



CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY REPRODUCTIVE SYSTEM

Third Conference

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Patho-Physiology of Male Reproductive Organs in Rodents and Non-Rodents

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Common sense in Toxicology/Pathology

- Histopathology techniques/**trimming**/staining and artifacts
- Identification of lesion and its criteria of grading
- Spontaneous lesions
- Proliferative *vis-à-vis* carcinogenic lesions
- Effects of management/environment factors

Spermatogenesis

- Spermatogenesis is essentially similar in all mammalian species.
- Following are the subtle changes
 - Germ cell loss,
 - Spermatid retention,
 - Inappropriate population of germ cells,
 - Identifying susceptibility of target cell population to relate the lesion/s to duration of dosing

Disturbances in Spermatogenesis

- Direct adverse effect on the seminiferous epithelium, sertoli cell or spermatogonial cells
- It may occur as a secondary response to
 - altered hormone levels,
 - altered vascular supply,
 - altered fluid balance within the testis/epididymides



Obstruction of fluid flow and resultant effects on testes and epididymides

- Blocked fluid flow from testes to epididymides leads to low epididymal sperm count and affects fertility adversely
- Increases testes-weight & marginal increase in testicular sperm count
- Seminiferous tubular dilation
- Atrophy of seminiferous epithelium

General Considerations for Pathologists

Effects of hormones

- Reduced weights of testes, accessory sex organs and impaired spermatogenesis

Relationship between Testes and epididymides

- Inhibition of sperm release from testes leads to raised spermatids in testes
- And lower epididymal sperm counts

Grading of Spermatogenic Germ Cell Degeneration

Severity grading of tubules affected

- (minimal) <5% of tubules affected
- (slight) 5–25% tubules affected
- (moderate) 25–50% tubules affected
- (marked) 50–75% tubules affected
- (severe) >75% tubules affected

Decreased testes weight

Causes:

- **Germ cell depletion**

Disruption of spermatogenesis due to effects on germ cells, Sertoli cells or hormonal imbalance or reduced blood supply

- **Contraction of Seminiferous tubular lumen**

Decreased production of seminiferous tubular fluid which may be due to inability of elongation of spermatids and/or decreased testosterone production

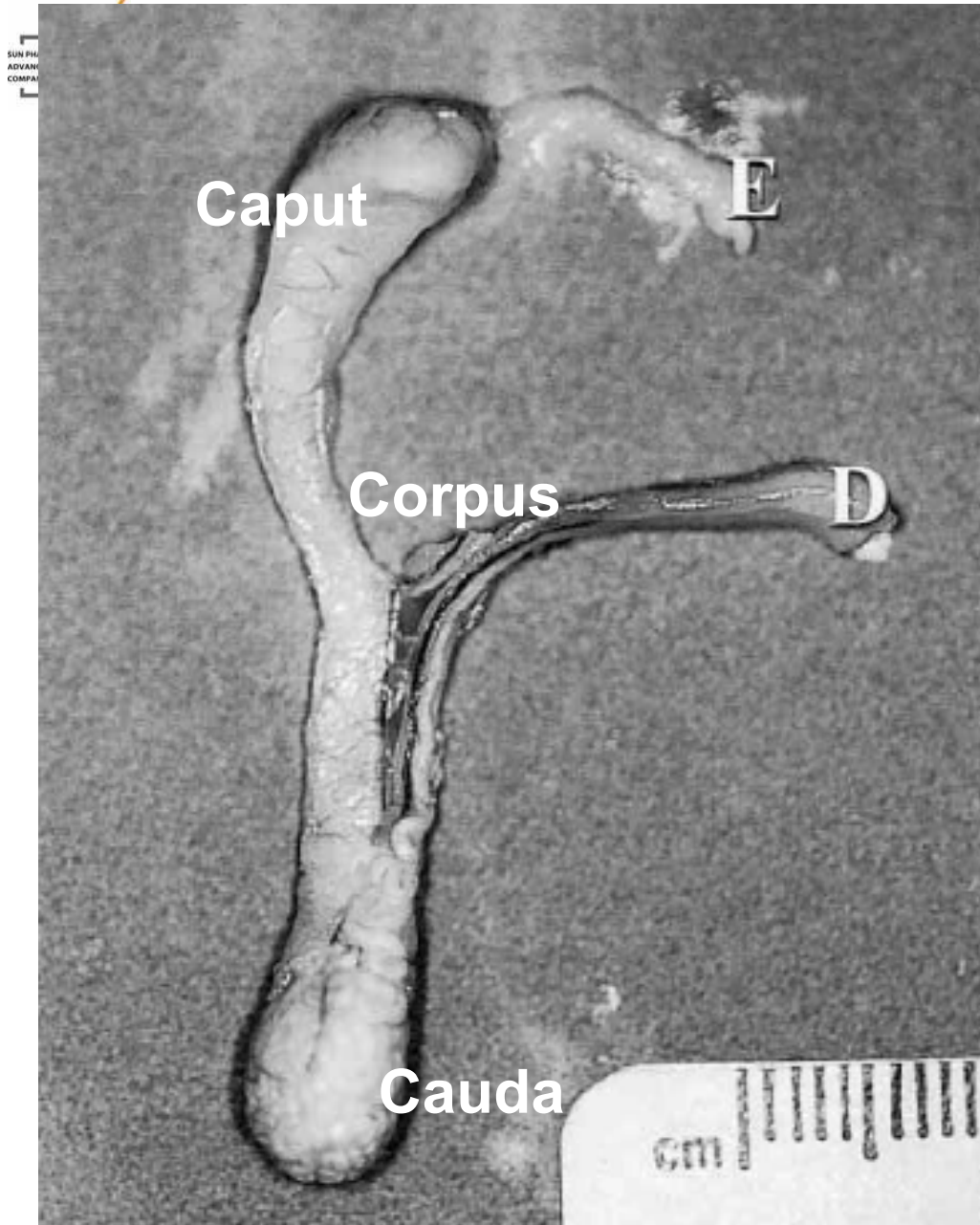


Microscopic Examination of Testes and Epididymedes

- **Influence of duration of dosing**
Cell and stage specific adverse effects are usually seen on spermatogenesis in studies of 2-4 weeks
- Depletion in maturation process resulting in germ cell loss affecting all stages of spermatogenesis in studies of 13 weeks or more
- Correlation is generally evident in organ weight changes and microscopic findings

Epididymis

- *Cellular composition and function of epididymis vary with region. Therefore it is important that that different regions are examined in one section.
- *A longitudinal section of caput, corpus and cauda should be selected for histopathological examination
- *Site-specific lesions in the epididymal epithelium, have been reported
- *Density of sperm and presence of germ cells within lumen of various segments of the epididymis reflect maturation process of spermatozoa

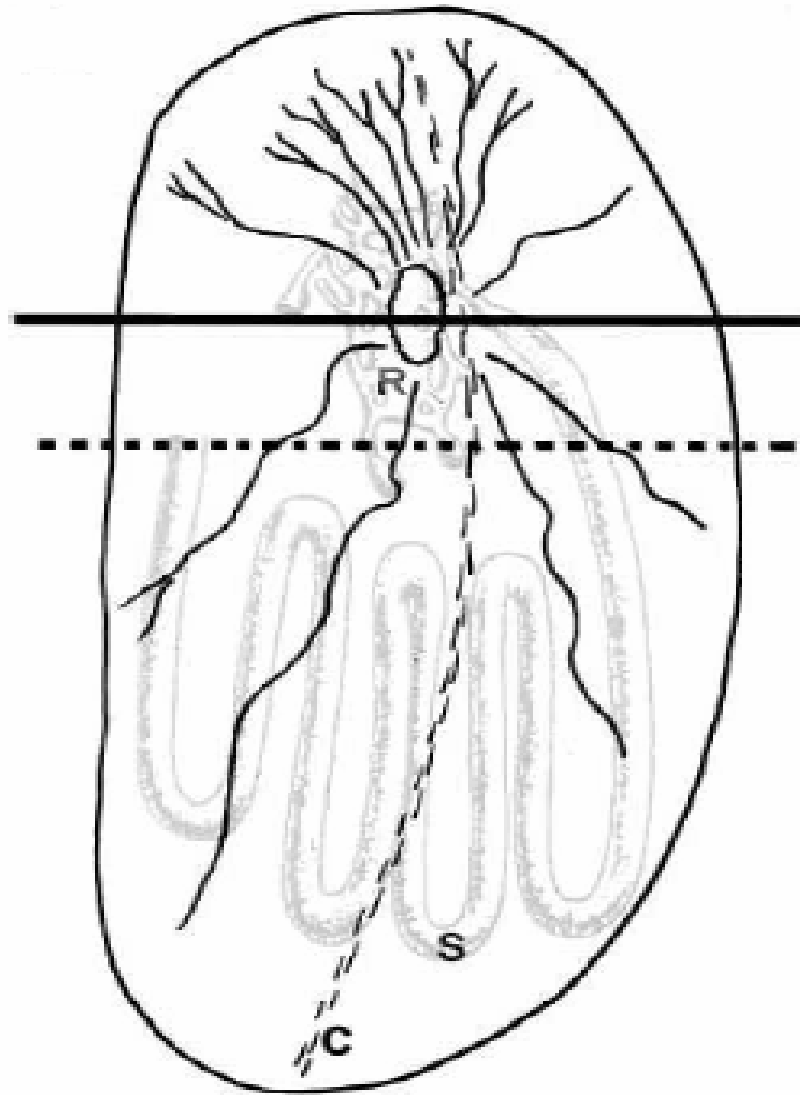


Epididymis

Efferent duct (E) attached to caput illustrating anatomy

Ductus deferens (D) attached to cauda

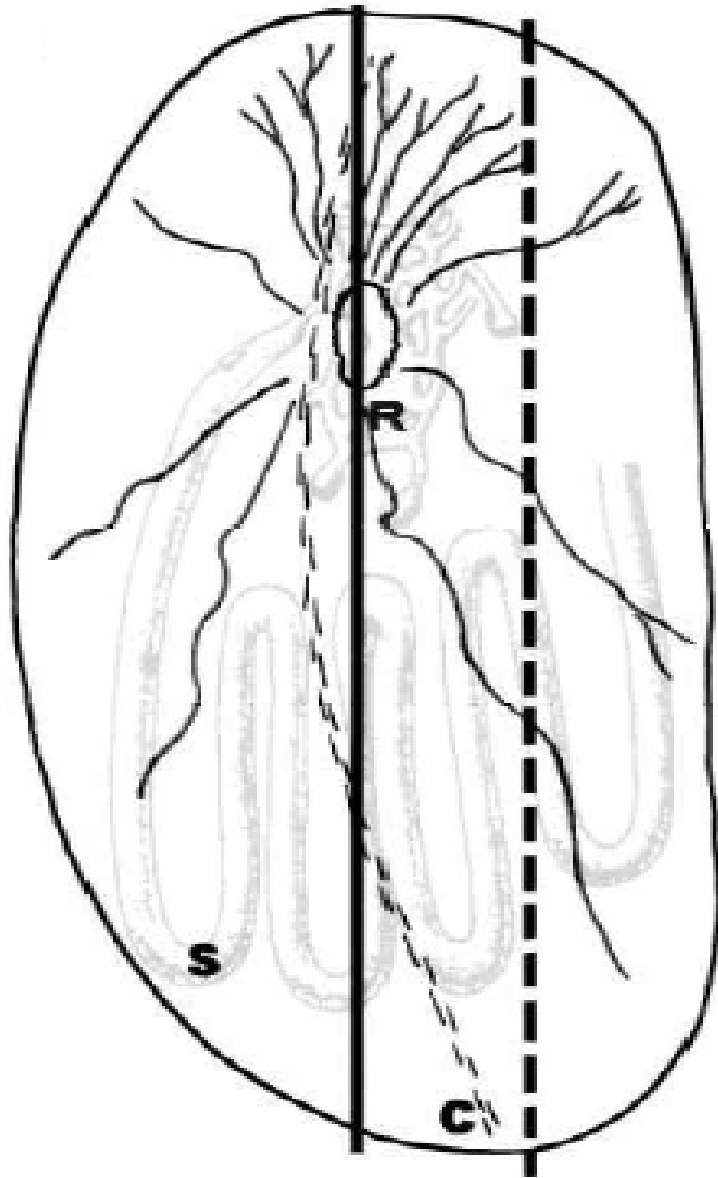
Identification of epididymal regions is distinct due to shape of caput, corpus and cauda



Orientation of T.S. of testes in rats. Solid line representing the surface for examination and dashed line the secondary surface. The section should contain the rete testis (R) and of seminiferous tubules (S).

Caudal view of Right Testis

- R = Rete testis
- S = Seminiferous tubule
- C = Epididymal attachment



Orientation of L.S. of testes in rats. Solid line representing the surface for examination and dashed line the secondary surface. The section should contain the rete testis (R) and of seminiferous tubules (S).

Caudal view of Left Testis

- C** = Epididymal attachment
- S** = Seminiferous tubule
- R** = Rete testis

**Photomicrograph of rat testis showing tubuli recti (T), rete testis (R).
Correct 'orientation' at trimming helps to locate and identify these areas
and avoid wrong diagnosis**



Spontaneous Findings

- Hypospermatogenesis,
 - * From oligospermia to aspermia
 - * Tubules generally smaller in diameter
 - * Often filled with pale eosinophilic cytoplasm of dead cells
 - * If the change is severe and unilateral, it is likely to be due to earlier a congenital lesion.
 - * Hypospermia - occasional tubules that has increased multinucleated spermatocytes and cellular debris along with spermatozoa

Multinucleated cells:

Small number of cells normally found in testes of control or untreated rats; increased in their numbers is an indication of toxicity.

Vacuolation

Vacuolation/s in sertoli cells is often an indicator of sertoli cell degeneration

It may be seen spontaneous in an occasional sertoli cell which may be scattered in multiple tubules

Spermatocoele or sperm granuloma

- Often marked dilation seen filled with sperm/germ cells, cellular debris and inflammatory cells and some times even multinucleated giant cells.
These granulomas become fibrotic or mineralize.
- If, it is located at rete testis, testicular fluid flow from one to several or all tubules may be obstructed causing luminal dilation and secondary pressure atrophy of seminiferous epithelium

Age

Age of Sexual Maturity

- Rat: >9 weeks
- Mice : >7 weeks
- Dog: 9-12 months
- Monkey: 4-5 years

Stress

- Malnutrition in adolescent rats is associated with hypogonadism and abnormalities in subsequent events of spermatogenesis
- In control group rats used in inhalation studies, increased incidences of testicular degeneration are noted as compared to the same strain of rat used for oral toxicity studies
- Or any other form of stress cause adverse effects in male reproductive system



Neoplasms of Male Reproductive Organs

Rat : Spontaneous incidences in %	C.river	Others
Interstitial (Leydig) cell Adenoma	7.17	10.5
Interstitial (Leydig) cell carcinoma	-	0.04
Teratoma, benign (Testis)	0.18	-
Mesothelioma, Malignant (Testis)	0.36	0.15
Mesothelioma, Malignant (Epididymis)	0.72	0.05
Adenocarcinoma (Epididymis)	-	0.05
leiomyosarcoma (Epididymis)	-	0.05



Testicular Neoplasms

Prevalence:

~ 3 - 20% In rats

~ <10% in mice

Majority interstitial cell tumors are found

Due to rare incidences and scanty information available on neoplasms of male reproductive organs, this presentation is derived from STP papers/references plus in-house data.

Interstitial cell tumors

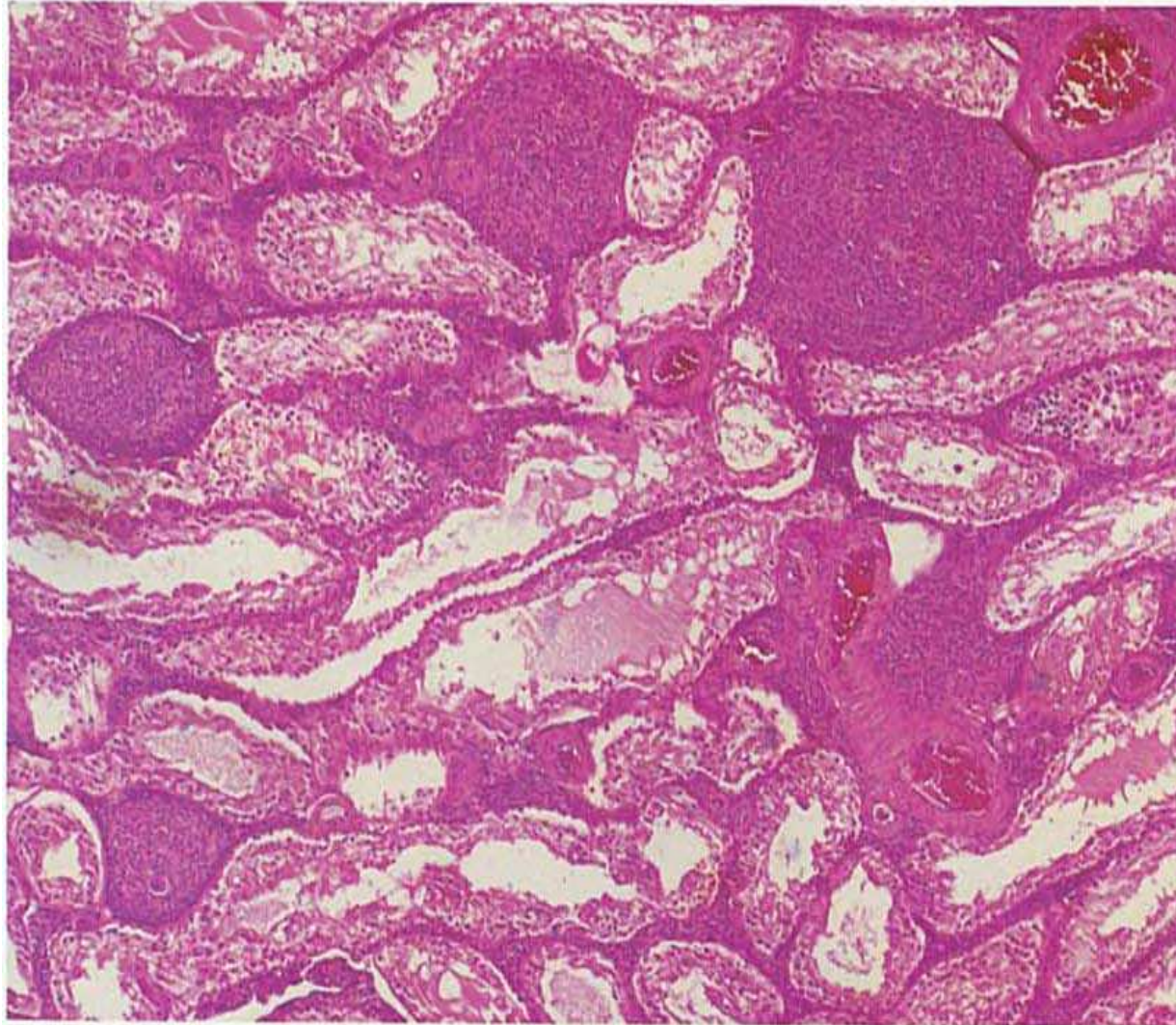
Gross appearance:

- ❖ **Circumscribed, yellow or white testicular masses, often hemorrhagic**
- ❖ **Can be bilateral**
- ❖ **Can be multiple within one testes**

Interstitial cell tumors

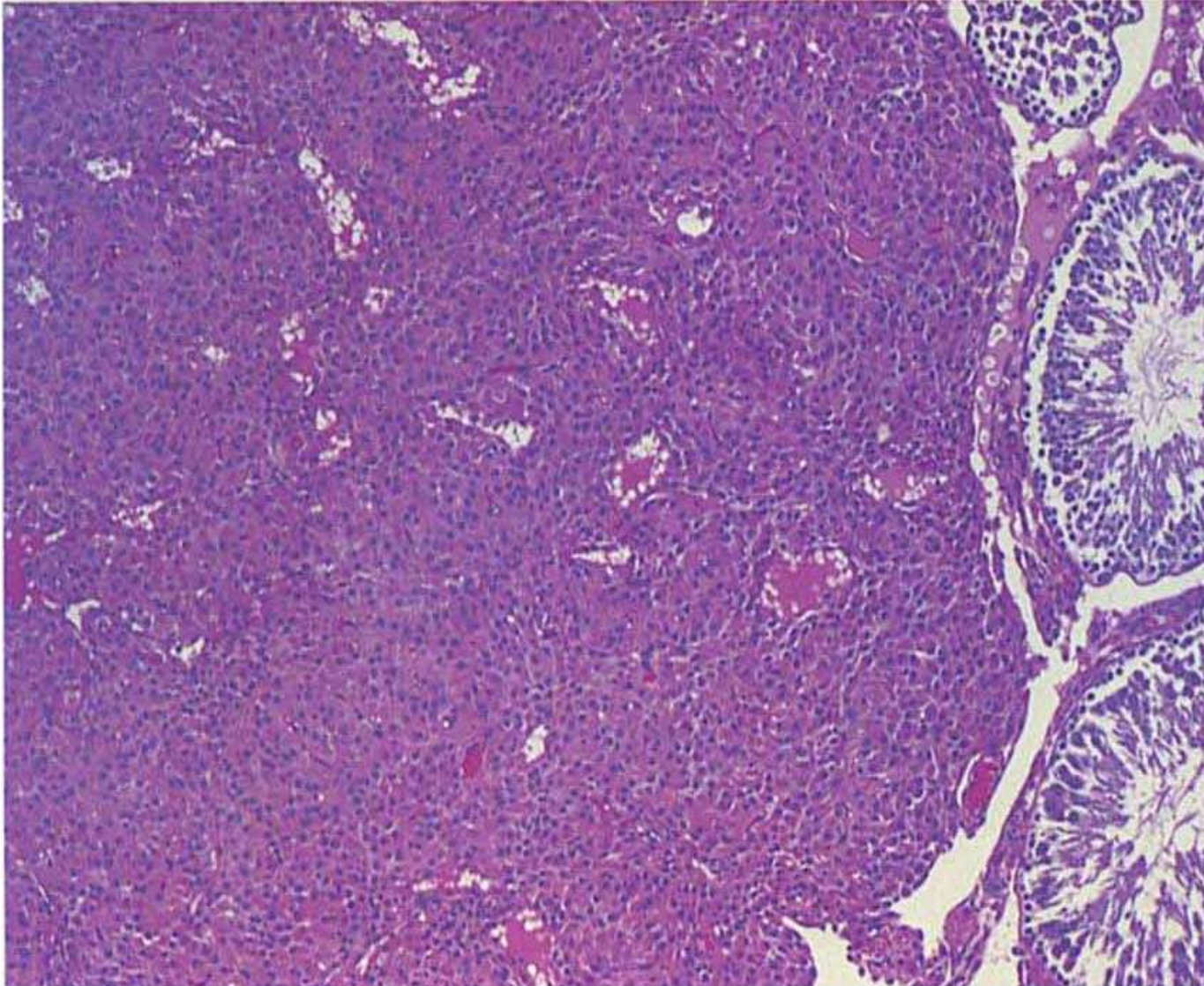


Multifocal Hyperplasia of Interstitial Tissue

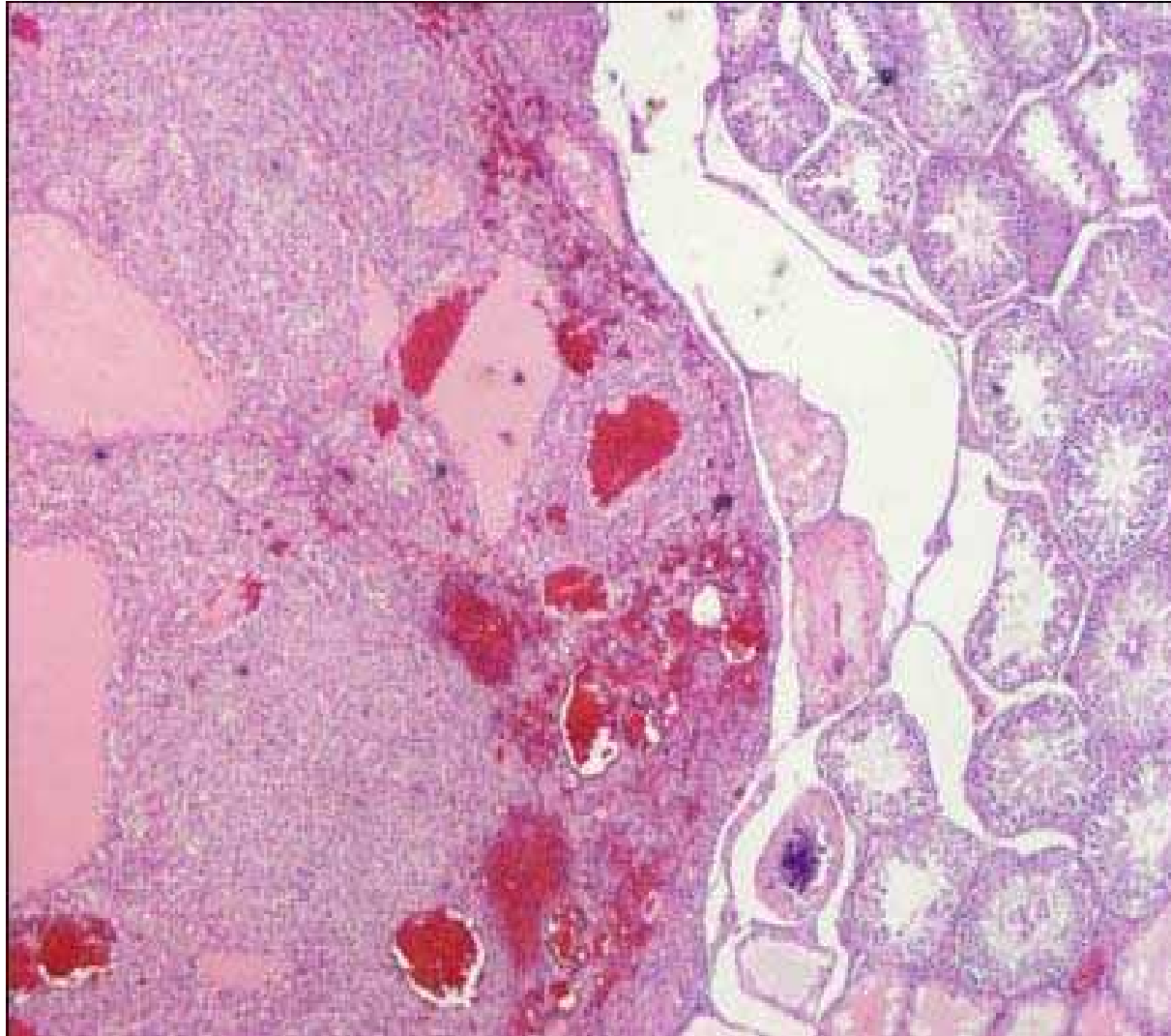


Rat 40X H&E

Testicular Adenoma



Testes: Cystic Adenoma

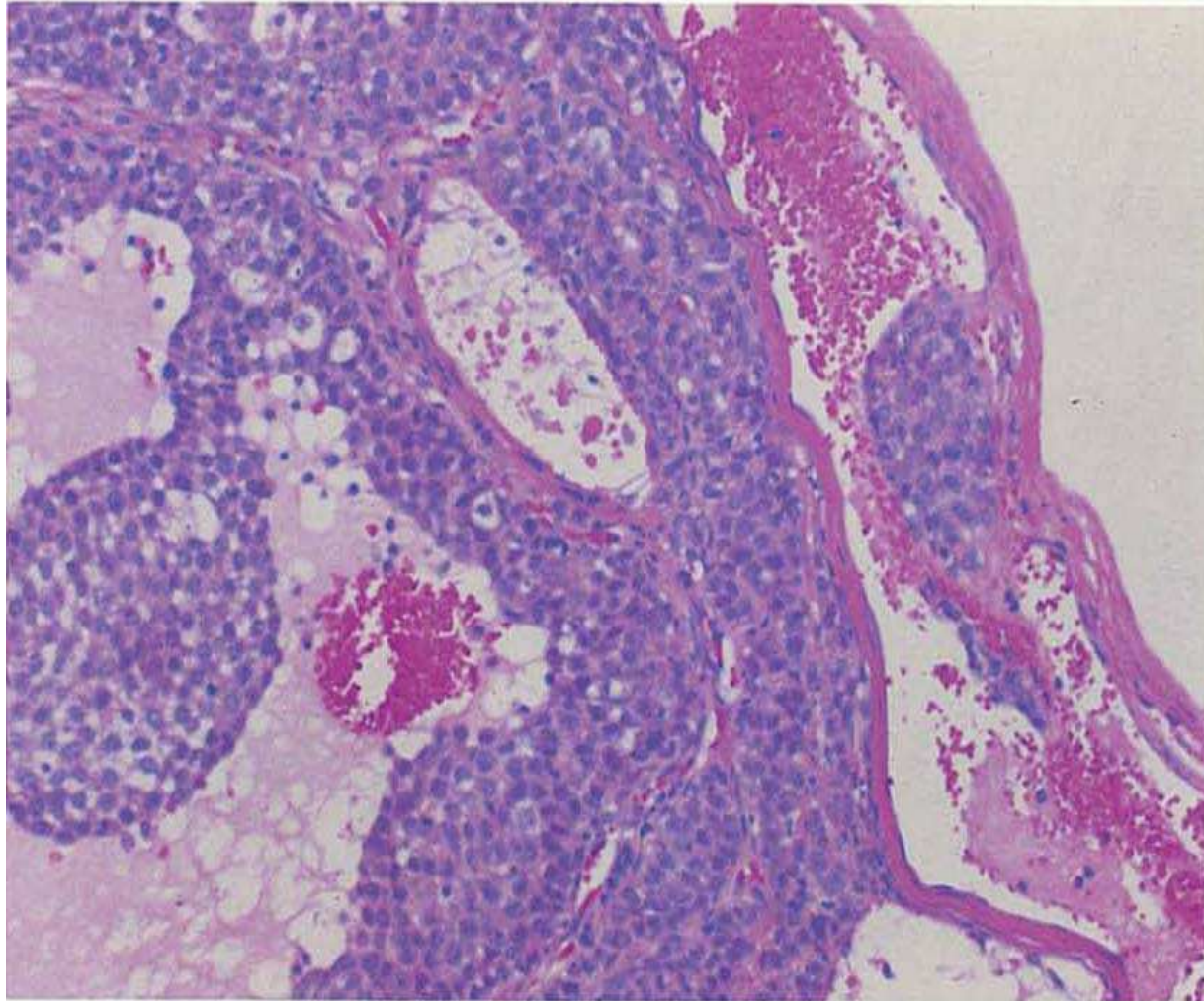


Interstitial (Leydig) Cell Carcinoma

Similar histo-anatomy of Adenoma but has.....

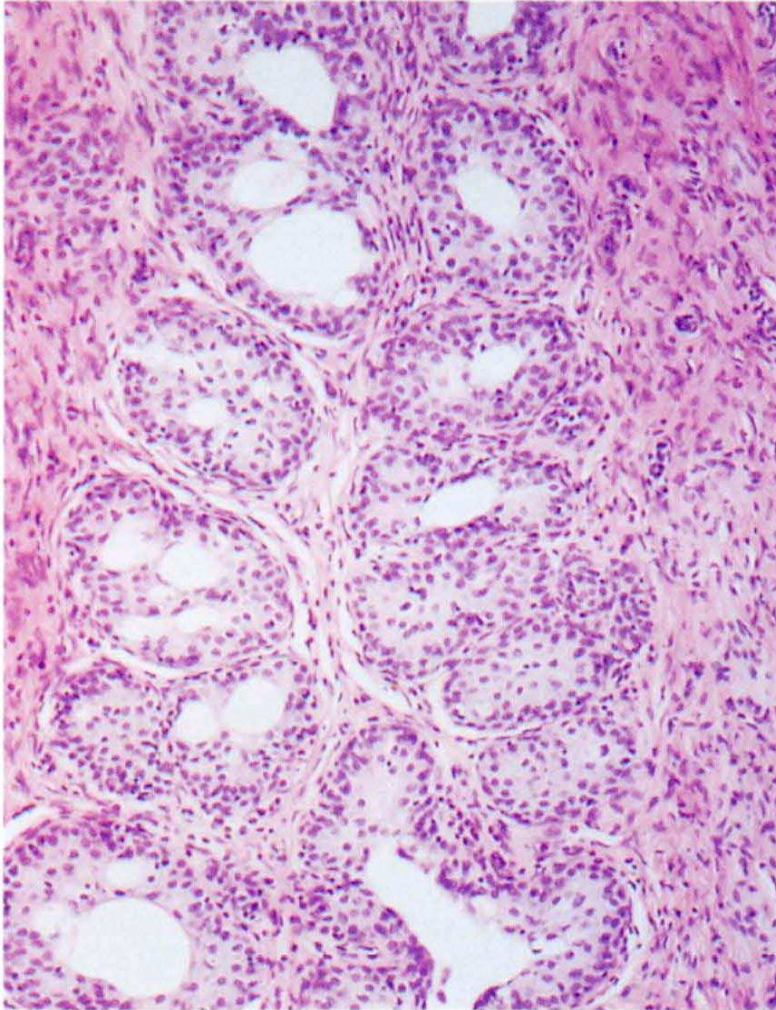
- ❖ Increased mitotic indices
- ❖ Hemorrhages or cysts may be prominent
- ❖ Invasion rare in other organs

Interstitial Cell Carcinoma



Tumor emboli in adjacent vessels 50X H&E

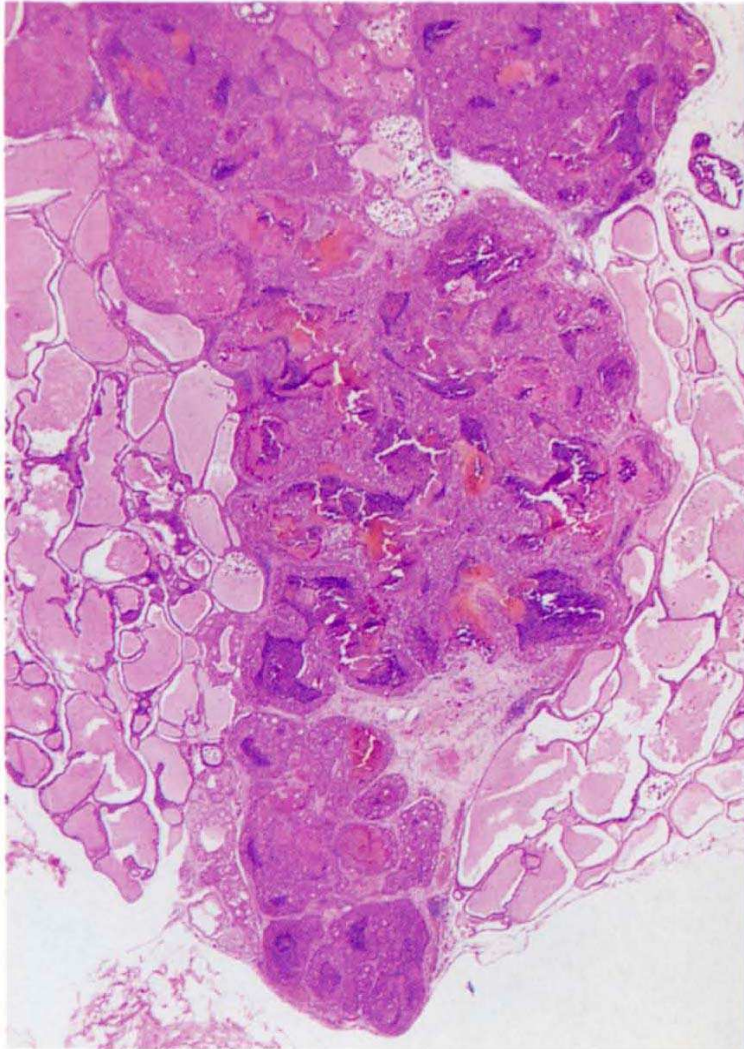
Squamous Metaplasia of coagulating gland in Rat



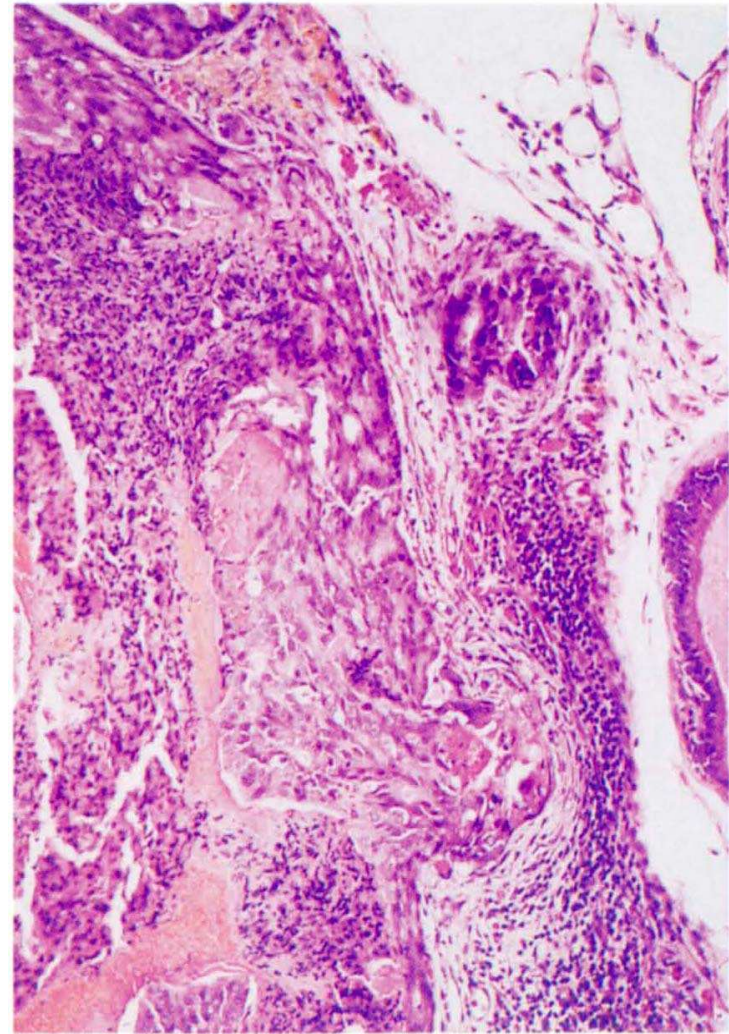
Squamous Metaplasia of prostate in Rat



Adenocarcinoma of prostate. Rat, H&E



Same Adenocarcinoma at 200x



Mesotheliomas

- Neoplastic focal complex papillary structures
- Prominent, vascularized stroma covered by one or more layers of cuboidal cells
- Generally spread throughout peritoneum

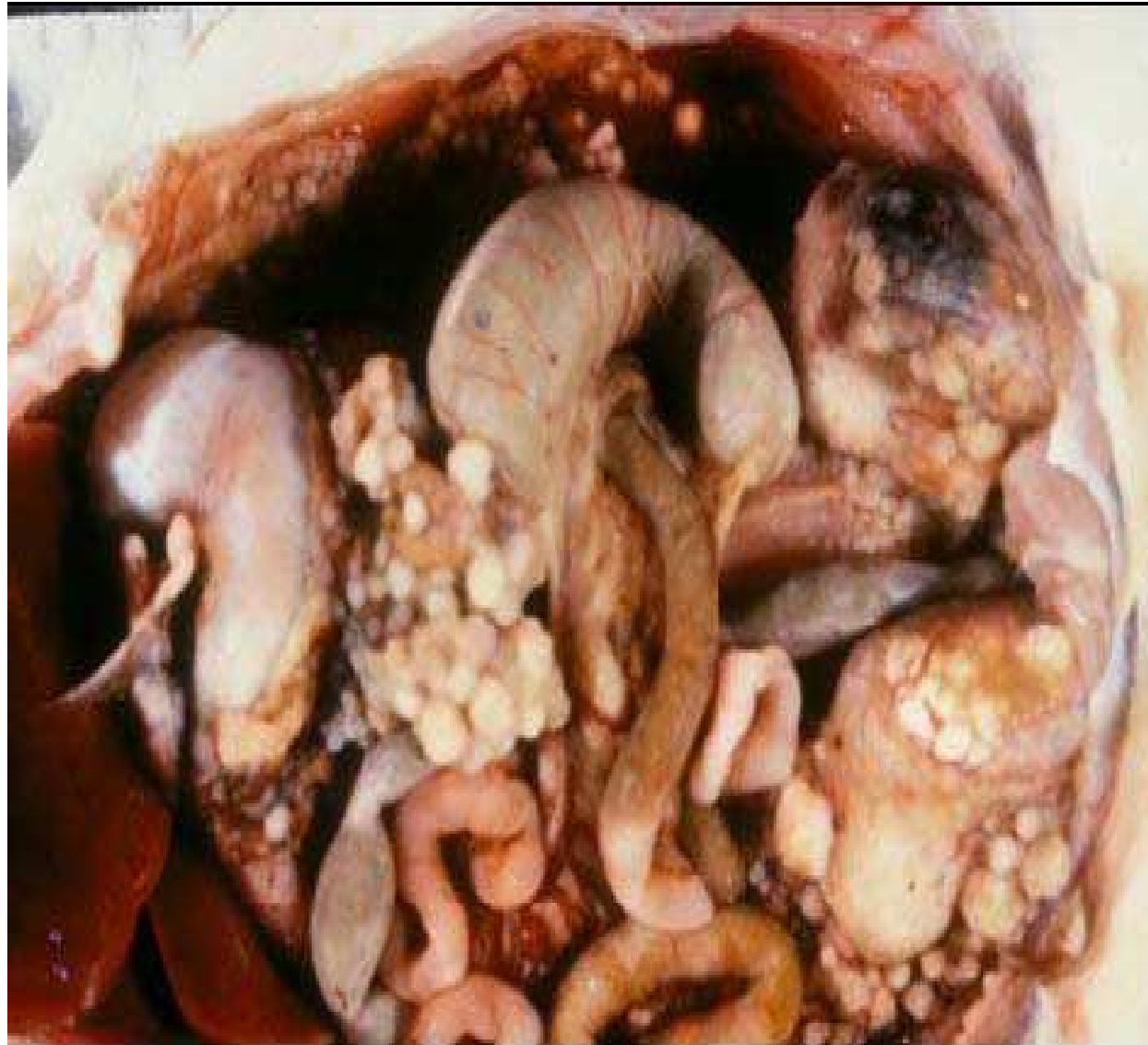
It should be distinguished from

- Mesothelial hyperplasia: Usually not visible grossly
- Microscopically, focal thickening, or occasional papillary projections seen, but no vascularization

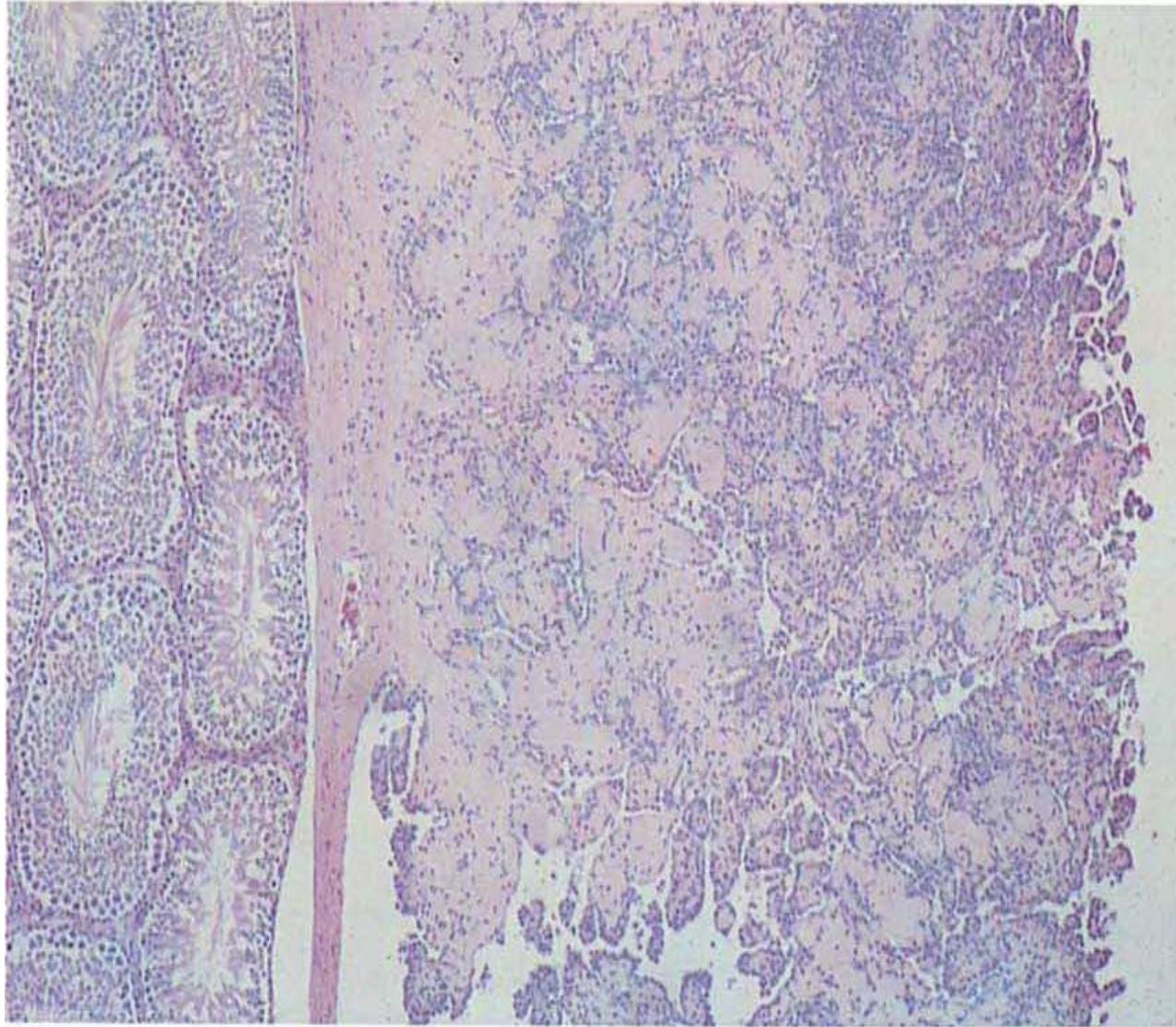
Mesotheliomas



Mesothelioma



Malignant Mesothelioma of tunica vaginalis: Rat



Pathognomonic lesions of mesothelioma ?

Although none of lesions are *pathognomonic* for Malignant pleural *mesothelioma* (MPM)

It is primary tumor of tunica vaginalis.

Pleural thickening has been classified as diffuse, nodular mass with very high number of mitotic indices.

(F Ökten – 2006)



Rete testis Hyperplasia, Adenoma and Carcinoma

Rete testis Hyperplasia

- Proliferative lesion rarely occurring in rats
- Consists of disorganized cystic tubules lined with a single layer of cuboidal or low columnar cells
- Papillary structure not formed in adenoma
- No encapsulation

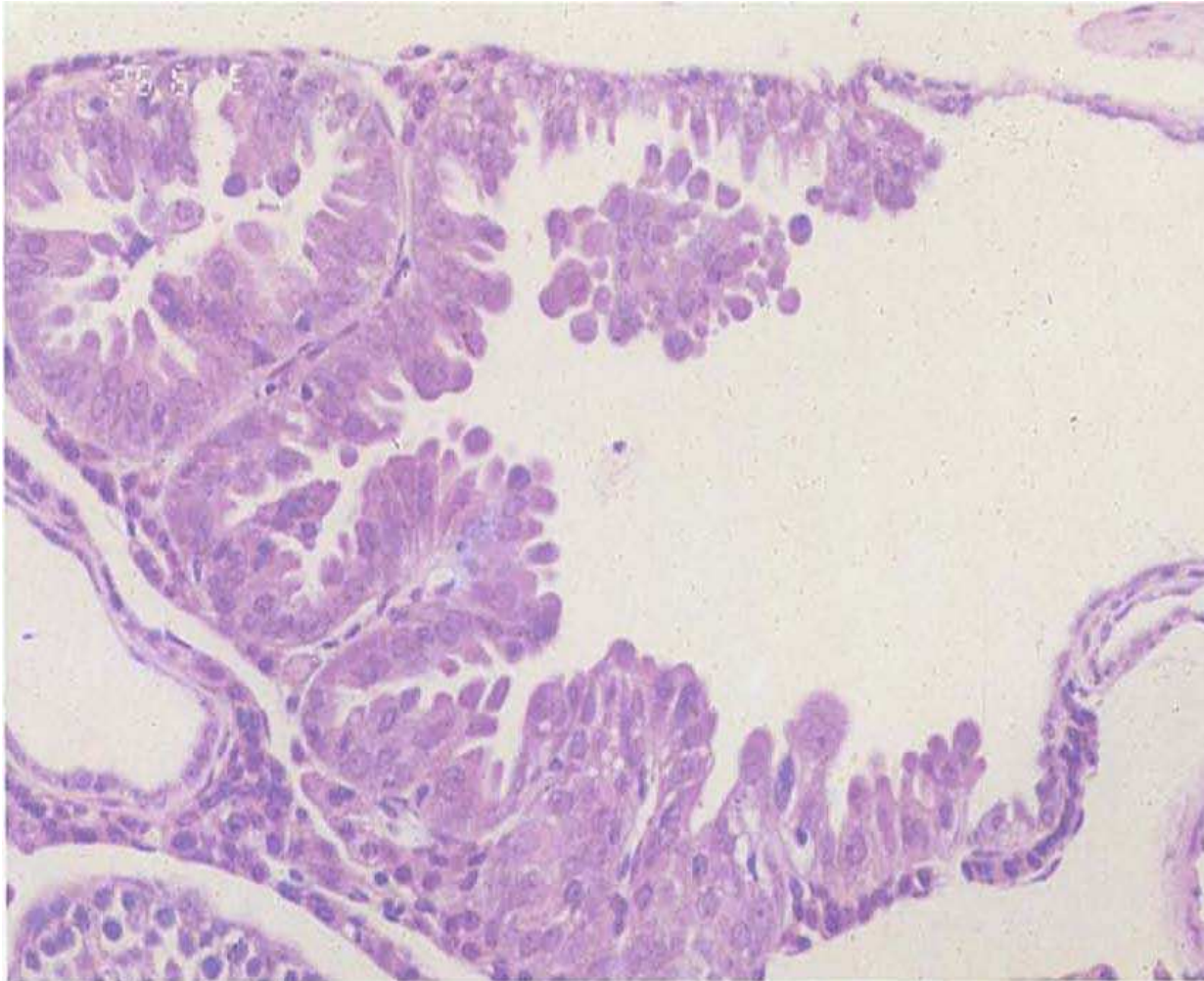
Rete testis adenoma

- Irregular dilated cystic tubules with single to multilayered cuboidal to low columnar cells
- Capsulation with compressed irregular periphery

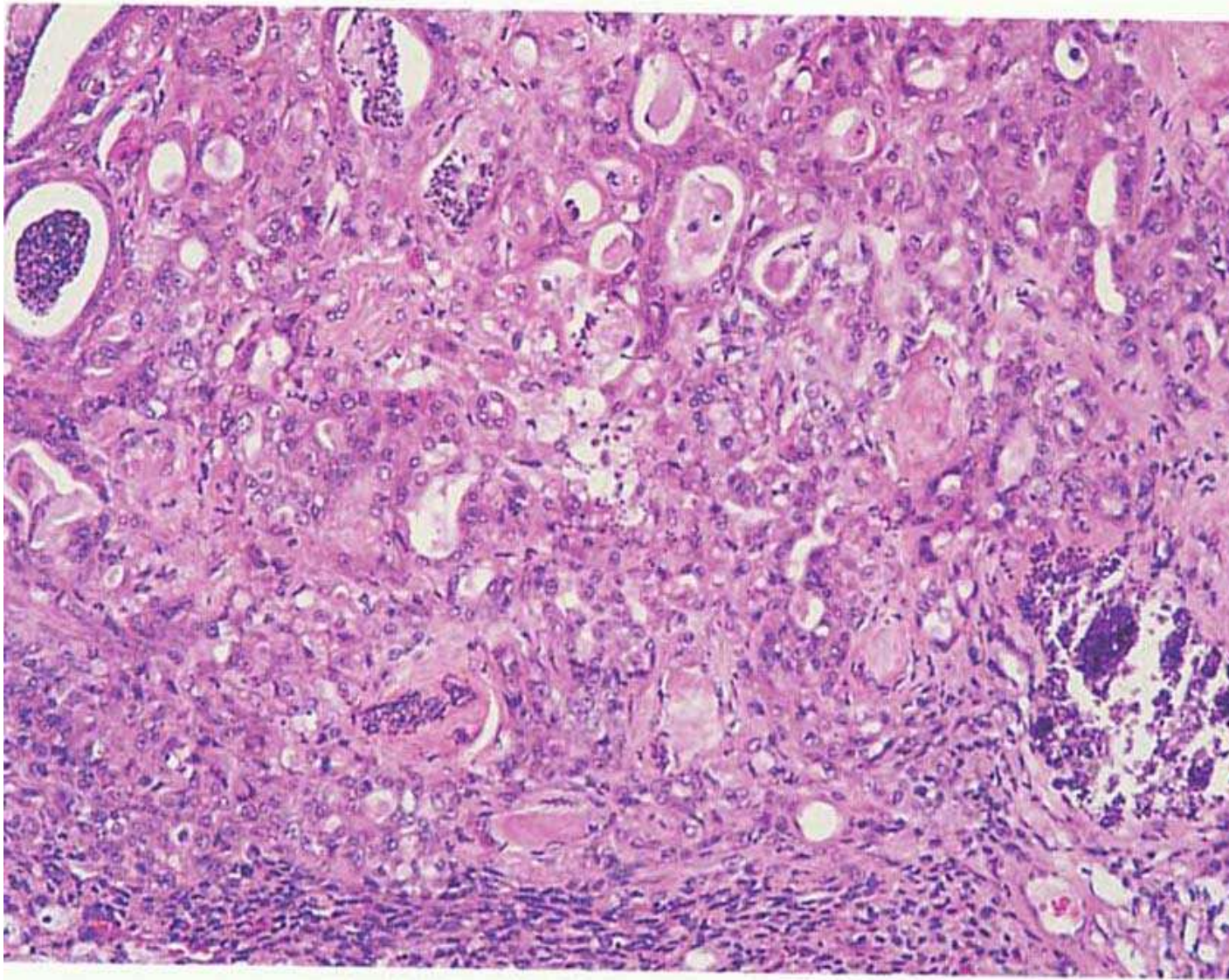
Rete testis carcinoma:

- Irregularly dilated cystic tubules with papillary projections, lined with cuboidal to stratified low columnar epithelium
- Interlobular connective tissue stroma
- Local invasion without distinct capsule
- Occasional hemorrhage and/or necrosis
- *Mitotic figures are rare*

Rete Testis Adenoma: showing papillary hyperplasia lined with cuboidal to low columnar epithelium, 66x H&E



**Rete Testis Carcinoma: Note irregularly dilated cystic tubules with cuboidal to stratified low columnar epithelium and connective tissue stroma.
No mitotic figures**



Sertoli Cell Tumors and Seminomas:

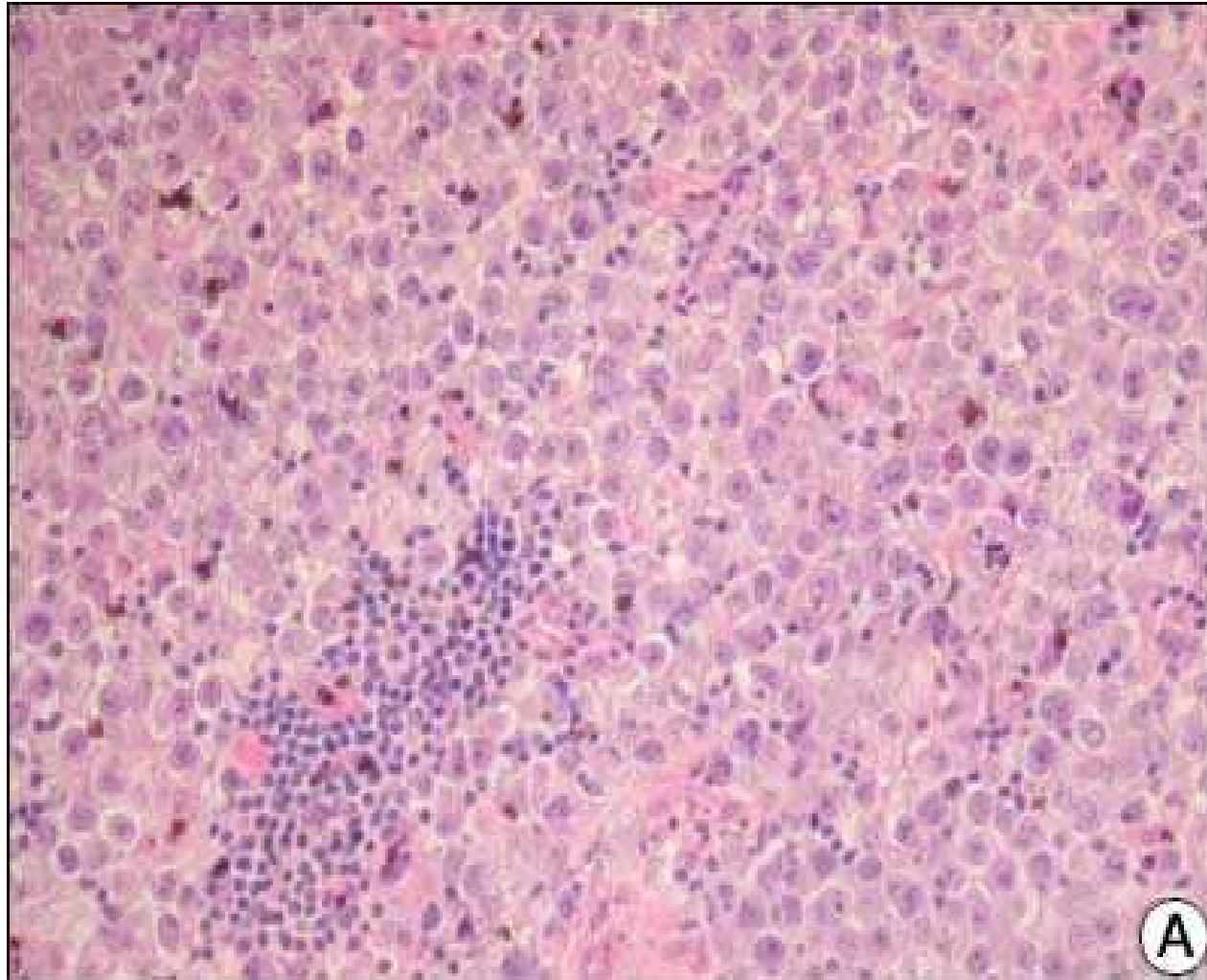
Sertoli cell tumors

- These rare in mice. In rats few cases have been reported in young rats
- In dogs it is considered to be of genetic origin
- In all species, the epithelium is of columnar cells palisaded along basement membrane
- Very few mitotic figure

Seminoma

- Seminoma also occurs rarely in rodents
- Neoplastic cell confined to seminiferous tubules
- High mitotic index, may be invasive

Seminoma: Comprised of seminoma cells with white blood cell-infiltrate



Lesion	Prostate	Coagulating gland	Seminal vesicle
Reactive Hyperplasia	Spontaneous	Spontaneous	Spontaneous
Functional Hyperplasia	Spontaneous	Not reported	Not reported
Atypical Hyperplasia	Rare	Rare	Spontaneous
Squamous Metaplasia	Spontaneous /Induce	Induce	Spontaneous
Seminal Vesicle type Metaplasia	Spontaneous	Spontaneous	Not applicable
Adenoma	Rare	Induce	Rare
Adenocarcinoma	Spontaneous	Induce	Spontaneous
Squamous Papilloma	Not reported	Rare (Duct)	Induce/ Rare (Duct)
Squamous cell carcinoma	Rare	Not reported	Not reported

CONCLUSION

- Male reproductive system (MRS) is complex and is influenced by hormonal cascade-effects
- Factors affecting MRS are age, environment (temp/humidity), nutrition, noise level, odors, pecking order (rabbit)
- Unlike in females, MRS does not have estrus cycle. Hence there is a monotony & paucity of meaningful studies/references on spontaneous lesions
- Finally pathologist is the 'key' to solve the jig-saw puzzle to identify adverse effects on MRS and prevent any such chemical/drug entering in market



*Thank You
for Your Attention*