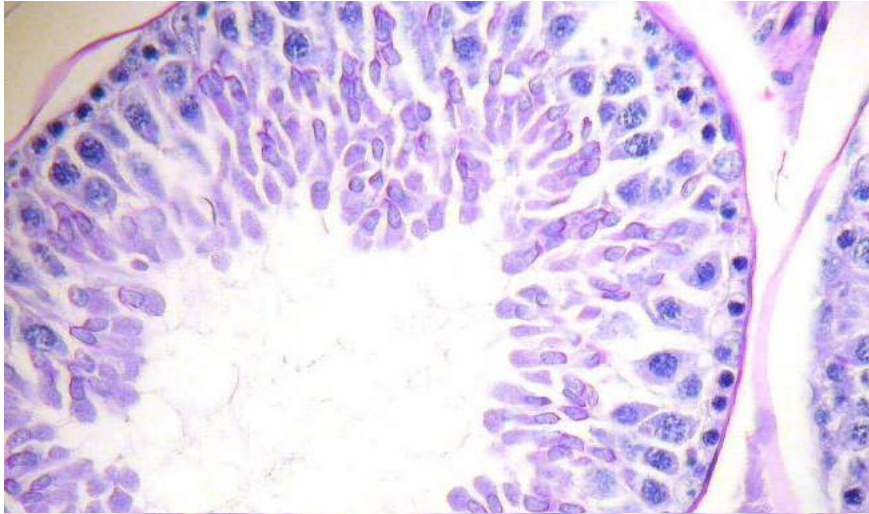
Stage IX



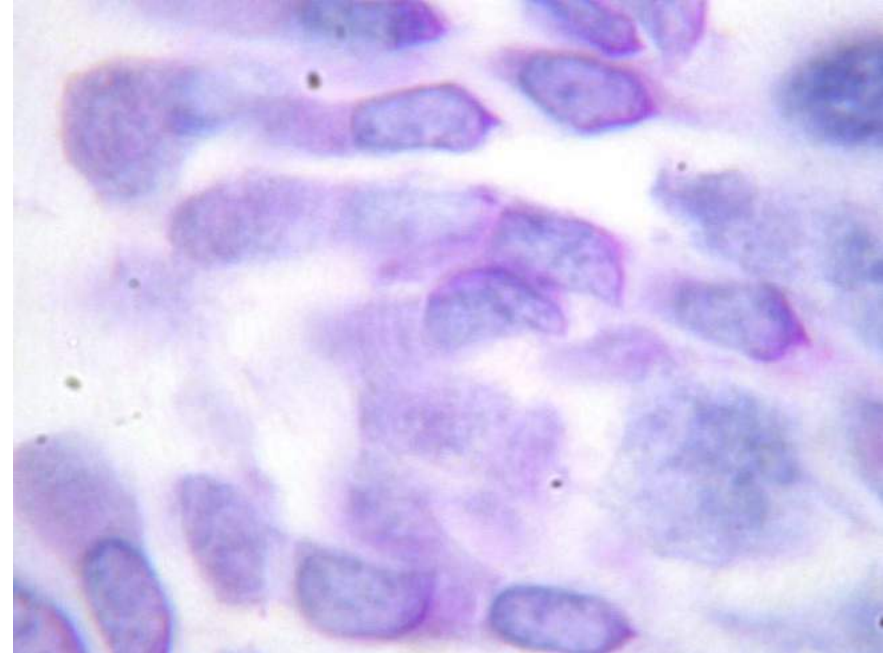
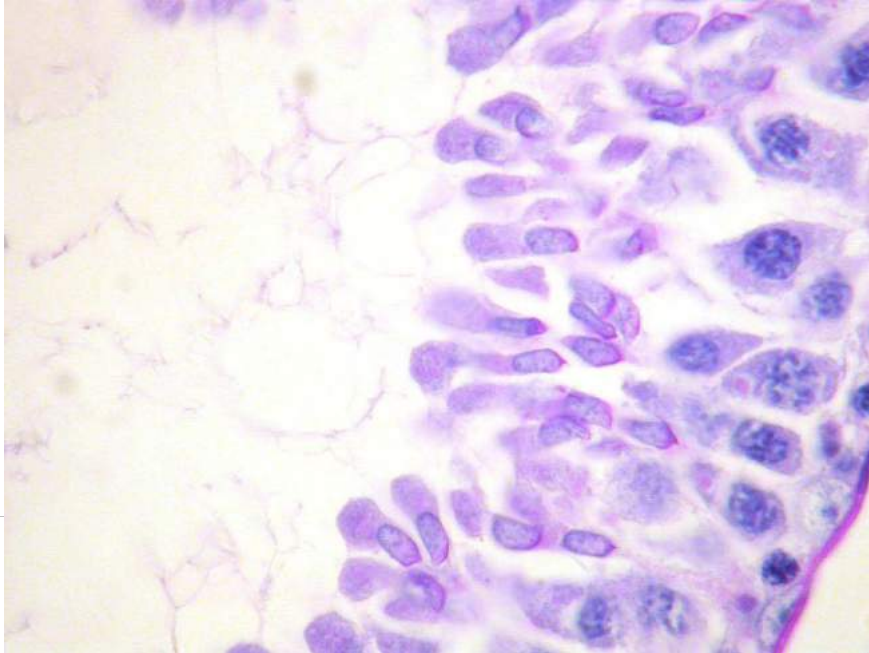
- ✓ No spermatids
- ✓ Nucleus of elongated spermatids tend to form ellipsoids
- ✓ Tail is forming



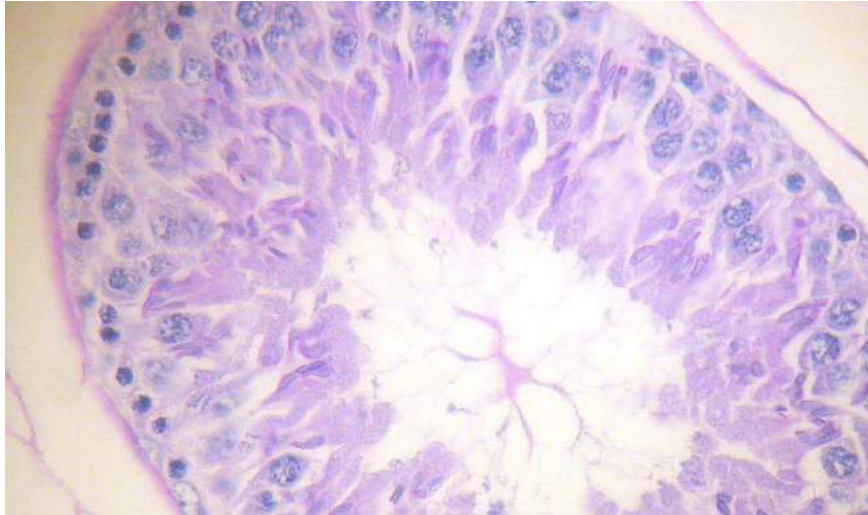
Stage X



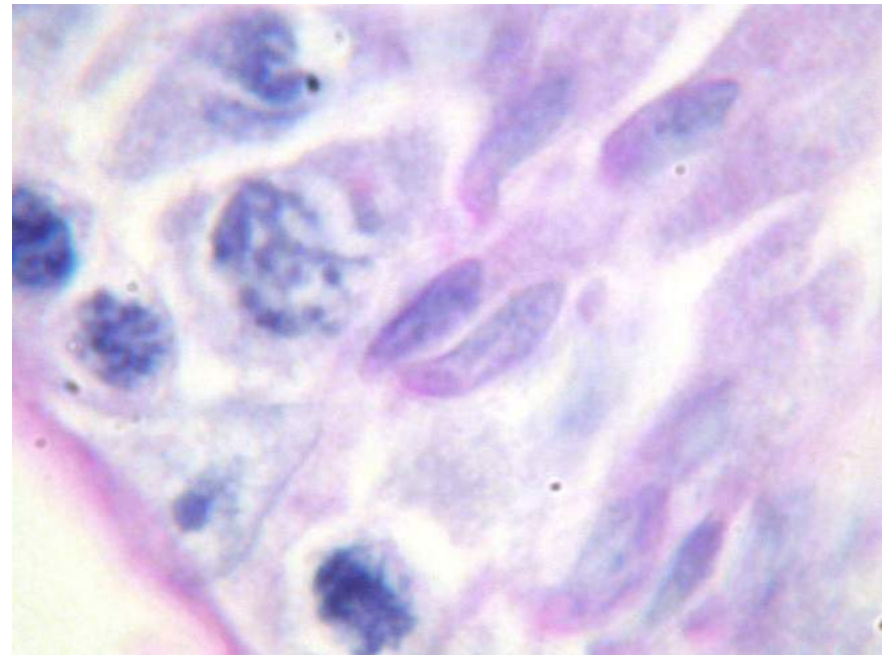
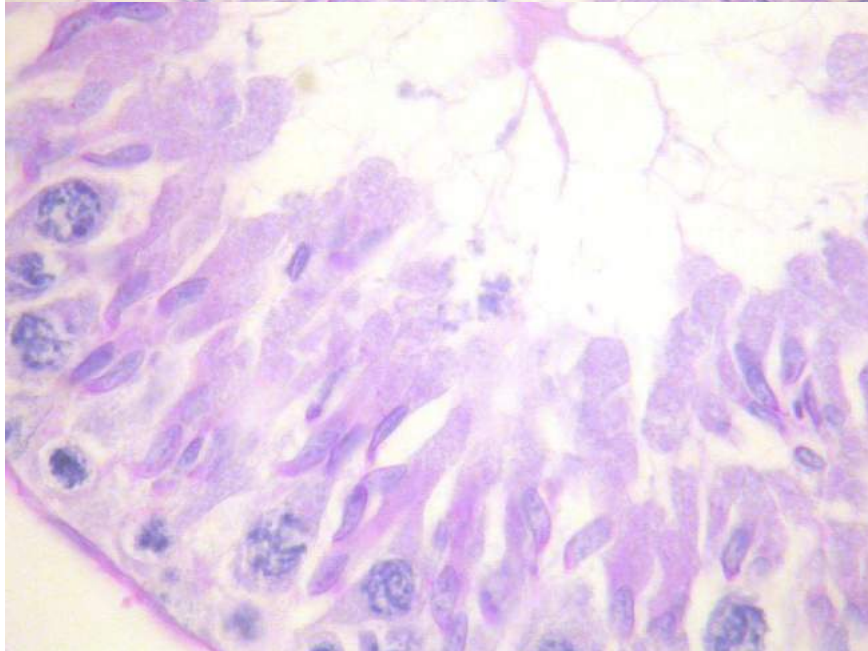
- ✓ Nucleus of elongated spermatids tends to form an angle
- ✓ Condensation of karyoplasma



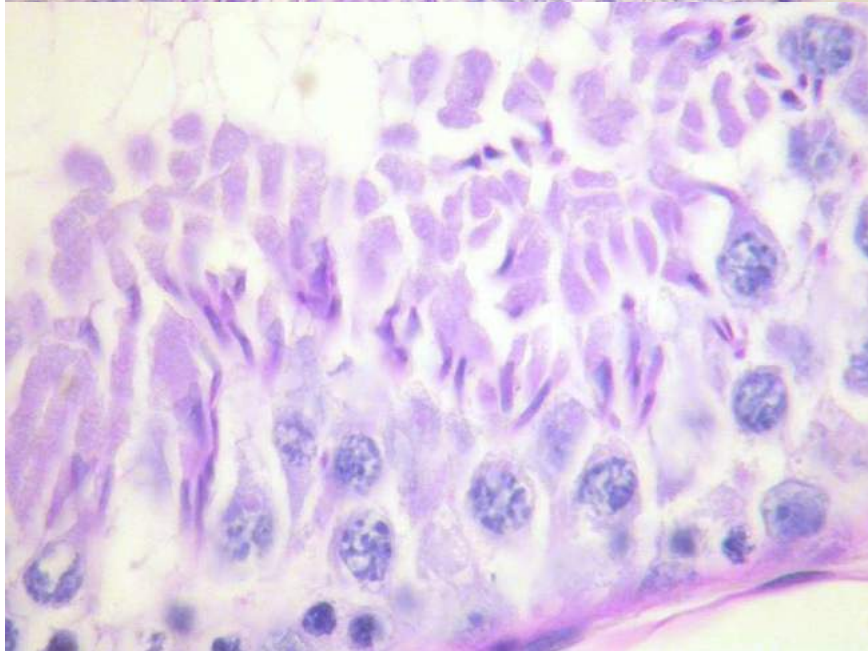
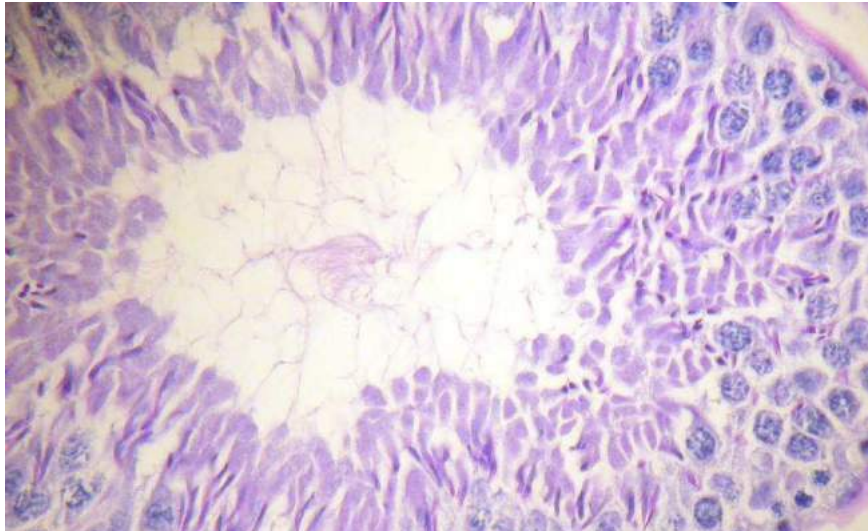
Stage XI



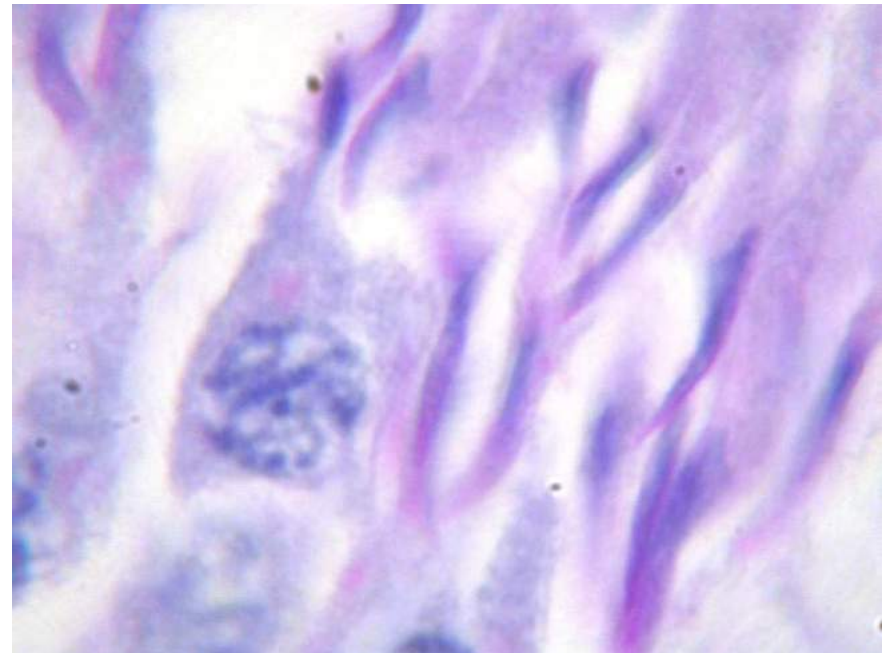
- ✓ Nucleus of elongated spermatids tend to become rectangular with pronounced condensation
- ✓ Large round p-spermatocytes



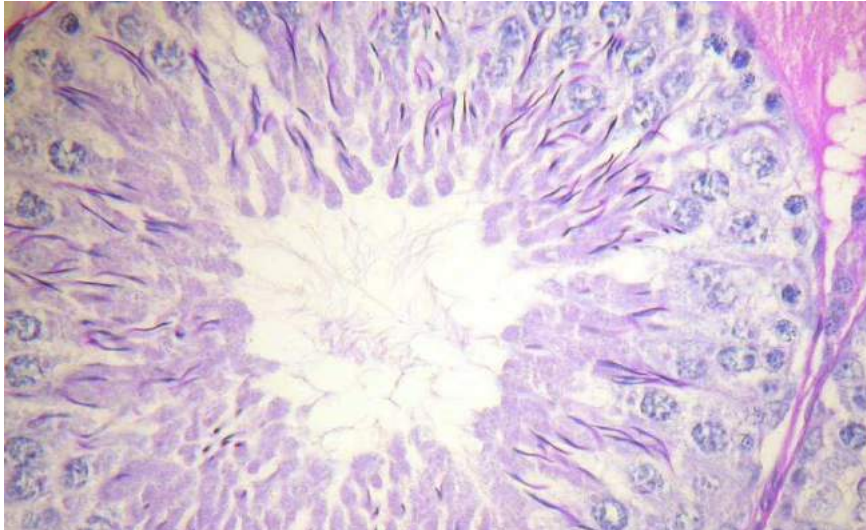
Stage XII



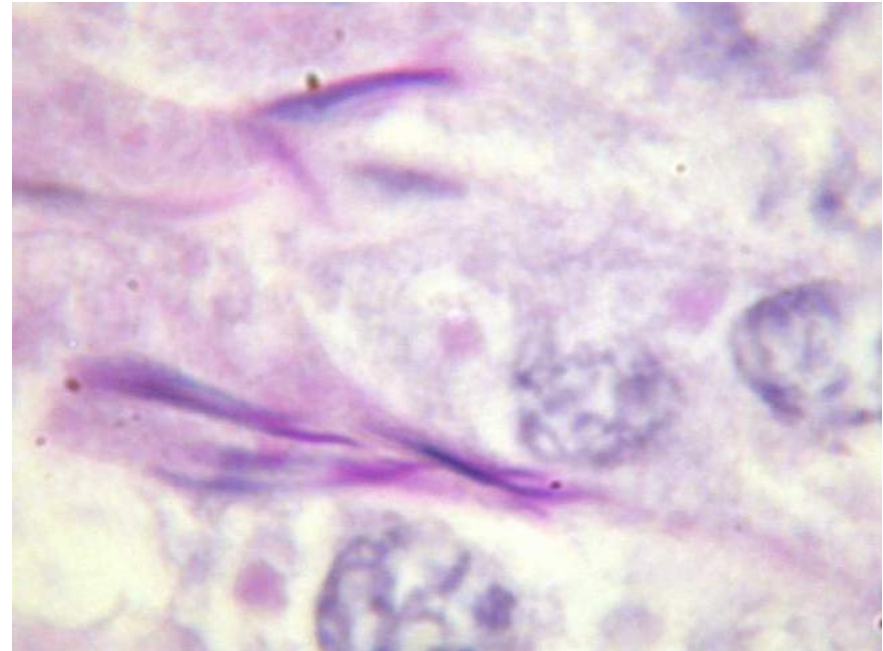
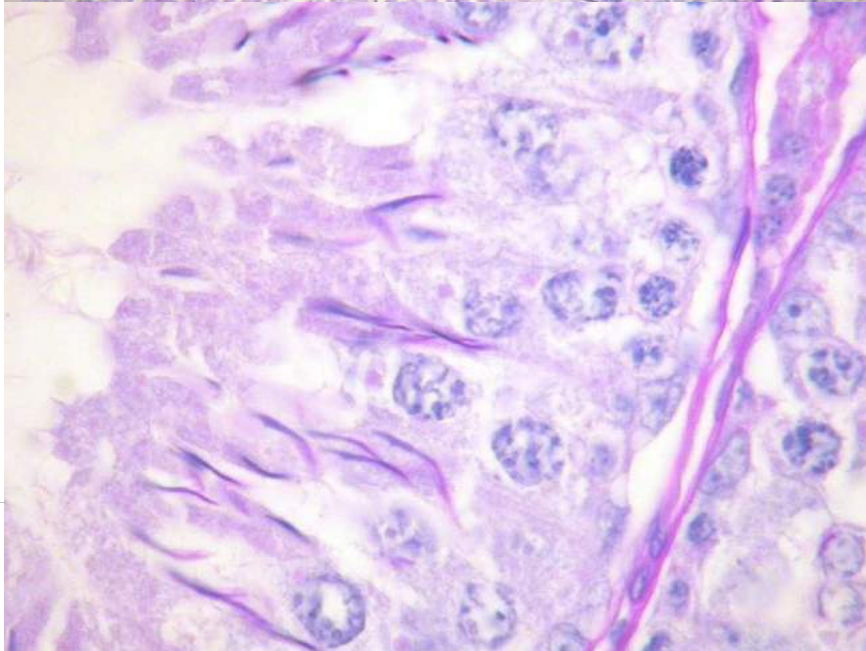
- ✓ Nucleus of elongated spermatids elongated and more condensed
- ✓ Acrosome vesicle large in round and large p-spermatocytes



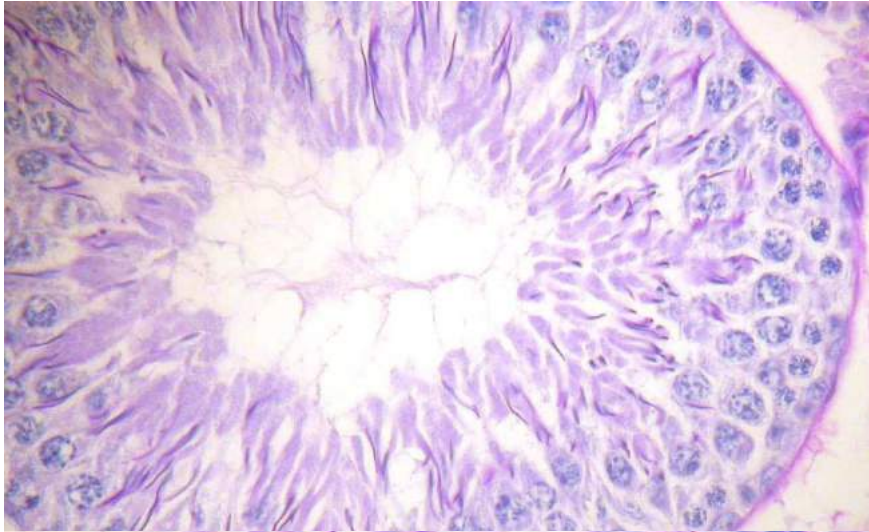
Stage XIII



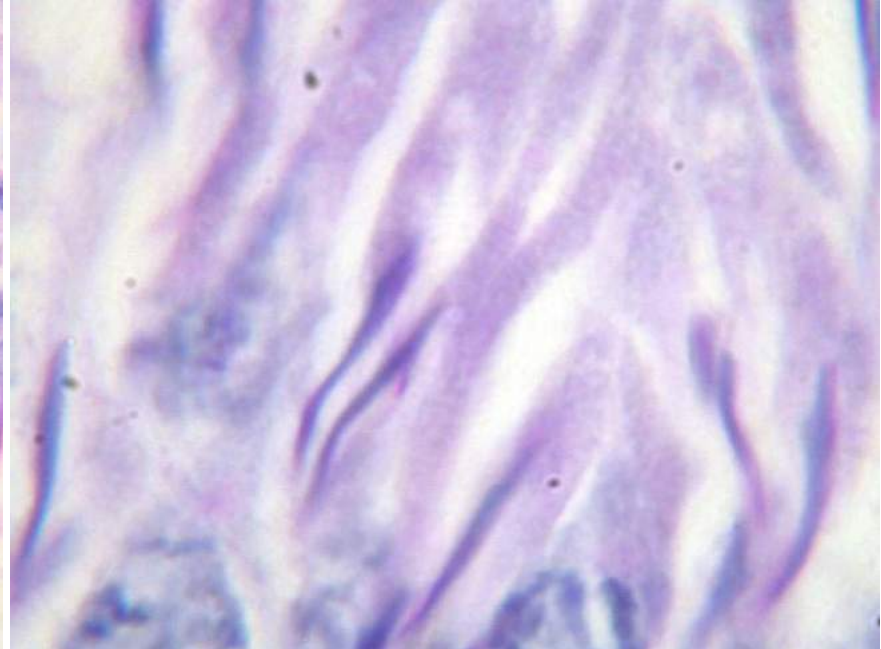
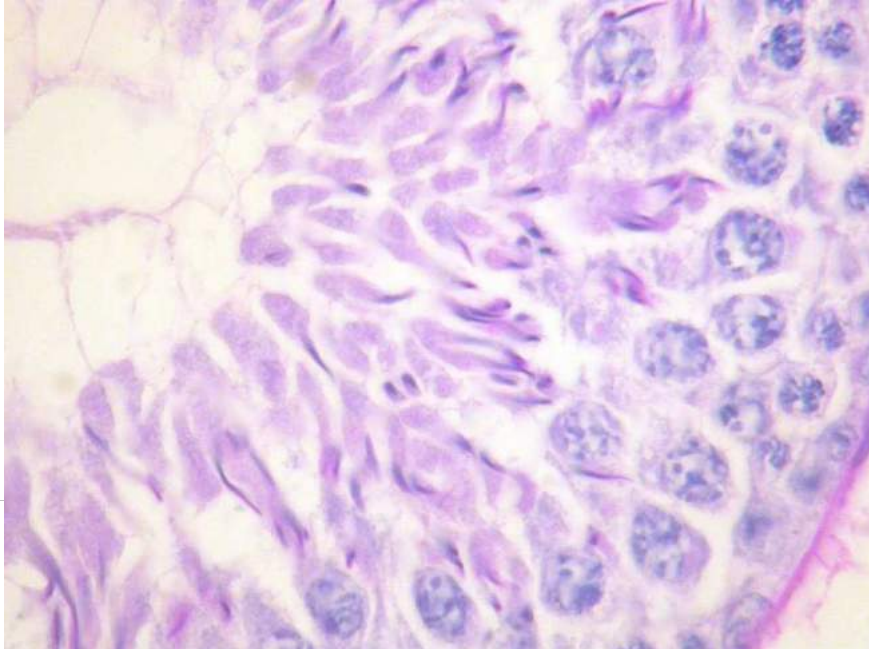
- ✓ Nucleus of elongated spermatids is forming hook-like head
- ✓ Acrosome vesicle in large round Di-spermatocytes



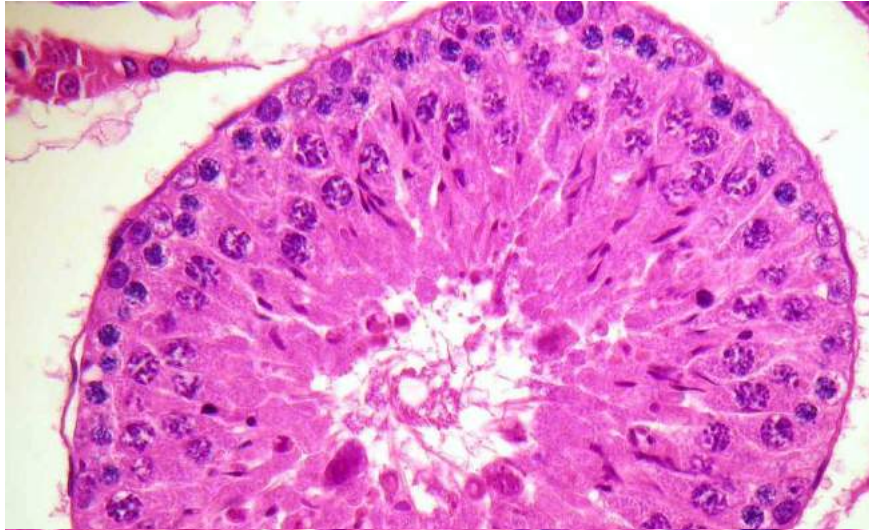
Stage XIV



✓ Nucleus of elongated spermatids completely condensed

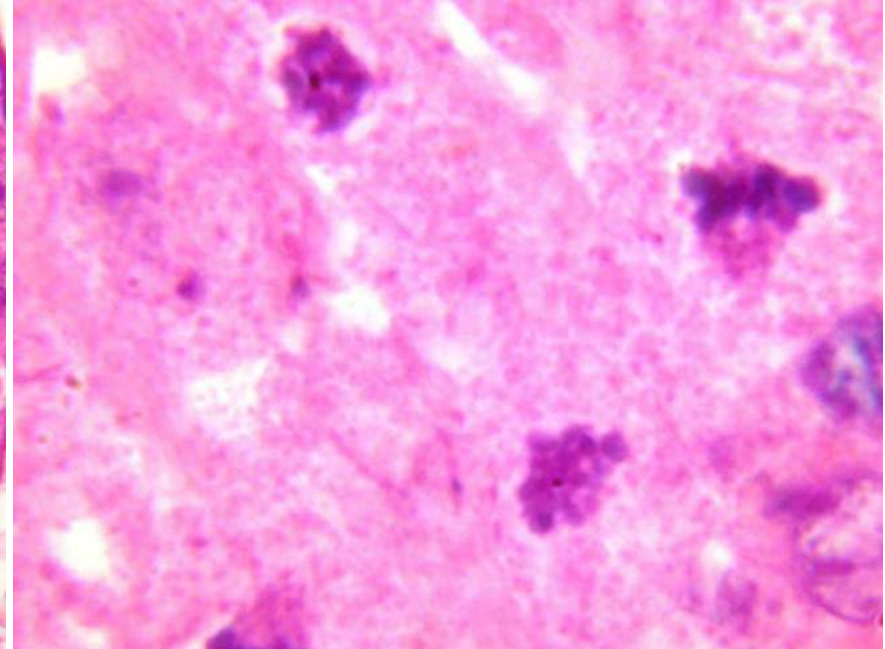
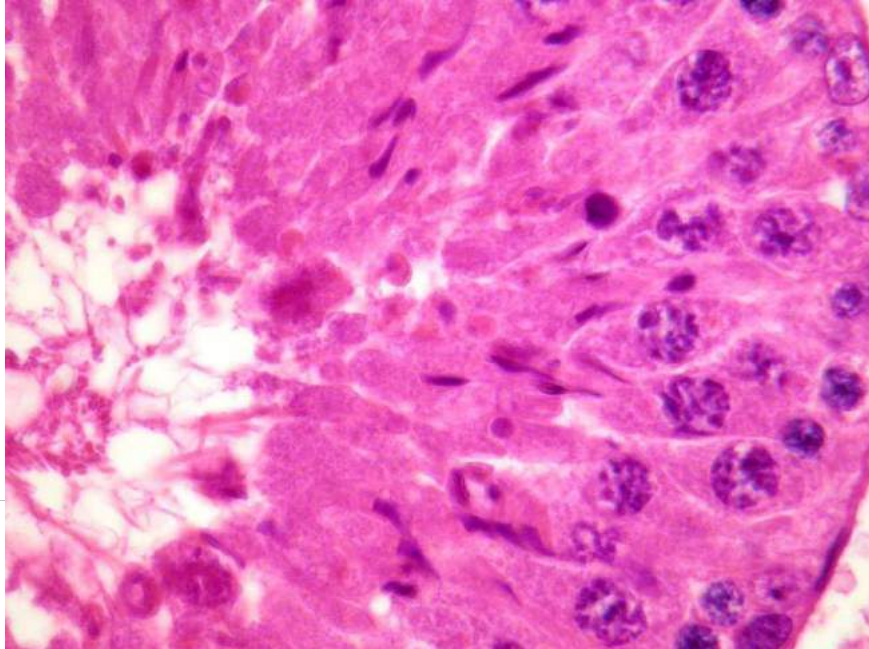


Staging: How to use?

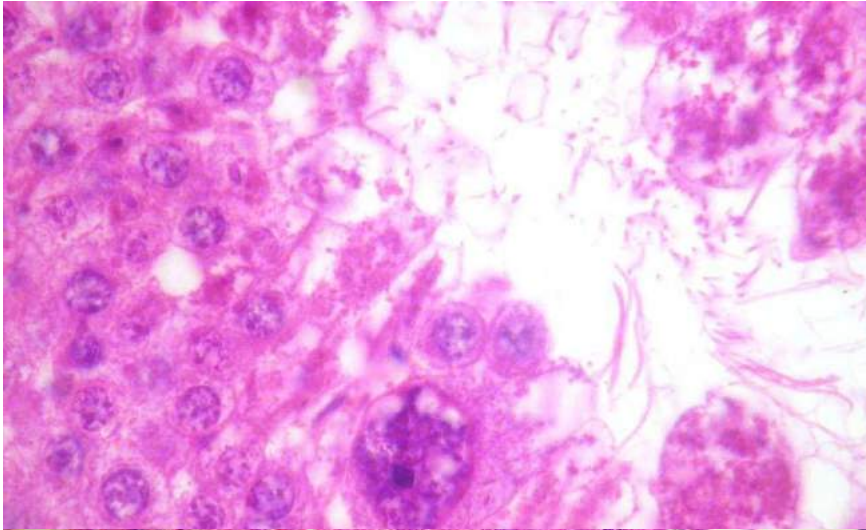


**A case:
28-Day Study
Terminal sacrifice**

Necrosis!

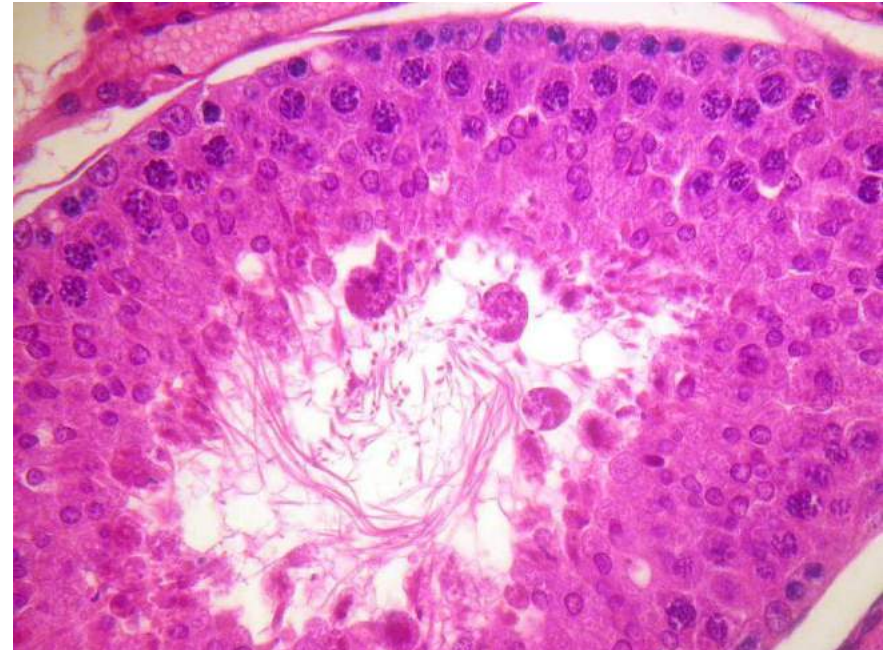
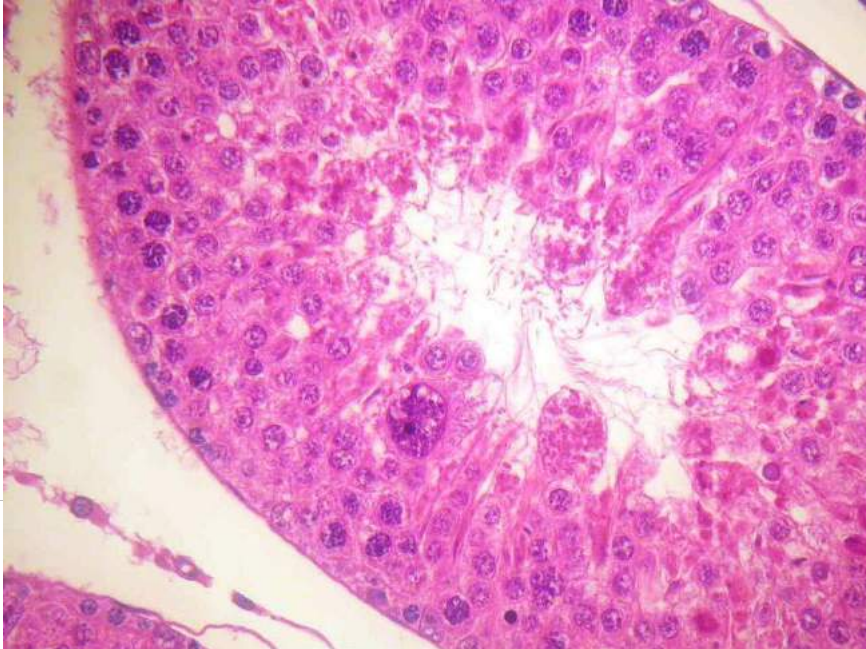


Staging: How to use?

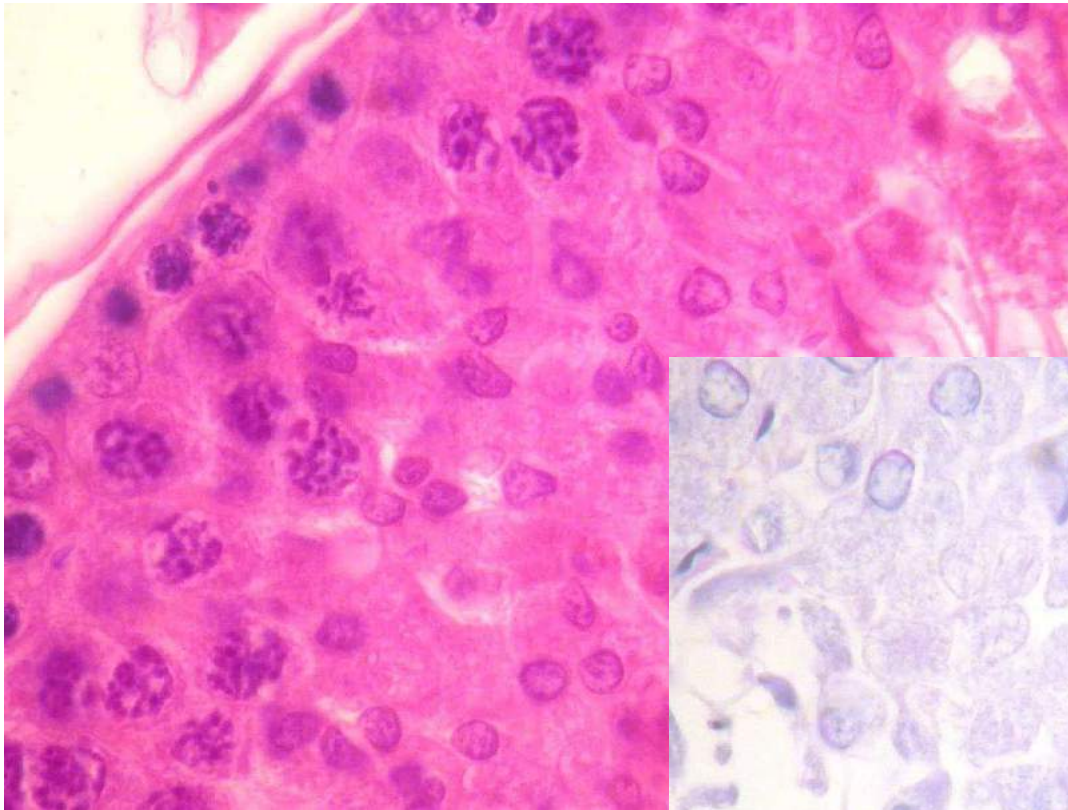


Necrosis and formation of giant cells!

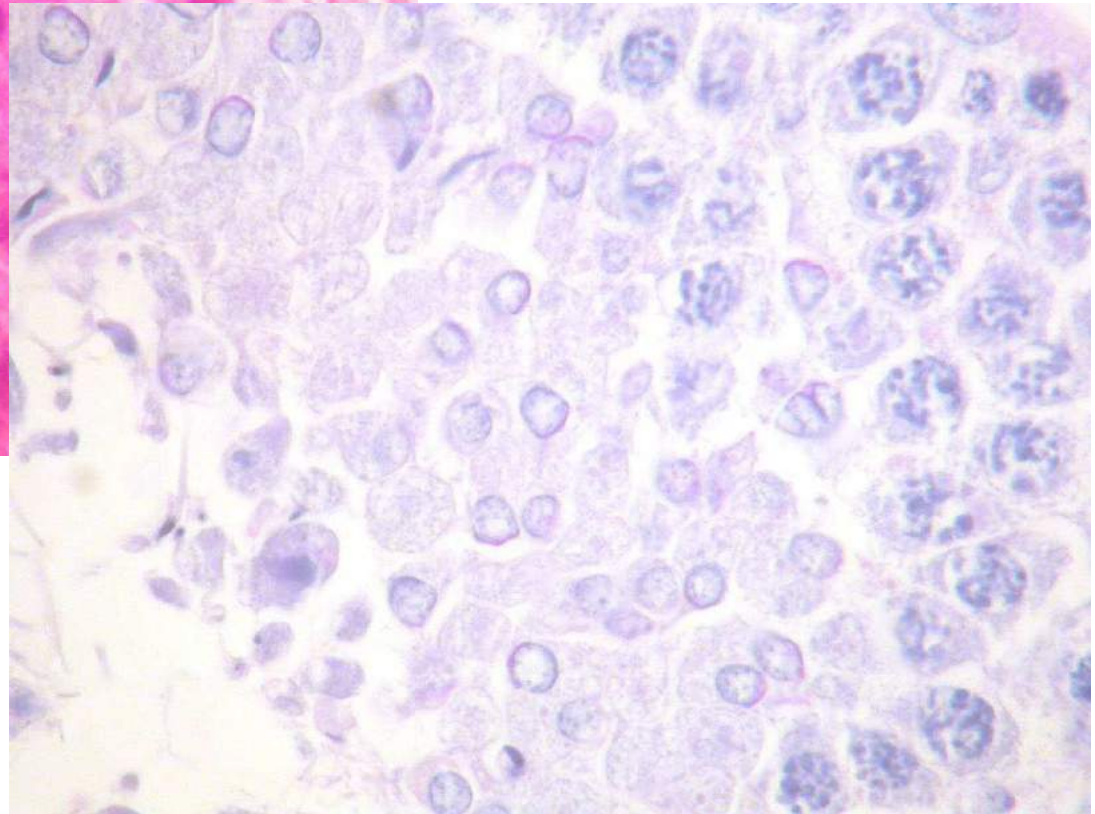
Which population???



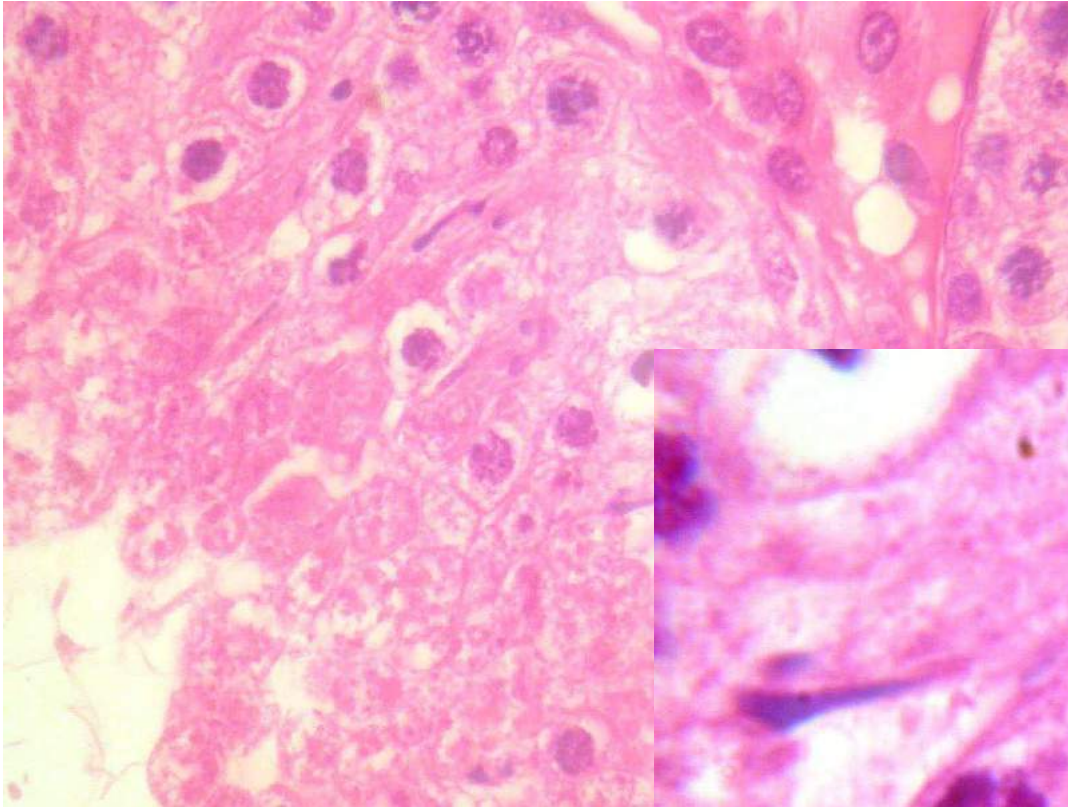
Staging; How to use?



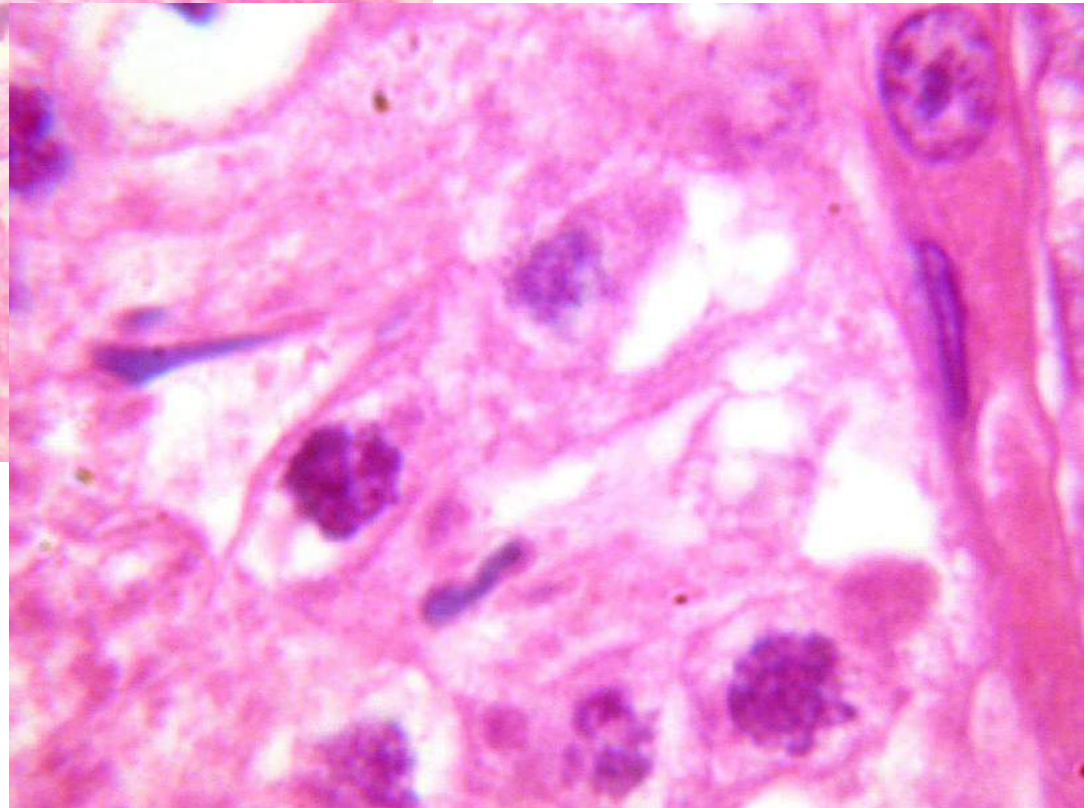
**Elongated Spermatids
in stage VII and VIII
with resorption!**



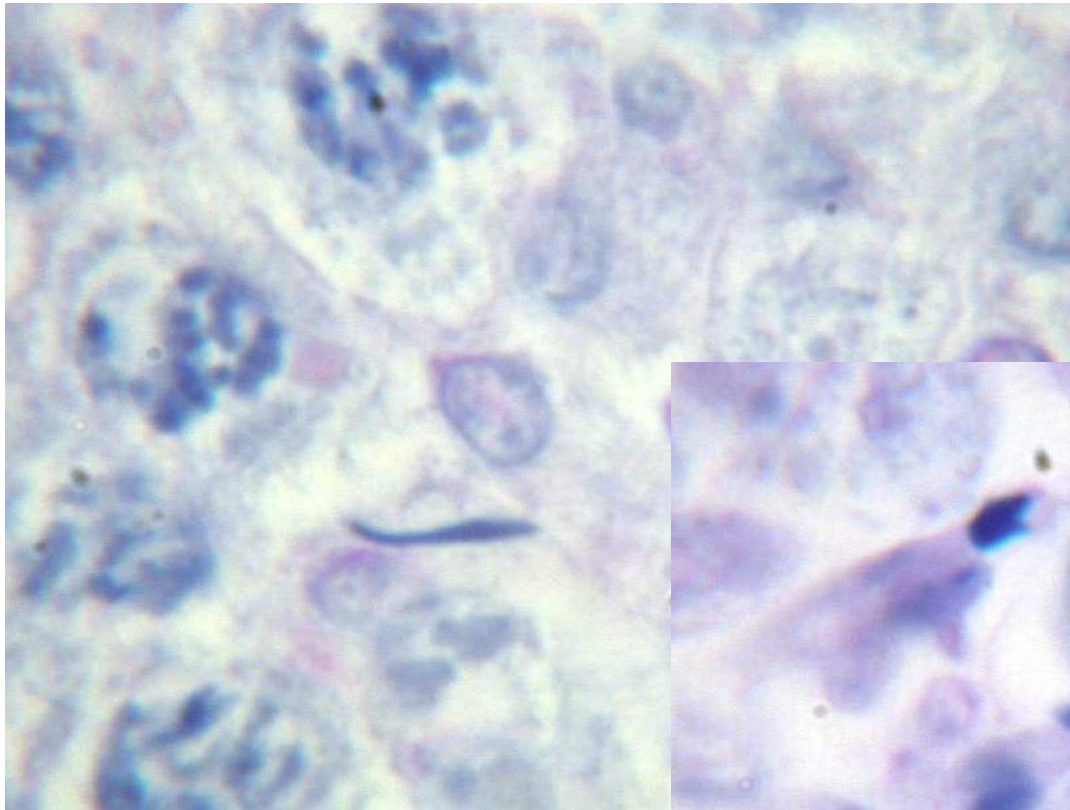
Staging: How to use?



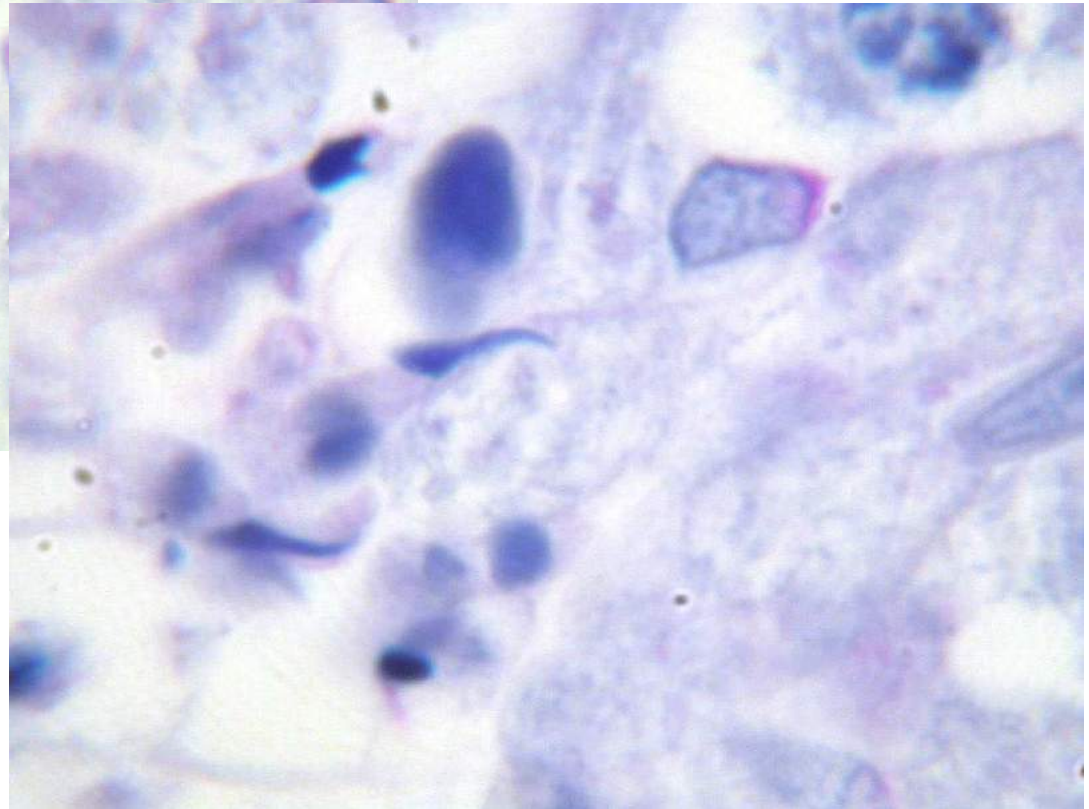
**Also necrosis in
round spermatids!
Stage?
Maturation arrest?**



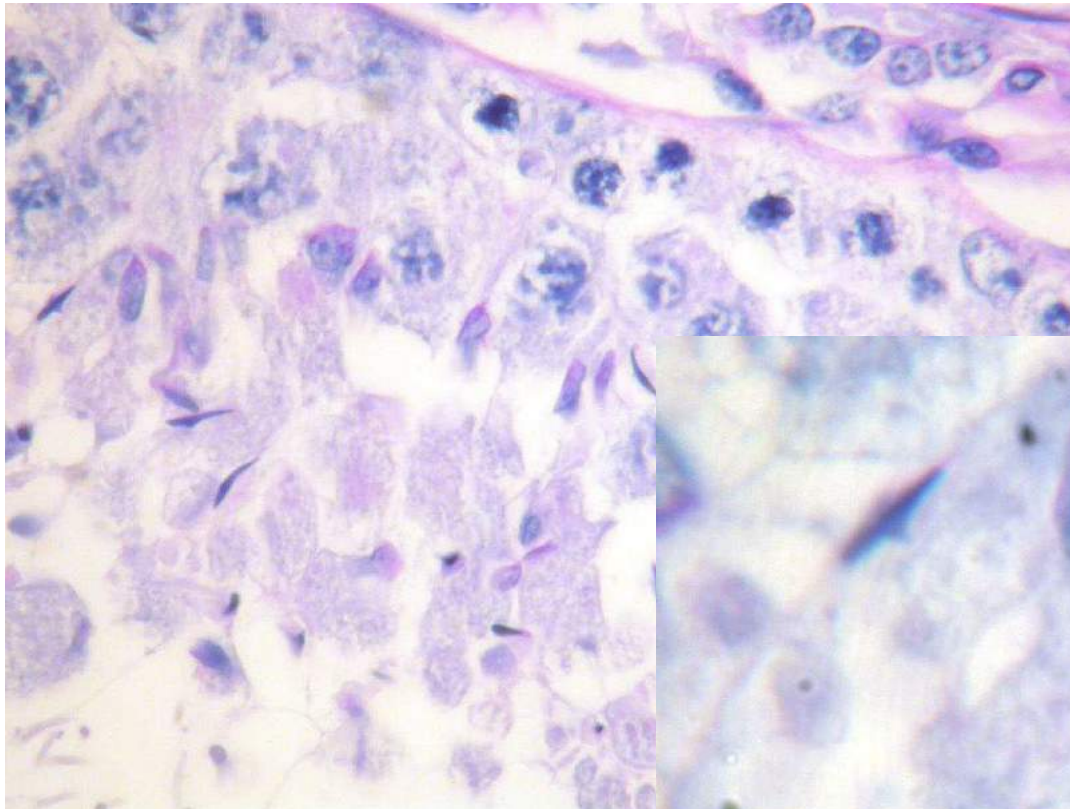
Staging: How to use?



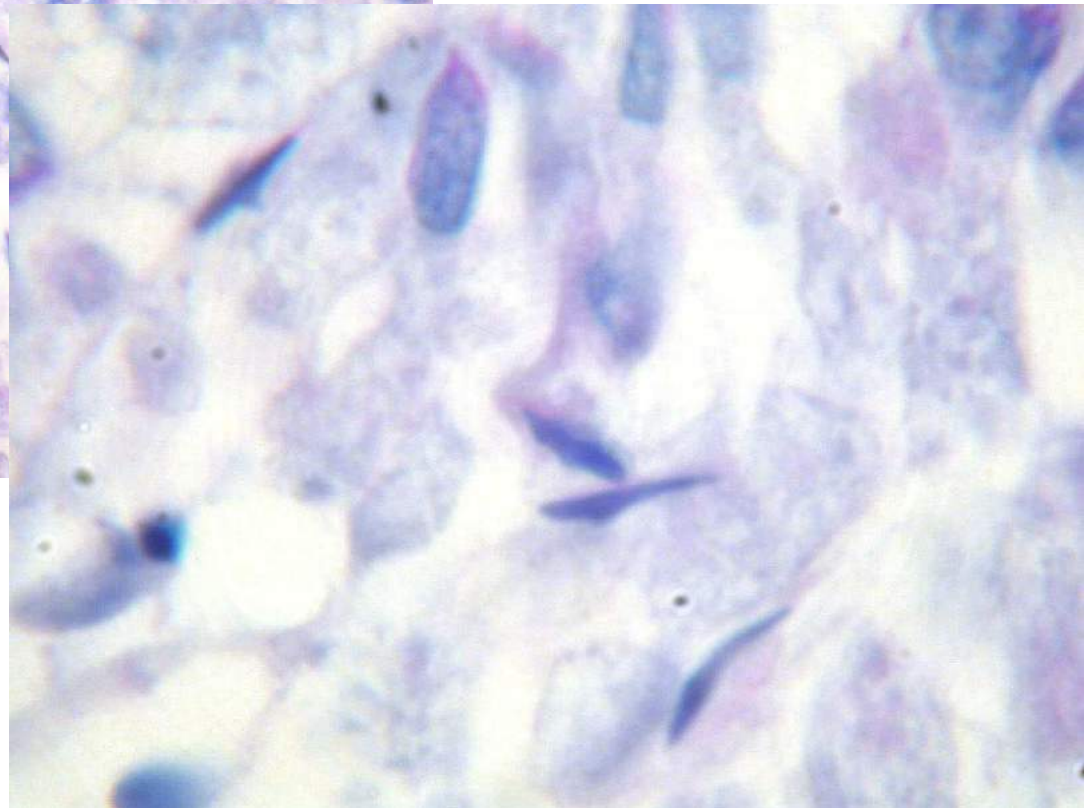
**Maturation arrest
in stage IX!**



Staging: How to use?



**Maturation arrest
in stage X!**



Other Species: Staging

Dog:

Acrosome ruptures during fixation

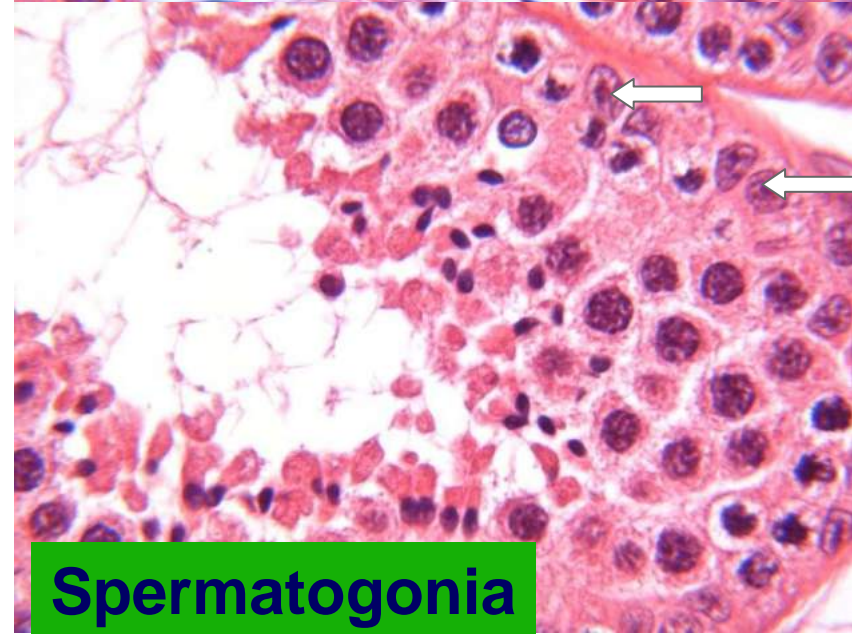
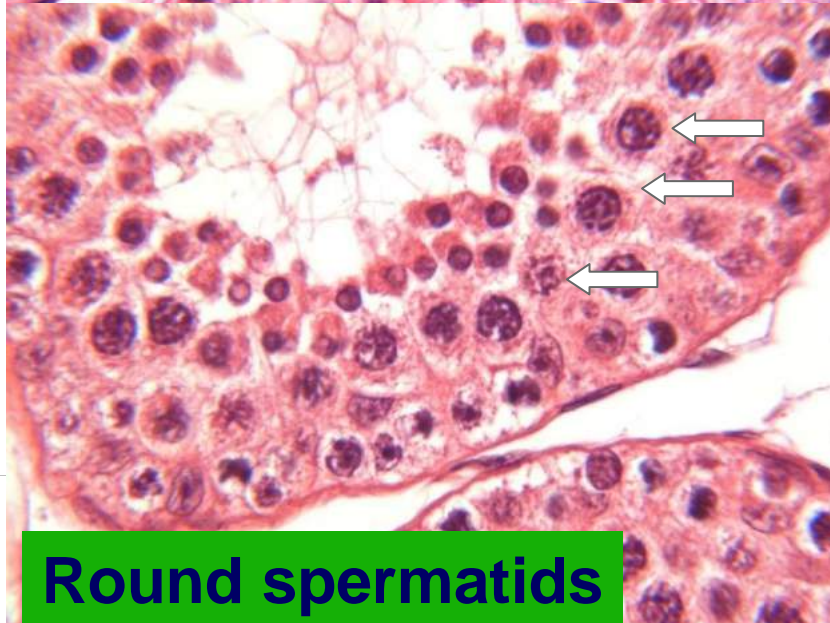
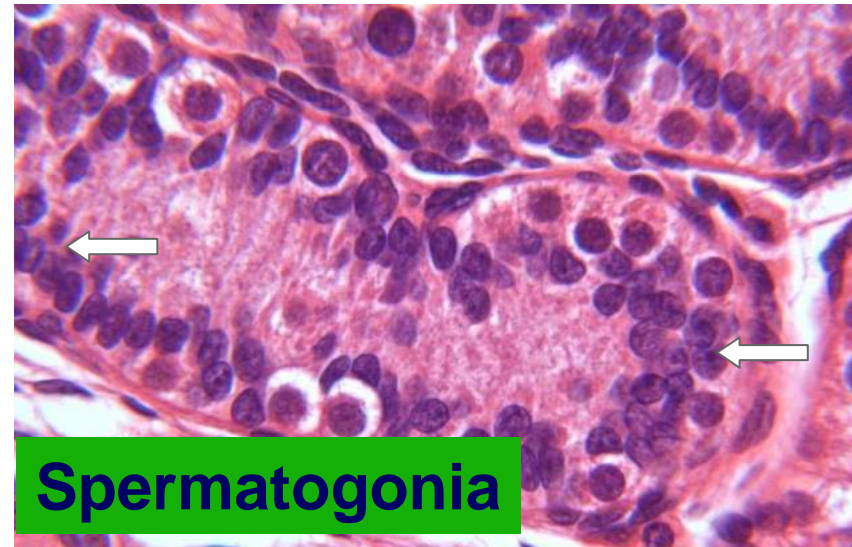
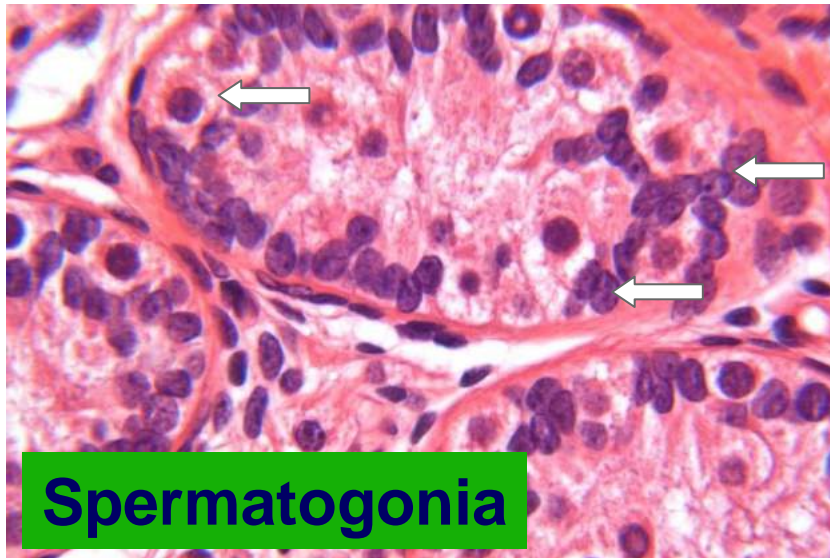
Cynomolgous:

In most toxicity studies immature

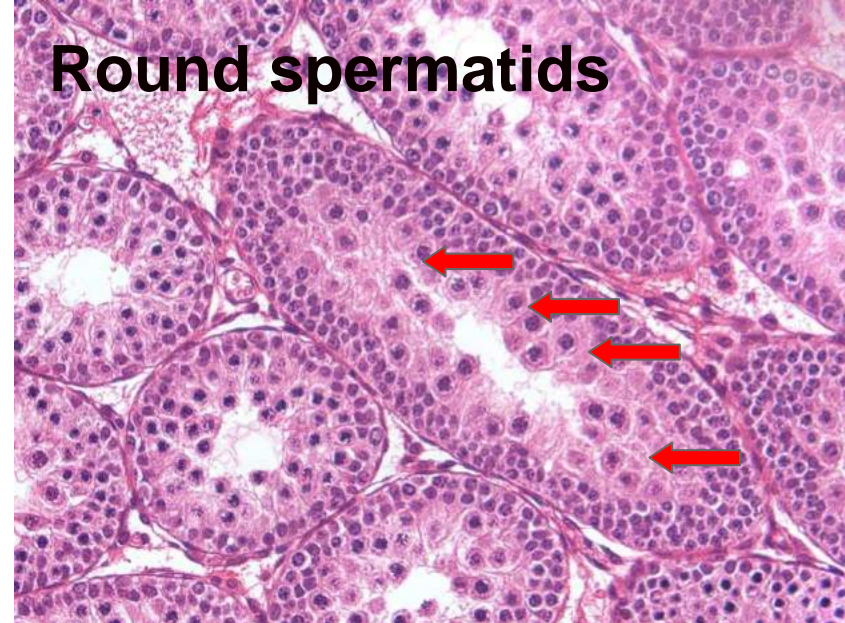
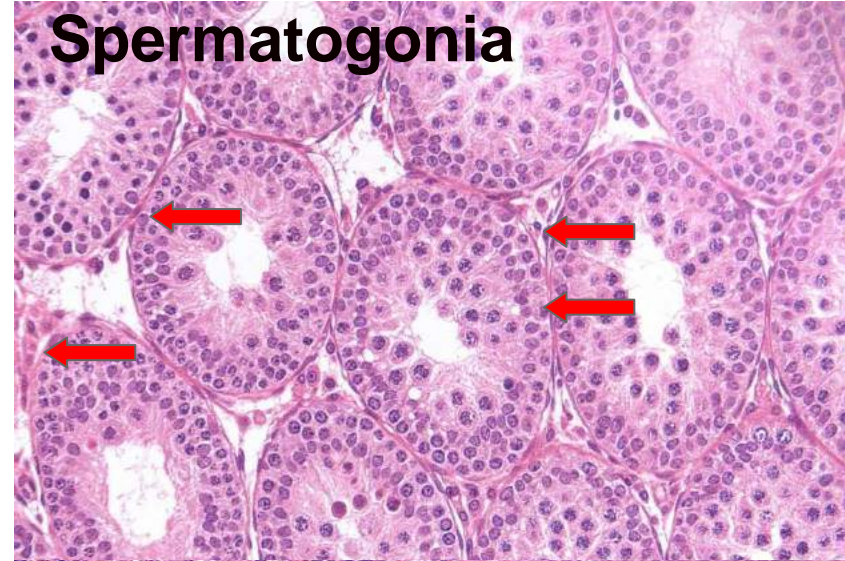
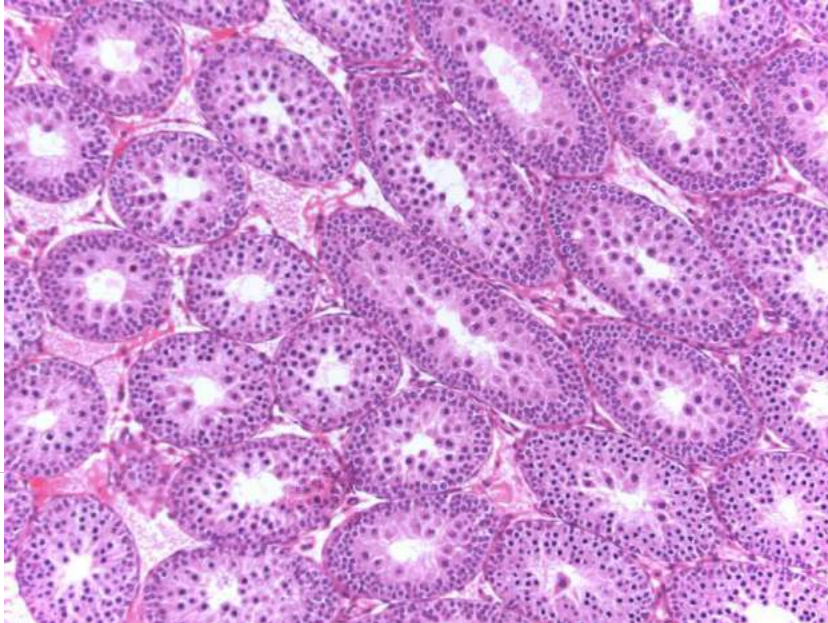
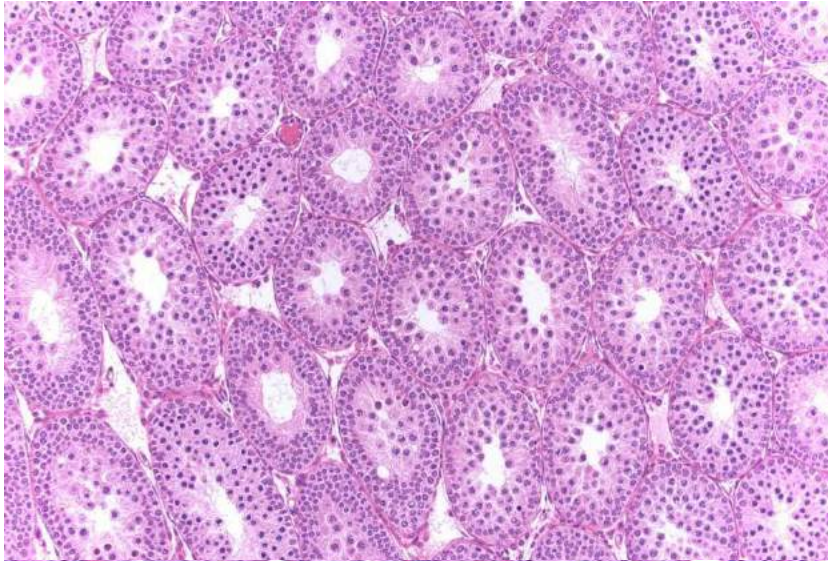
Human:

Approximately 4 stage on one tubular section due to spiral arrangement

Immaturity: *Cynomolgus*



Immaturity: Rat



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

Testes	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Numbers of rats examined	772					
Cellular debris	12	1.55	0.79	4.54	0.00	40.00
Tubular vacuolation	41	5.31	6.42	21.02	0.00	100.00
Tubular degeneration	19	2.46	1.88	5.80	0.00	30.00
Multinuclear sperm	1	0.13	0.04	0.46	0.00	5.00
Mononuclear foci	1	0.13	0.17	1.83	0.00	20.00

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

Males	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Numbers of rats examined	1011					
Anomaly	1	0.10	0.13	1.14	0.00	10.00
Hypoplastic testis	1	0.10	0.13	1.14	0.00	10.00
Tubular ectasia	1	0.10	0.13	1.14	0.00	10.00
Hemorrhage	1	0.10	0.09	0.76	0.00	6.67
Cellular debris	3	0.30	0.43	3.80	0.00	33.33
Mononuclear cell foci	4	0.40	0.35	3.04	0.00	26.67
Multinuclear giant cell	2	0.20	0.26	1.60	0.00	10.00
Hypospermiogenesis	1	0.10	0.06	0.57	0.00	5.00
Tubular degeneration	40	3.96	4.25	7.77	0.00	40.00
Sertoli cell vacuolation	7	0.69	0.84	5.09	0.00	40.00
Spermatid retention	2	0.20	0.26	2.28	0.00	20.00
Granuloma	1	0.10	0.13	1.14	0.00	10.00
Leydig cell hyperplasia	1	0.10	0.06	0.57	0.00	5.00

104-Week

Males	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Numbers of rats examined	3617					
Dilation/rete testis	1	0.03	0.04	0.28	0.00	2.00
Capsular dilatation	2	0.06	0.03	0.24	0.00	1.67
Spermatocele	18	0.50	0.51	1.14	0.00	5.00
Congestion	21	0.58	0.60	2.34	0.00	15.71
Hemorrhage	5	0.14	0.16	0.50	0.00	2.00
Edema	182	5.03	4.54	8.85	0.00	37.37
Vascular hyalinosis	3	0.08	0.09	0.34	0.00	1.43
Amyloidosis	1	0.03	0.04	0.28	0.00	2.00
Mineralization	164	4.53	4.60	3.73	0.00	13.13
Angiopathy	7	0.19	0.20	1.41	0.00	10.00
Oligospermia	5	0.14	0.13	0.55	0.00	3.00
Aspermia	16	0.44	0.60	2.14	0.00	12.00
Maturation arrest	3	0.08	0.09	0.45	0.00	2.86
Sperm stasis	48	1.33	1.37	3.65	0.00	20.00
Giant cells	17	0.47	0.48	1.20	0.00	4.29
Tubular degeneration	812	22.45	22.04	15.55	0.00	99.00
Mononuclear cell foci	5	0.14	0.13	0.58	0.00	2.86
Granuloma(s)	18	0.50	0.54	2.12	0.00	14.49
Periarteritis/arteritis	327	9.04	8.81	8.81	0.00	40.58
Inflammation	1	0.03	0.04	0.28	0.00	2.00
Fibrosis	4	0.11	0.11	0.44	0.00	2.02
Leydig cell hyperplasia	120	3.32	3.43	4.52	0.00	22.00