CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
REPRODUCTIVE SYSTEM

Third Conference

ORGANIZED BY SOCIETY FOR TOXICOLOGIC PATHOLOGY IN INDIA (STPI)

OCTOBER 29-31, 2010

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Non proliferative lesions of Male reproductive system in rats

Shekar Chelur

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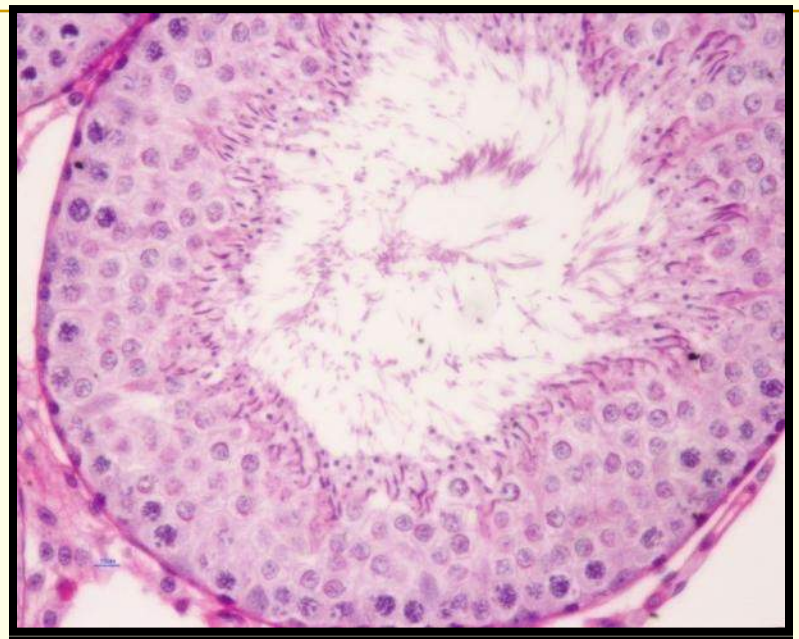
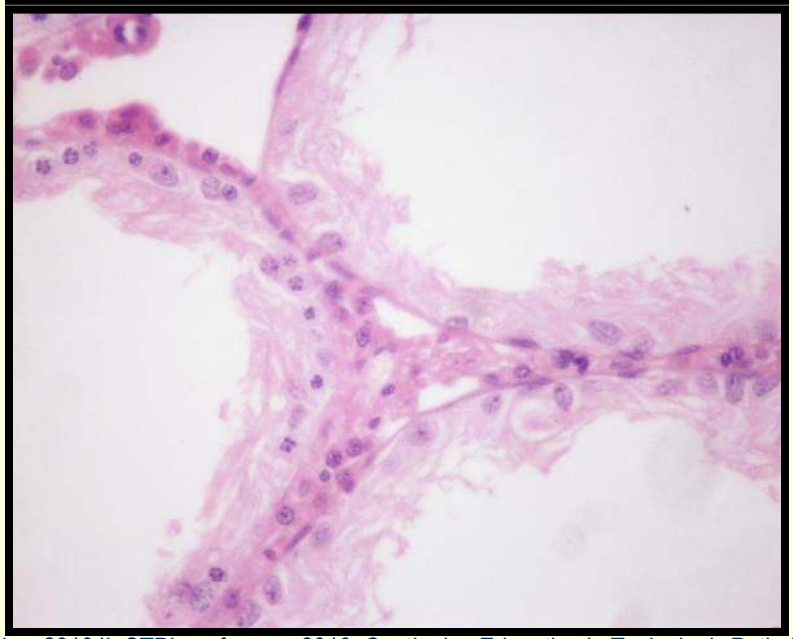
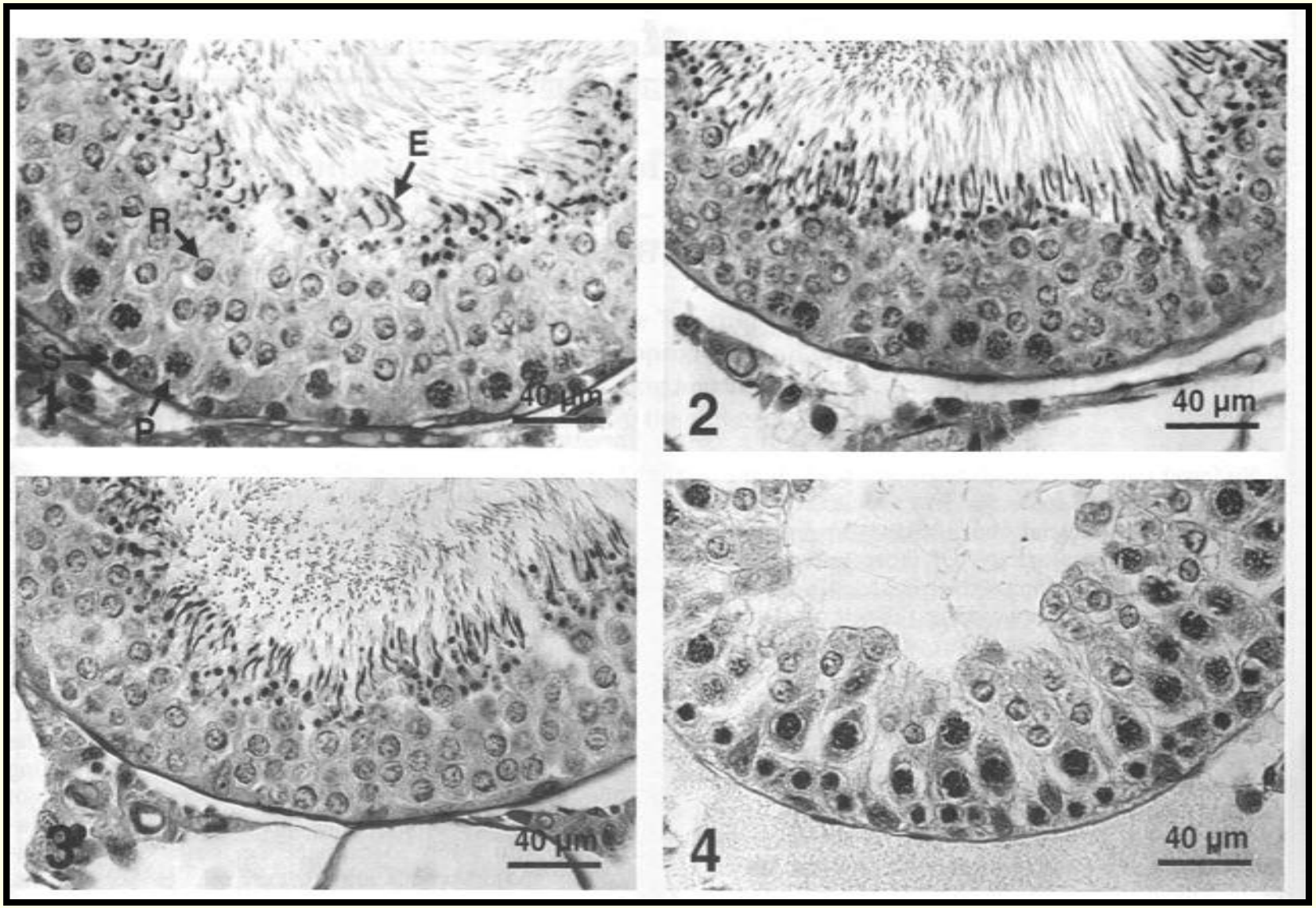
End result- Disruption of spermatogenesis





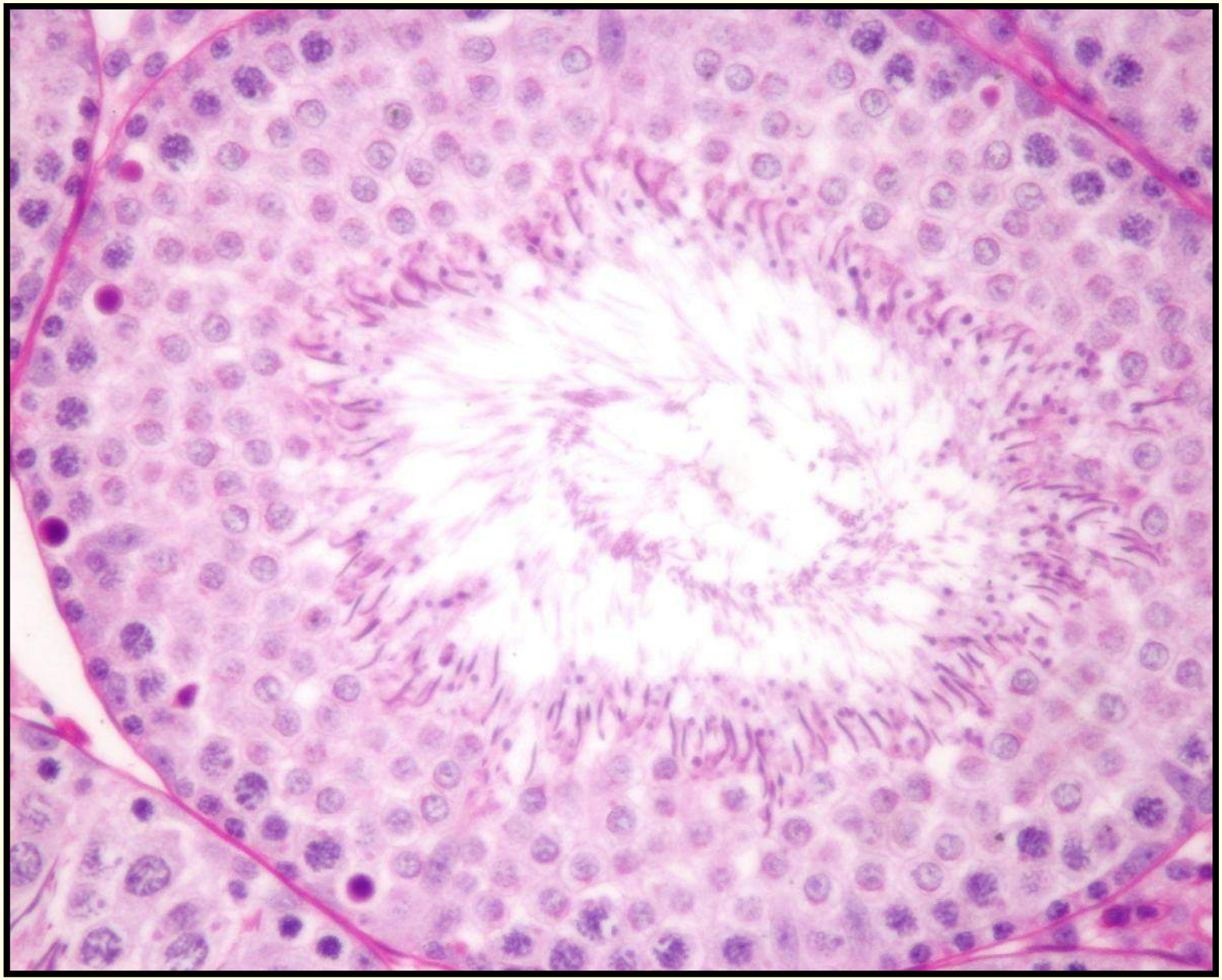
Spermatogonia death

Spermatogonia degeneration

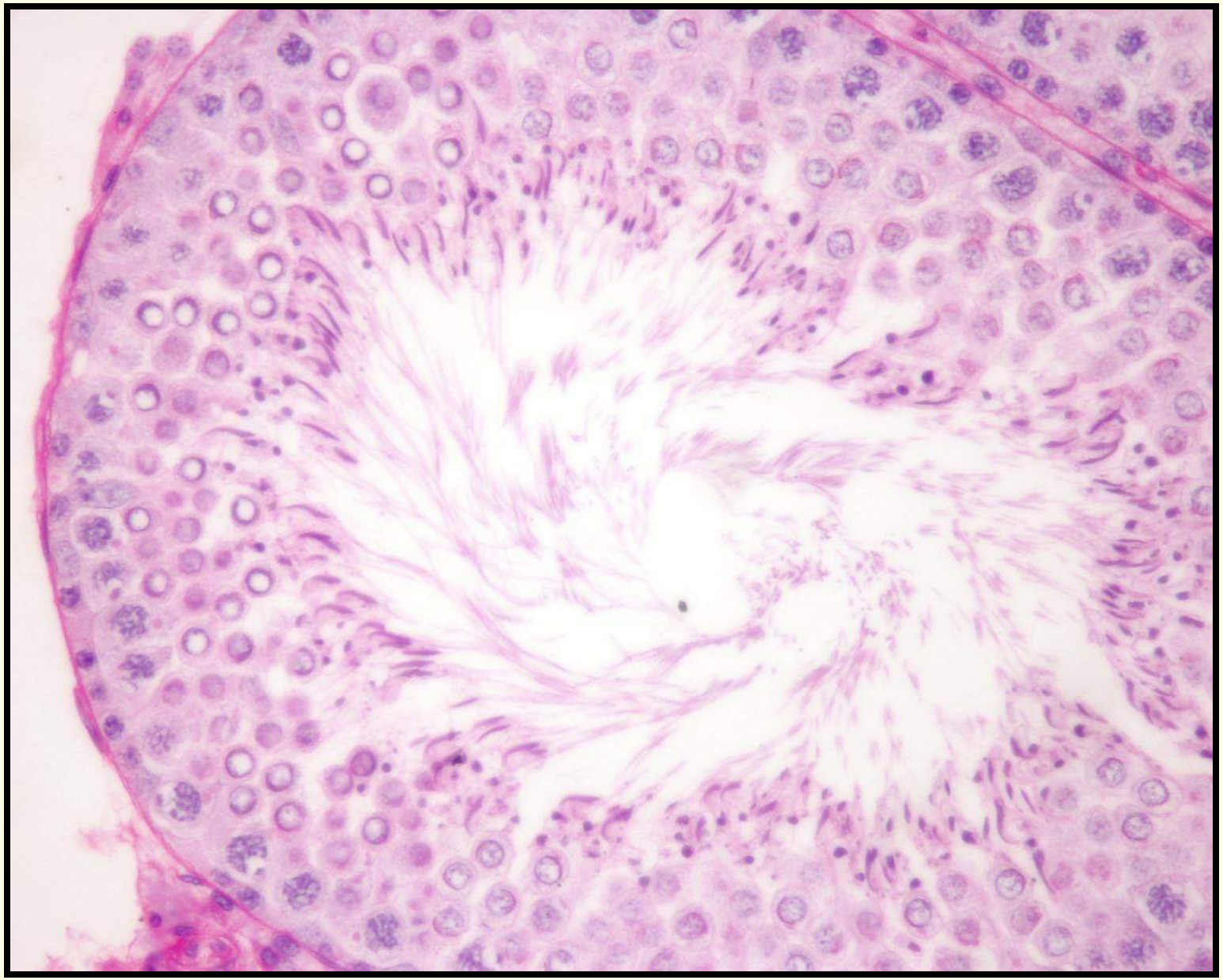


Progression of Drug-Induced Testicular Toxicity, Daniel Morton, *Toxicol Pathol 1999; 27; 380*

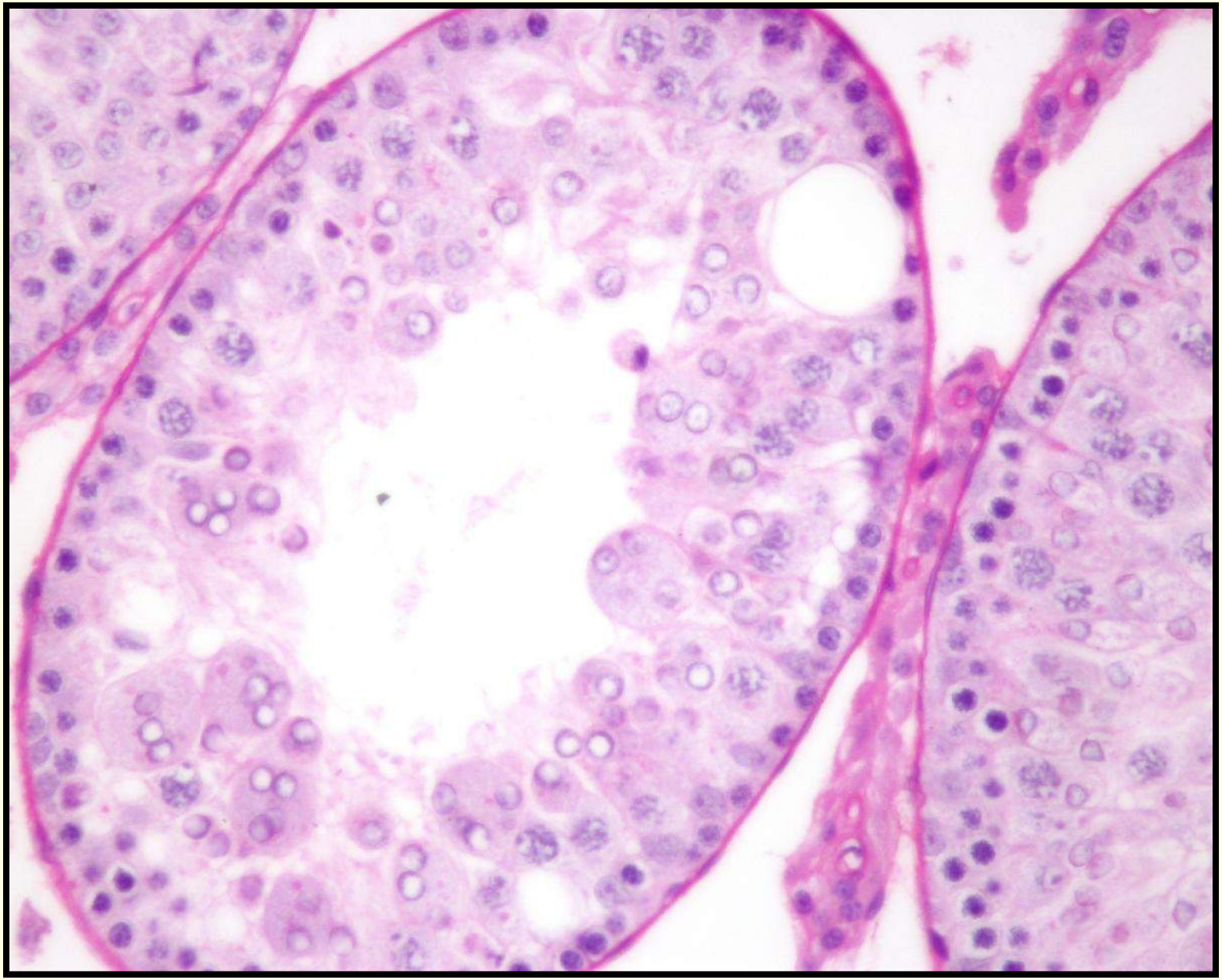
Pachytene spermatocyte death



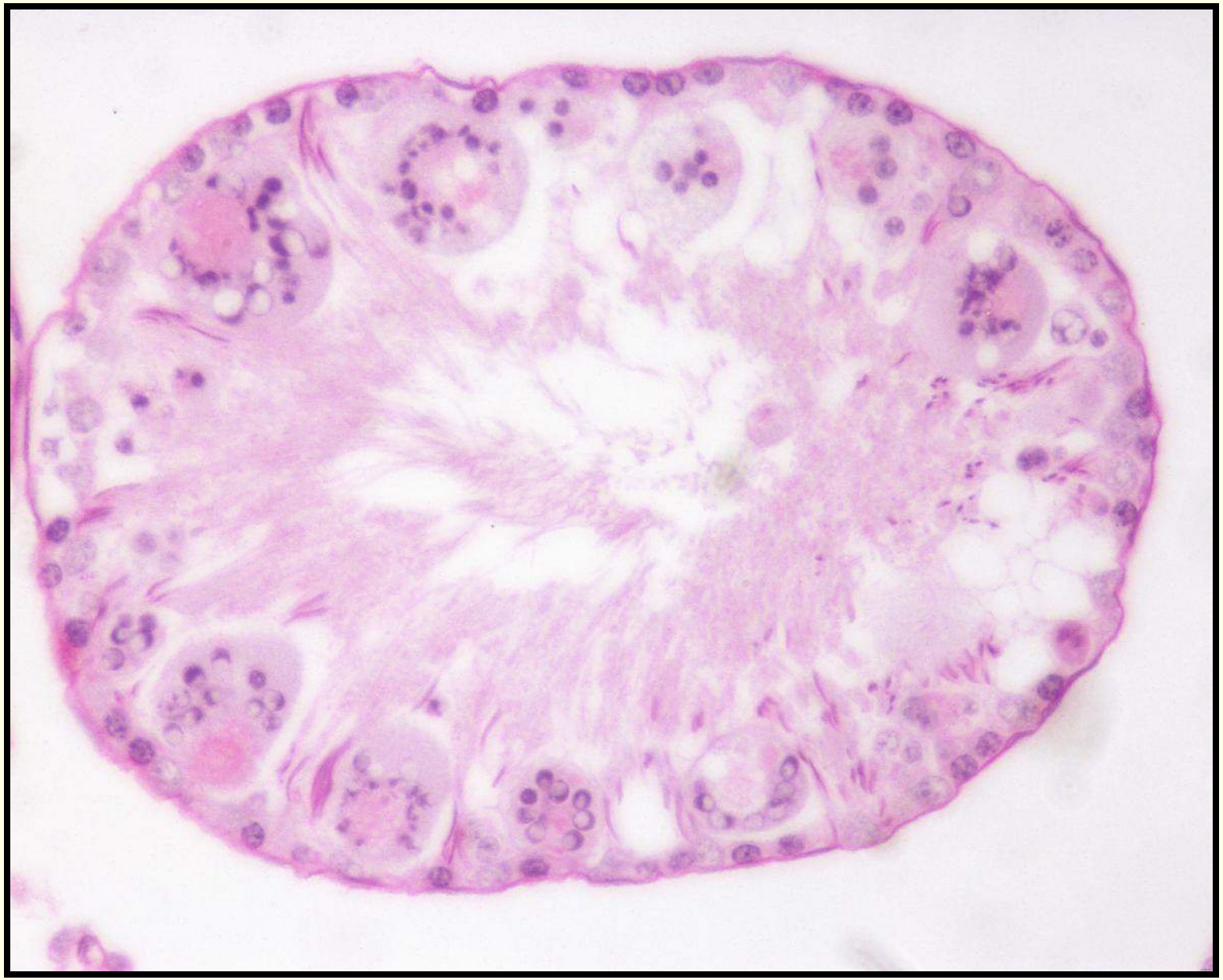
Round spermatid death



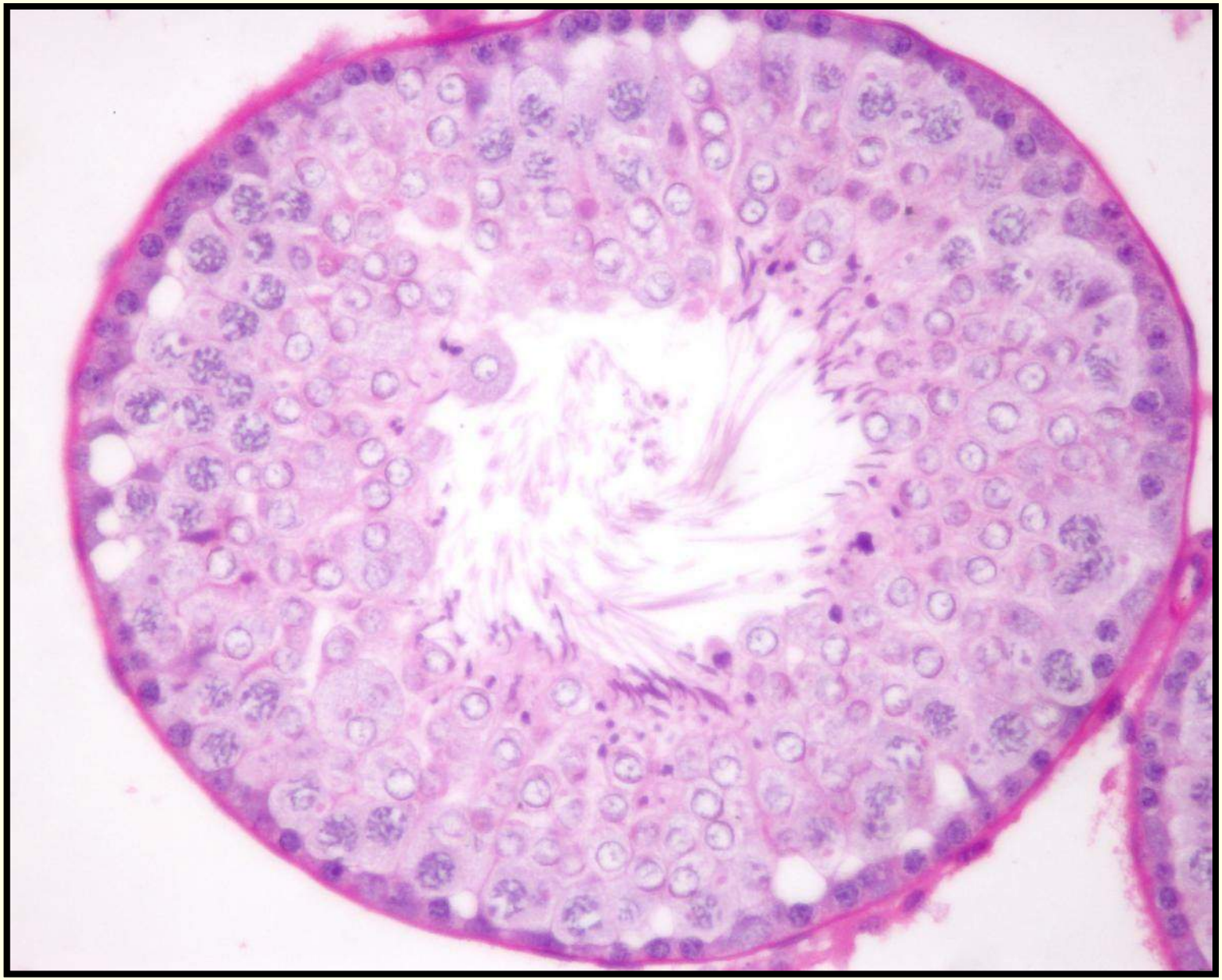
Round spermatid death



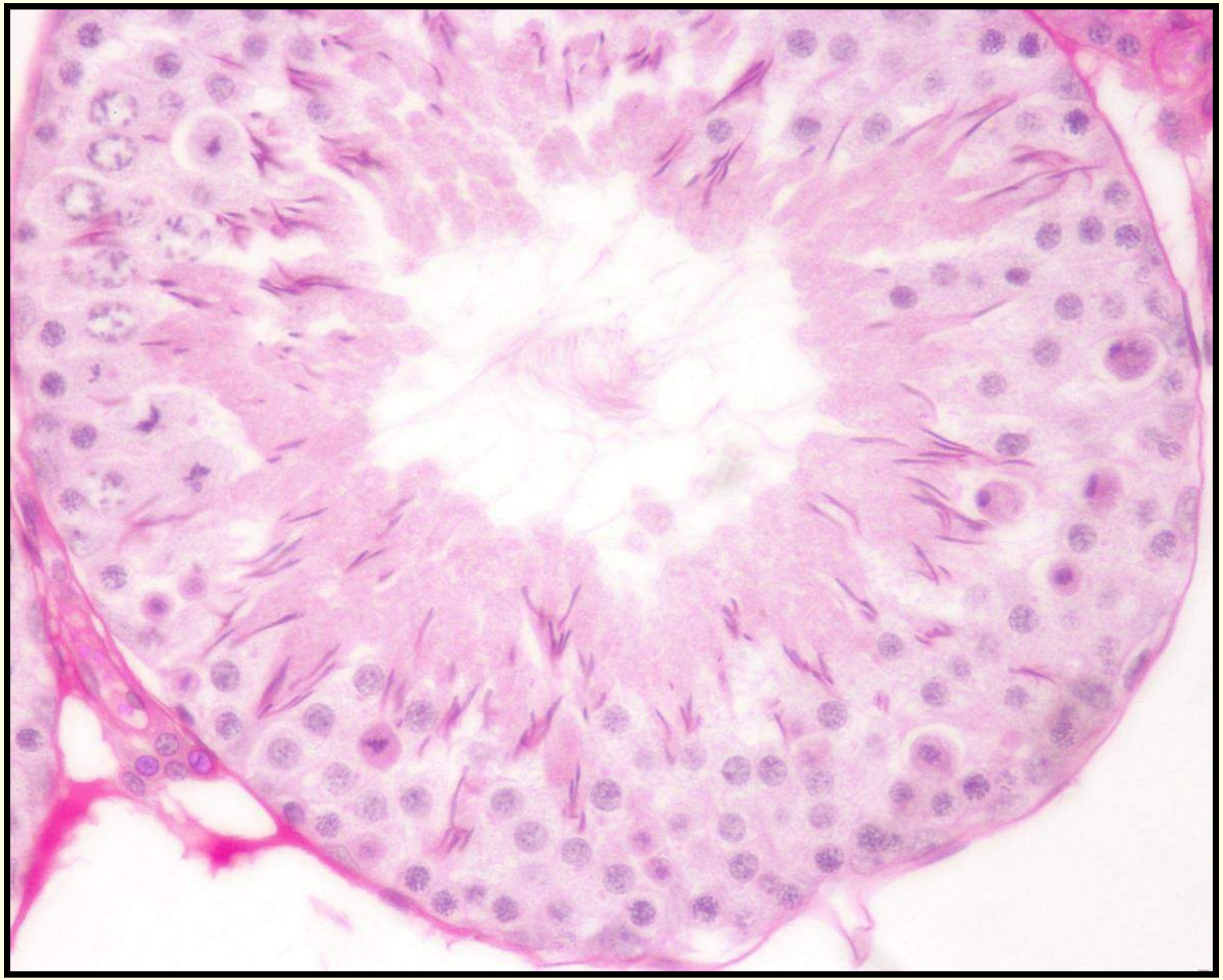
Syncitial cells



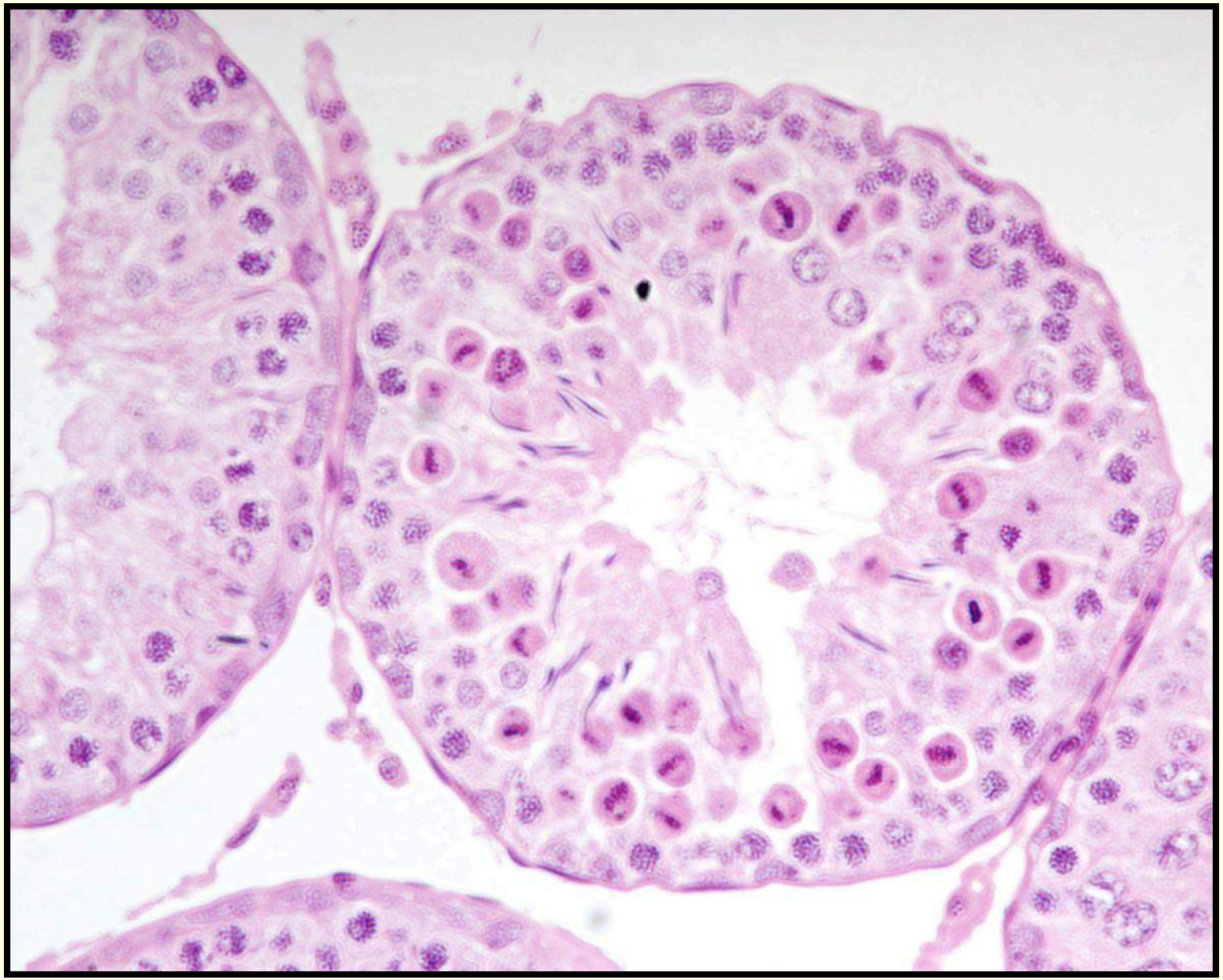
Loss of Germ Cell Layer



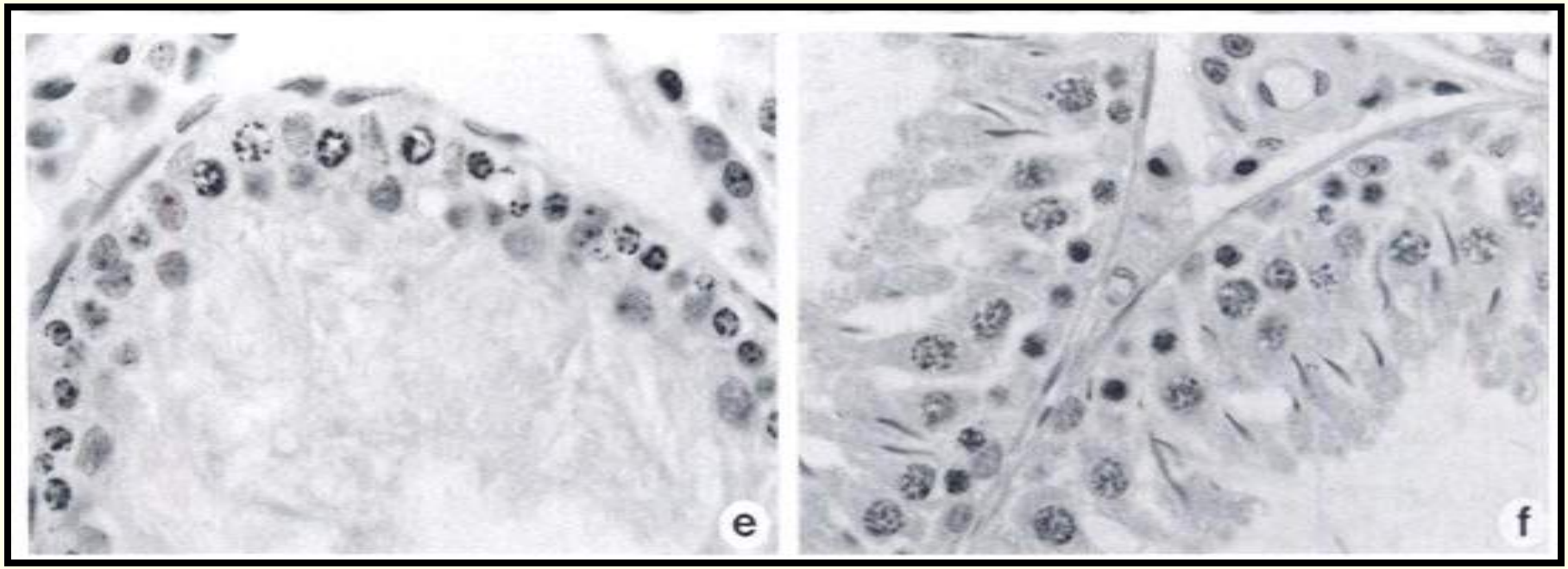
Meiotic germ cell death



Meiotic germ cell death



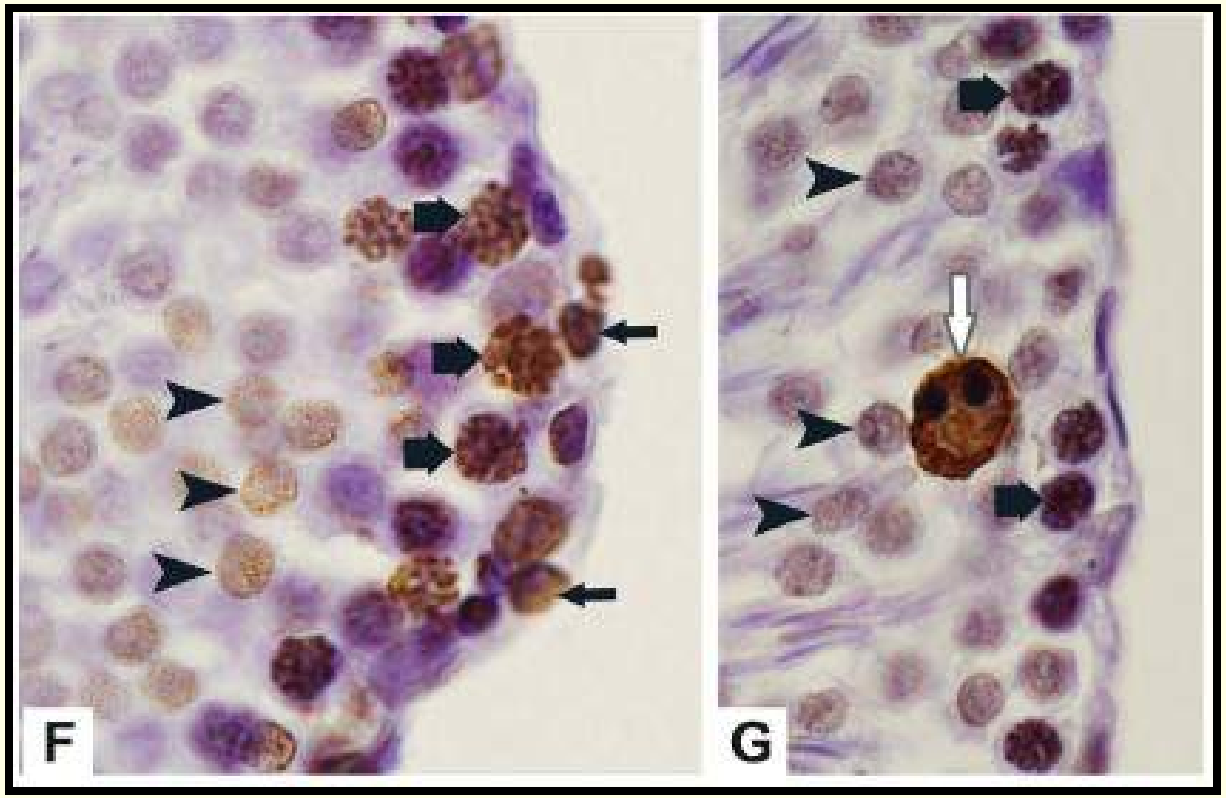
Vitamin A deficiency



(e) Cross section of a seminiferous tubule, 10 days after retinol-acetale treatment, showing B spermatogonia in mitosis.
(f) Cross section of seminiferous tubules 41 days after retinol acetate treatment, all in epithelial stage XII.

The Origin of the Synchronization of the Seminiferous Epithelium in Vitamin A-Deficient Rats after Vitamin A Replacement, ANS M.M. VAN PELT1 and DIRK G. DE ROOIJ, BIOLOGY OF REPRODUCTION 42, 677-682 (1990) 677

Detection of cell death



Spermatogonia (thin arrows), primary spermatocytes in different stages (thick arrows) and round spermatids (arrowheads) are TUNEL-positive. A giant multinucleated cell derivative from round spermatids (white arrow) is also positive

Structural alterations in the seminiferous tubules of rats treated with immunosuppressor tacrolimus, Breno H Caneguim, *Reproductive Biology and Endocrinology 2009, 7:19*

Testes - Germ cell toxicity

- Almost and always- Cell specific and Stage specific
- Death is predominantly through apoptosis
  - Spontaneous or induced
- Cell death and phagocytosis can be complete with in 24 hours- Cell depletion
- **Spermatogonia** death in stage XI-XIV and stage I
- Stem cell spermatogonia are less vulnerable
  - Early stages- base of tubule
  - Later stages- round up and slightly displaced from base of tubule
  - Stains heavily
  - Busulfan, Bleomycin
- Phagocytosed by sertoli cells
- No clear example of arrest of cell
- Temporary arrest – Vitamin A deficient rats- A1 spermatogonia
- Short duration, reversal on retinol adiministration, synchronisation

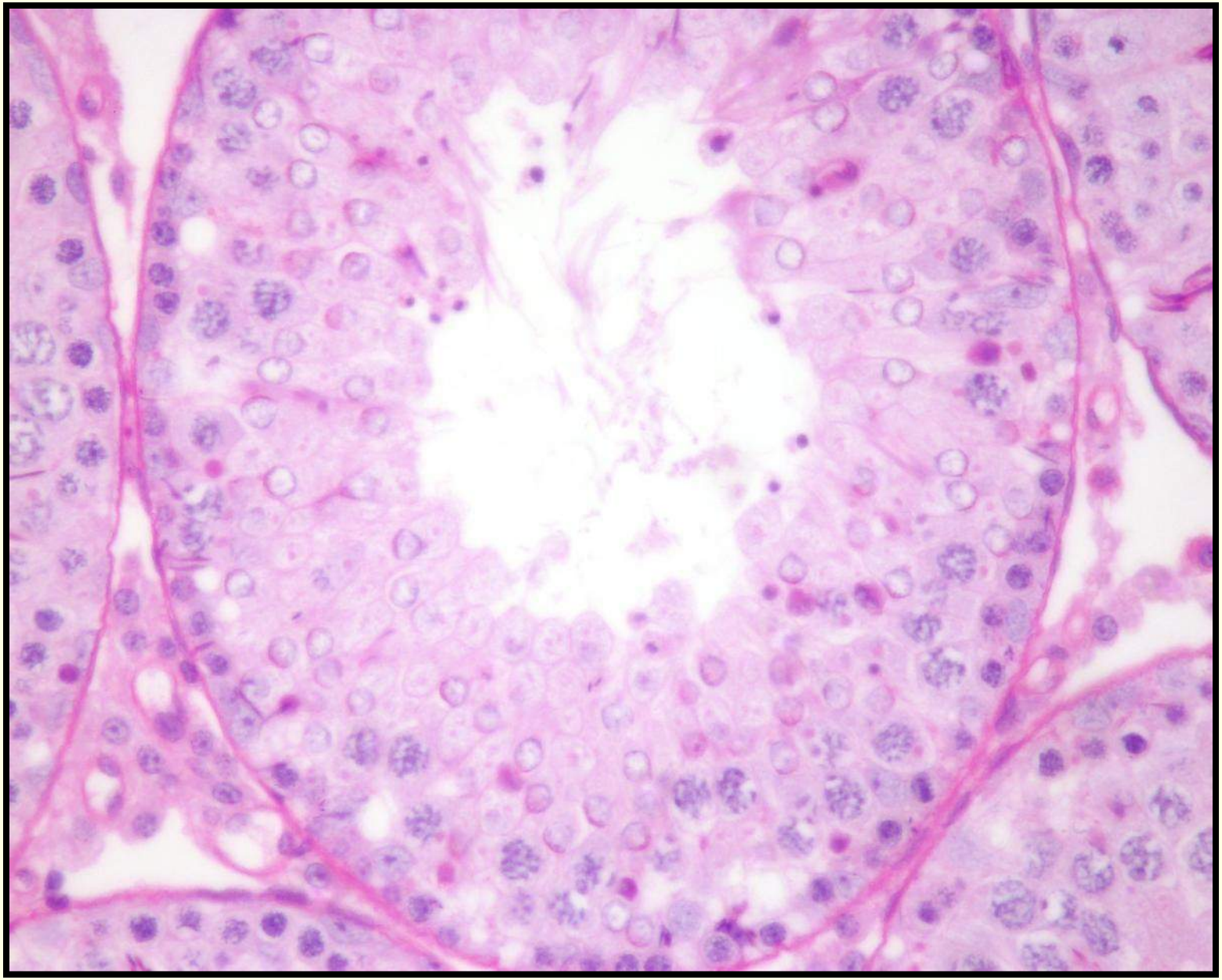
Testes - Germ cell toxicity

- **Primary spermatocyte** degeneration
  - 2 Methoxy Ethanol, Dinitropyroles
- Preleptotene spermatocytes– Base of tubules
  - Pyknotic and densely stained
- Leptotene and zygotene spermatocytes –no reports
- Pachytene spermatocyte- Easy to identify -Stage VII
  - Cells shrink, unstained crescentic intracellular space around portion of surface or full
- Spermatocyte degeneration in metaphase of second meoitic division – Stage XIV
  - Seen on normal animals
  - Characteristic feature- both chromosome and spindle fibers stain intensely
  - Microtubule destructing agents- Colchecine, Vinblastine, Taxol
- Round spermatids – Step 7 at Stage VII
  - Developing acrosome- pyknotic, distarted, irregularly infolded nucleus
  - Cytoplasma intensely stained as degeneration progressses
  - Acrosomal contents less heavily stained
  - Multinucleate syncytium (Symplast, multinucleate giant cell)– less rapidly phagocytosed by sertoli cells
  - Ethane methane sulphonate, Methyl Chloride

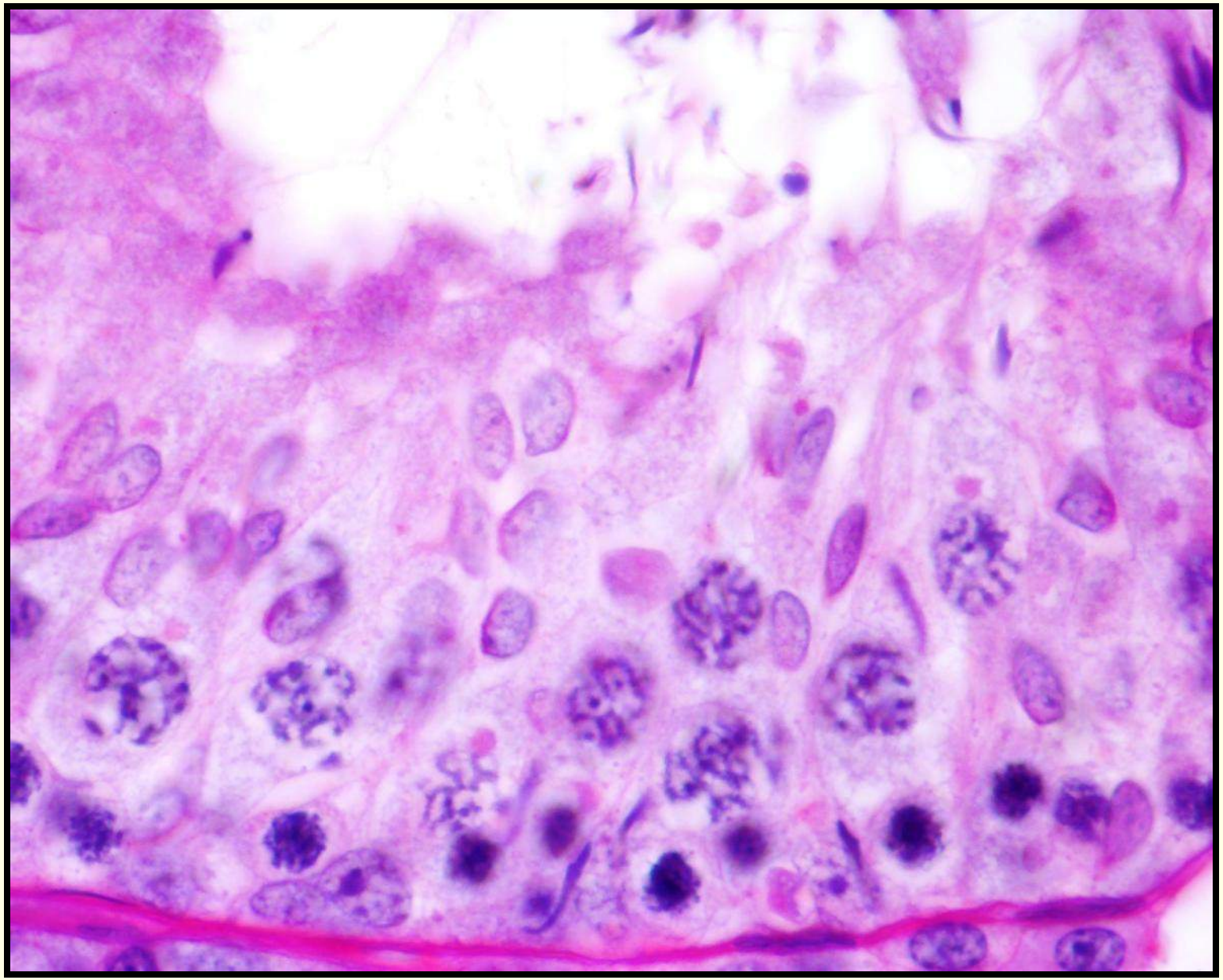
Testes - Germ cell toxicity

- Elongating spermatids- Step 19-Stage VII
  - Characteristic shape
  - Position near tubular lumen
  - Increase in density of entire cell
  - Boric acid, Dibromo Acetic acid
- Blood vessels damage - irreversible
  - Cadmium Chloride, 5 Hydroxytryptamine, Histamine
- Sertoli cells are highly resistant for cell death
- slow cycling stem cell spermatogonia more resistant than differentiating (committed) spermatogonia
- spermatogonia basal compartment (outside the blood-tubule barrier) are exposed to any xenobiotic that enters the interstitial fluid, spermatocytes, which also undertake DNA synthesis and meiotic division are protected by blood testis barrier

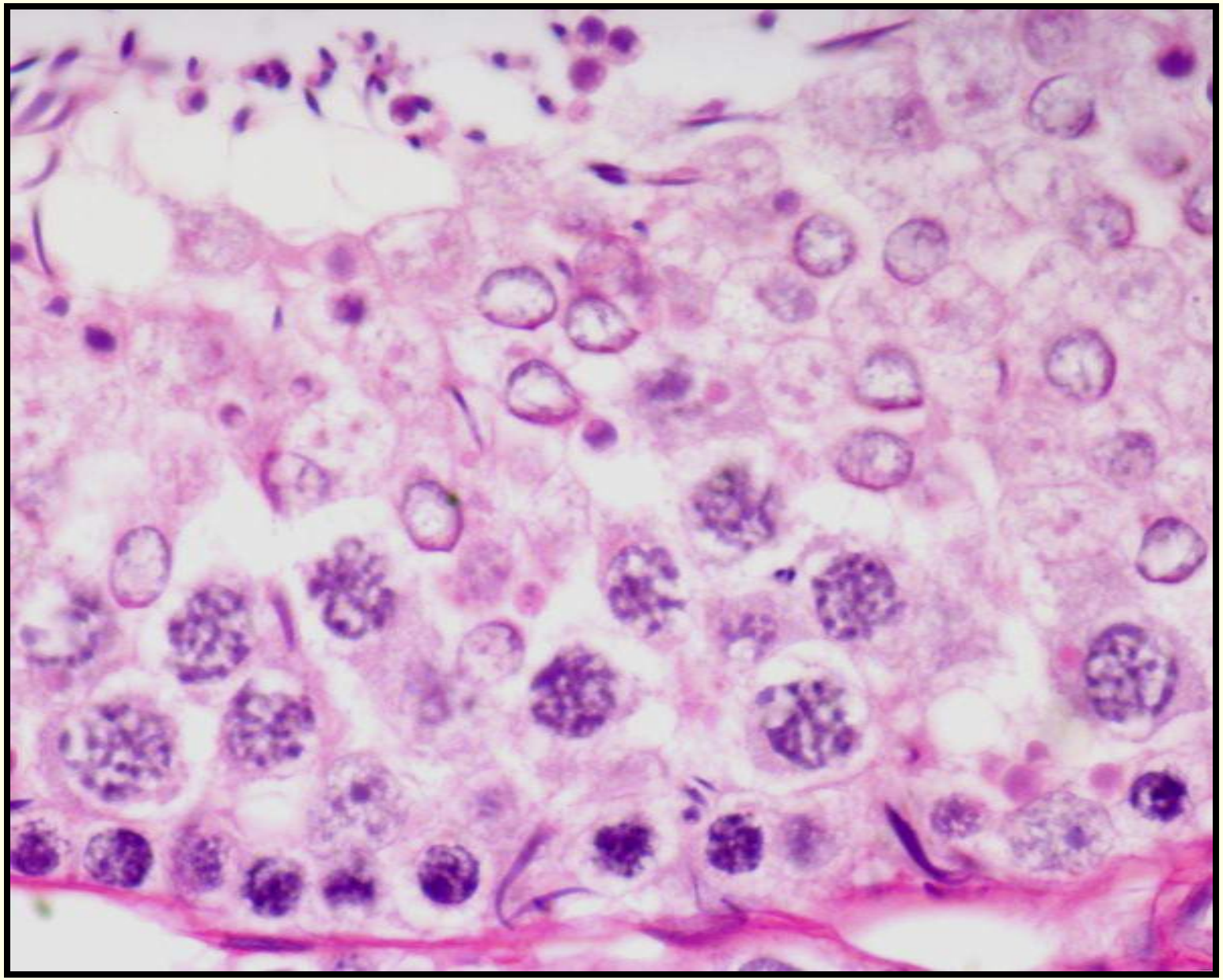
Sperm retention



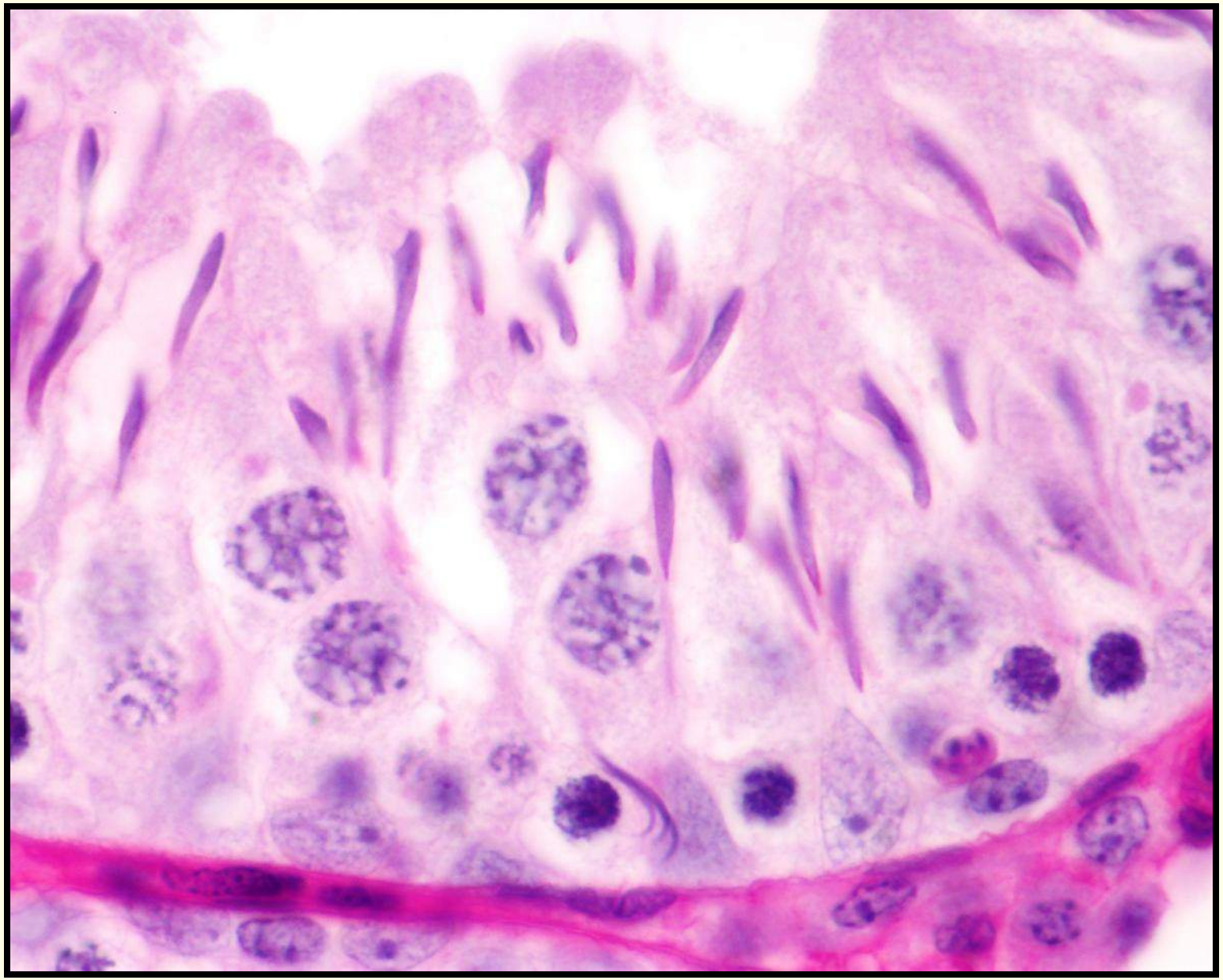
Sperm release defect



Spermatid retention – Stage VIII



Spermatid retention – Stage XII

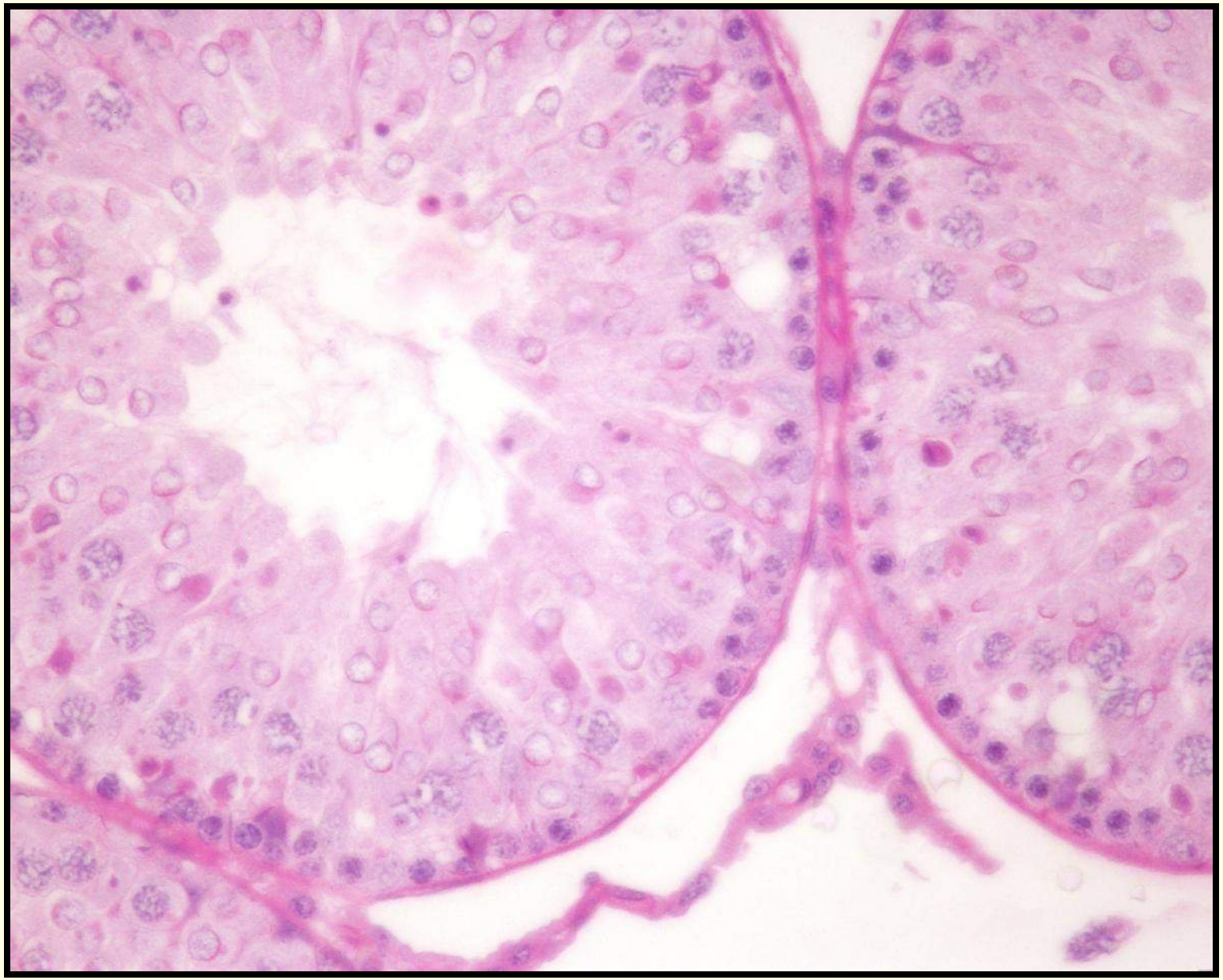


Residual body defects

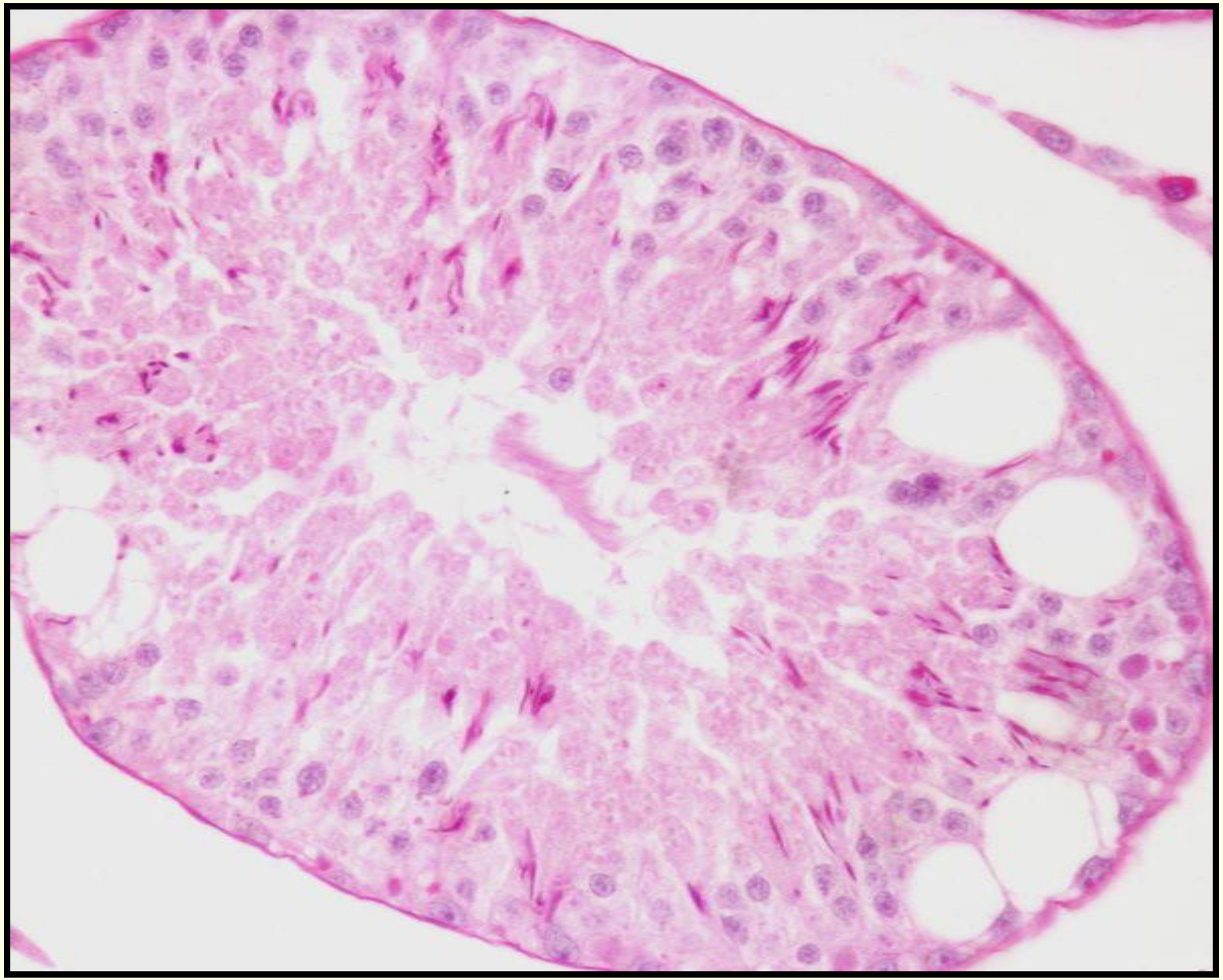
Sperm release defects

- Retention of step 19 spermatids in stage VIII-XII
  - Luminal
  - Basal
  - Can it be in any other stage?
- Retained spermatids per sertoli cell
- Defect in spermatid or defect in sertoli cell
  - Even good spermatozoa can be retained and phagocytosed- Hormone deprivation
- **Residual bodies**
- Decent and phagocytosis- stage 9-11
  - Stage 12 with spermatid retention?
- Formation and behaviour of residual body can be altered
  - Abnormal shape, size
  - Tubular lumen and epididymal lumen

Sertoli Cell Vacuolation



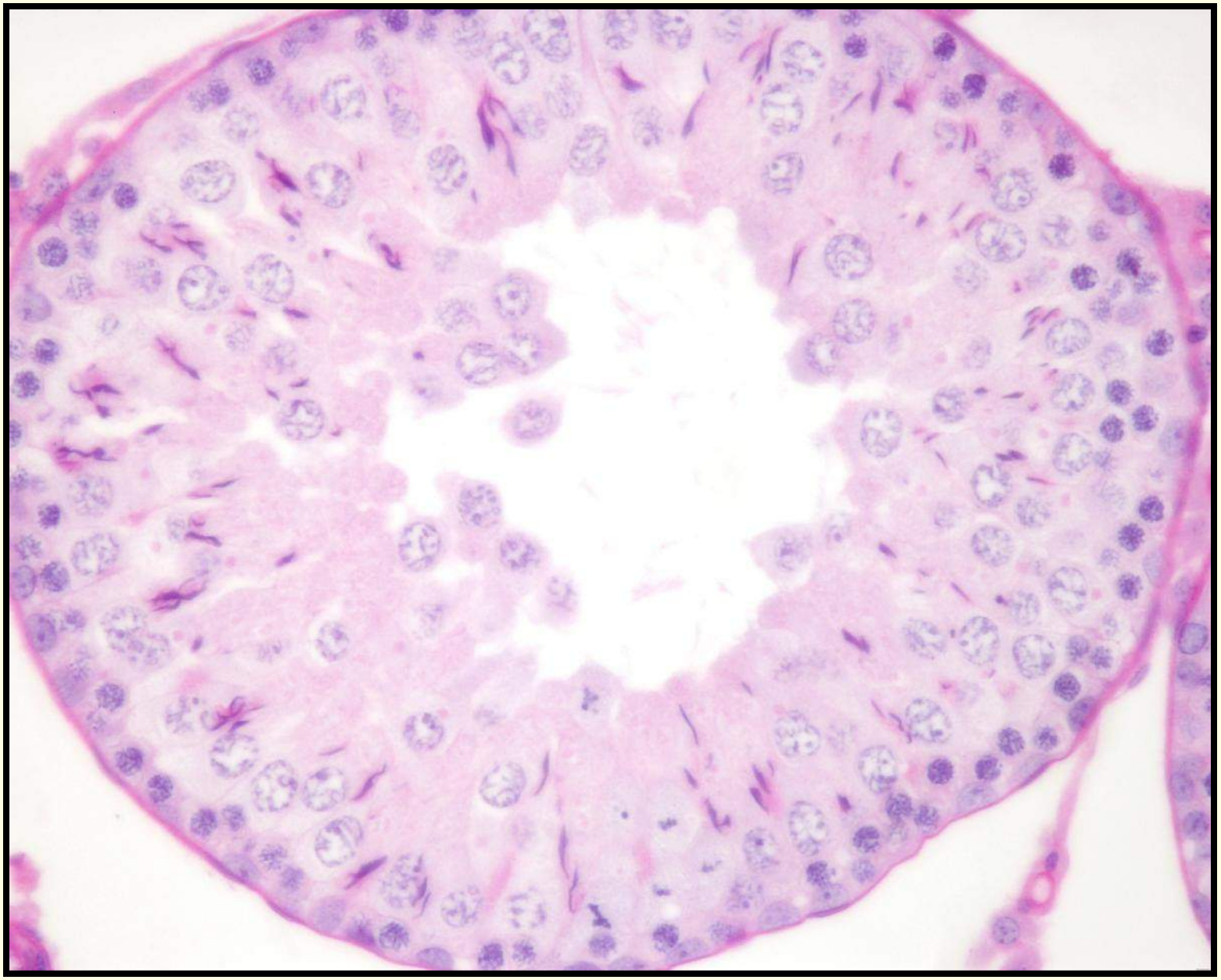
Tubular Vacuolation



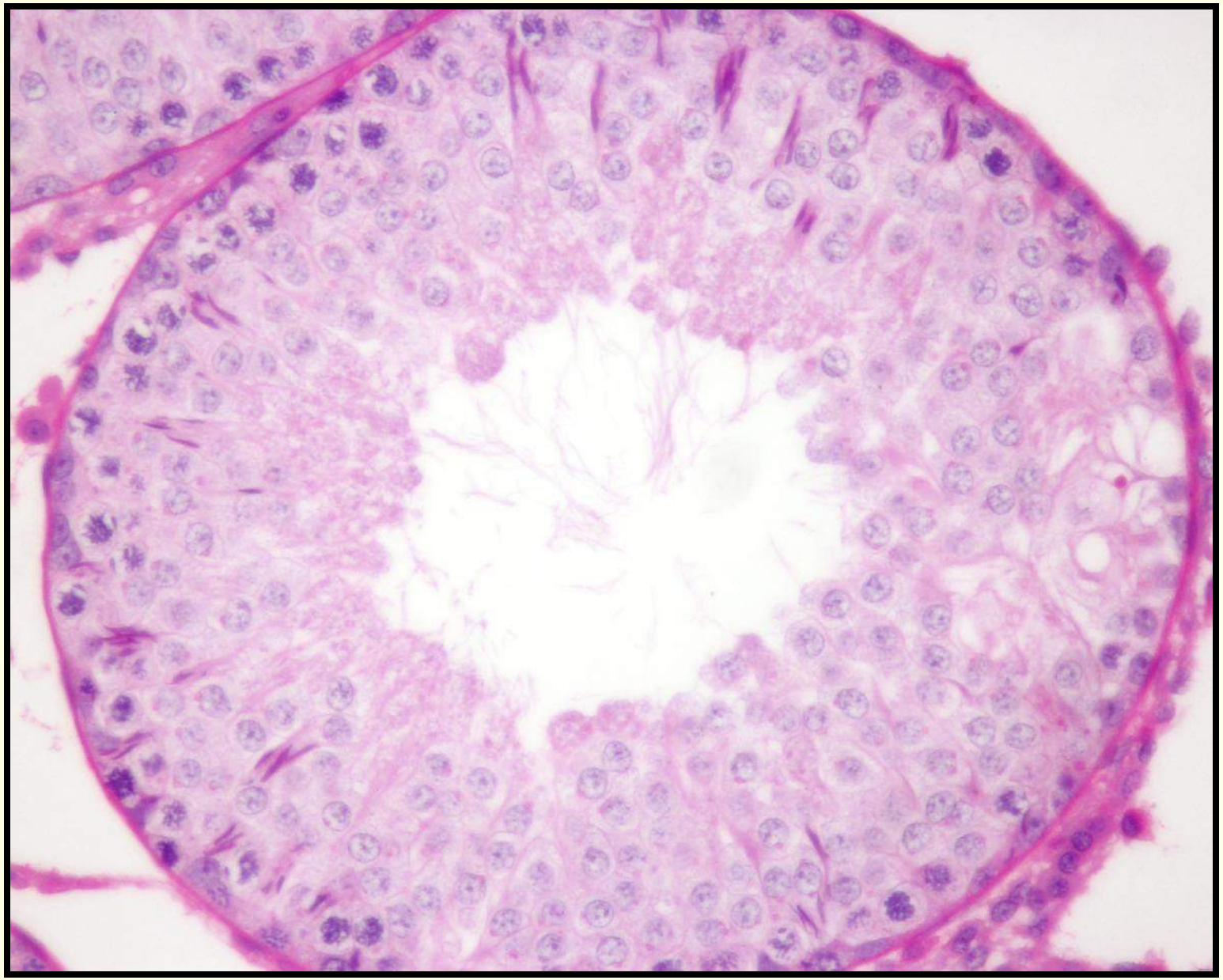
Sertoli Cell

- Sustentacular cell or Nursing cell
  - Blood testes barrier- tight sertoli - sertoli junction
- Vacuolation –Single or Multiple or Microvacuolation
  - Dilatation of smooth endoplasmic reticulum
  - Fixation artifact- osmotic shrinkage at basement membrane
- Rate of phagocytosis differ throughout the cycle or is affected by treatment
- Death and depletion of sertoli cells is rare- ischemia
  - Pthalate esters, 2,5 hexanedione, 1,3 dinitrobenzene- first affected
- Number of unique structures and functions of sertoli cell are targets- Protein and fluid secretion, cytoskeletal alterations, metabolic disturbances

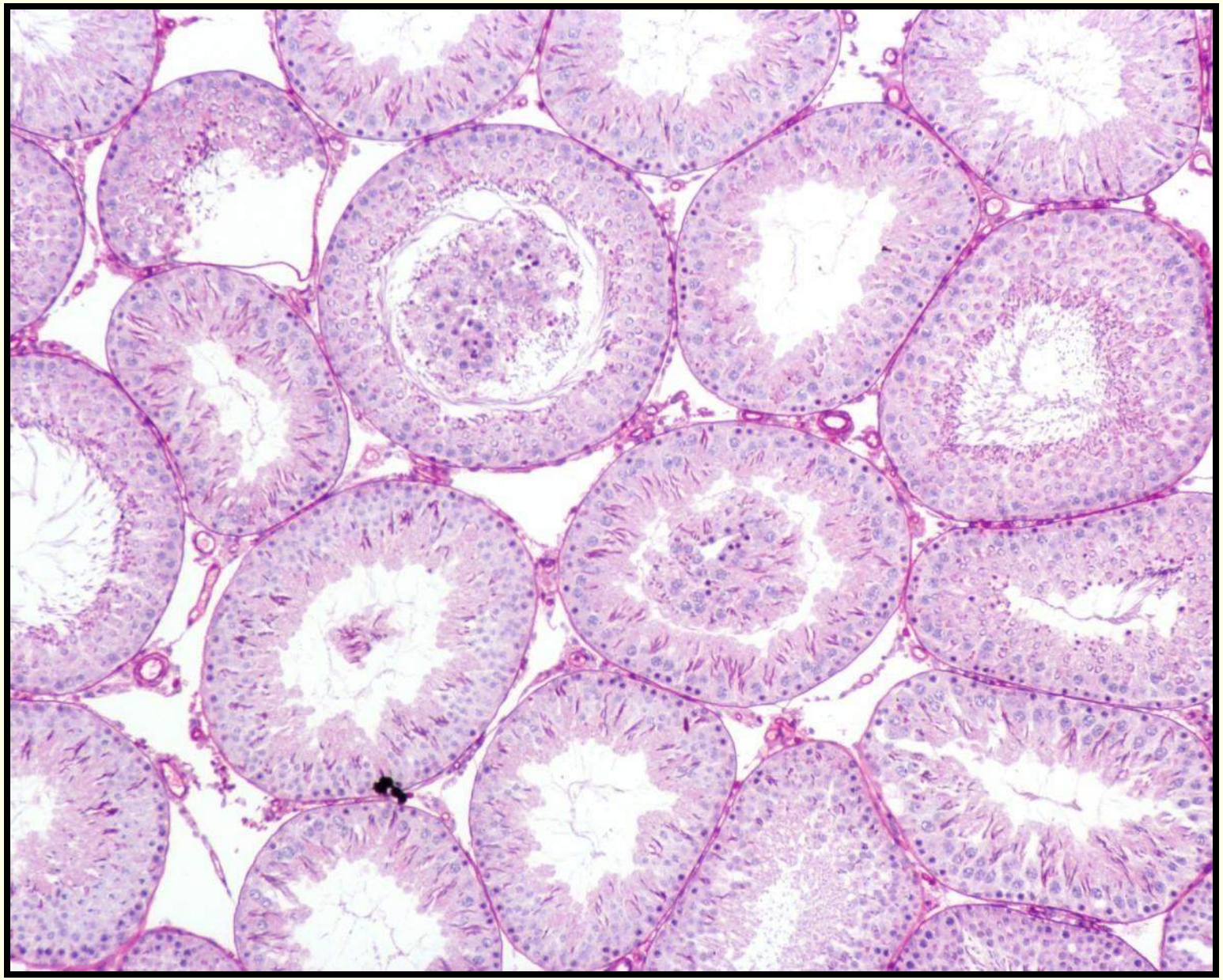
Germ cell exfoliation



Focal exfoliation



Cellular debris- Trauma



Sertoli and Germ Cell exfoliation

- Shearing of sertoli cell cytoplasm by cytoskeletal disrupting agents
- Loss of adhesion b/n sertoli cell and germ cell
- Breakage of intercellular bridges
- Effect of prolonged treatment
- Trauma, handling and cutting of testis before fixation

- Hormones of lack of hormones do not affect spematogenesis by speeding up or retarding germ cell development

Morphometry

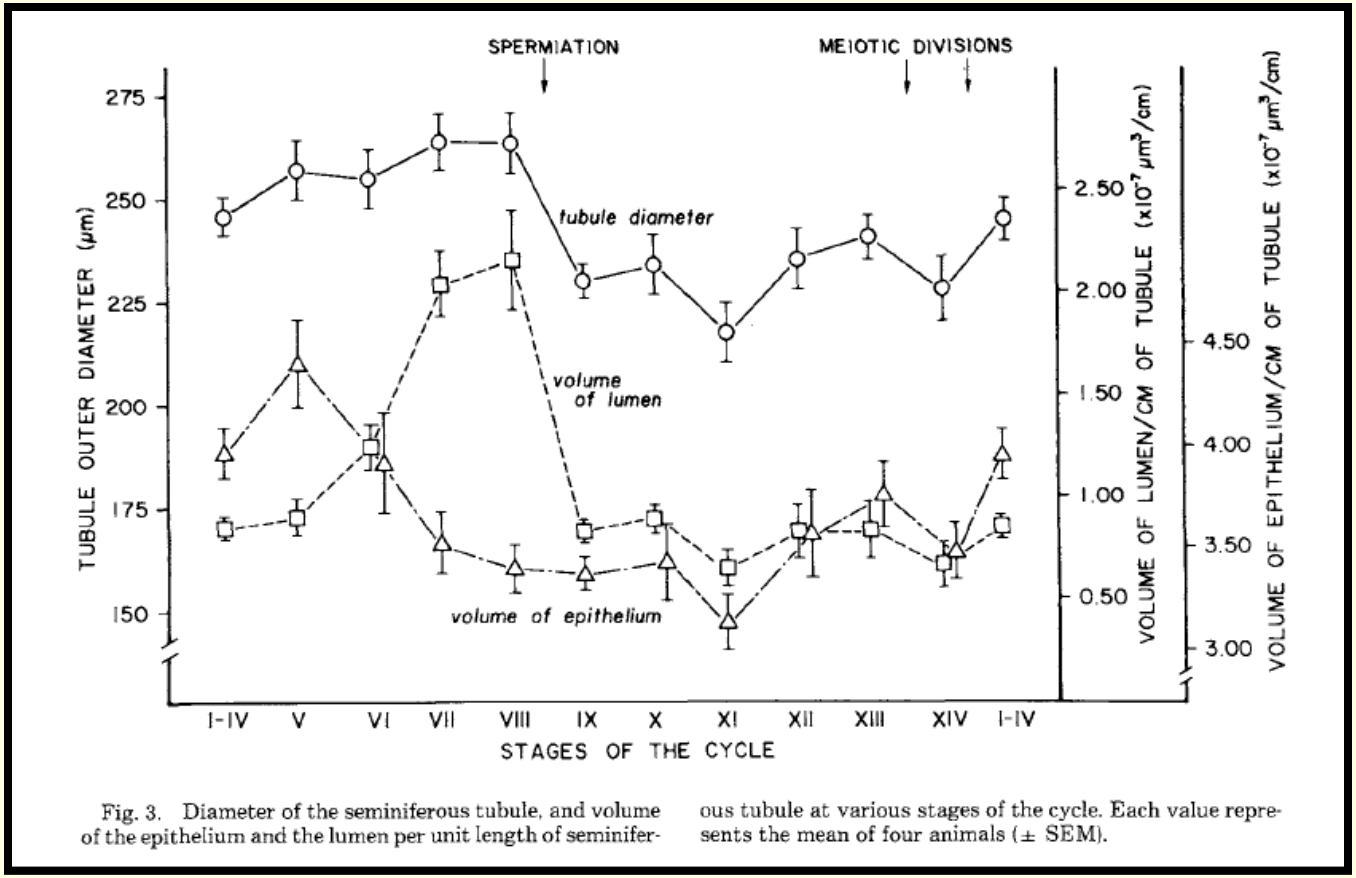


Fig. 3. Diameter of the seminiferous tubule, and volume of the epithelium and the lumen per unit length of seminiferous tubule at various stages of the cycle. Each value represents the mean of four animals (± SEM).

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Mean	13.7	5.3	2.3	4.9	6.8	7.5	20.9	7.6	3.0	3.2	3.0	8.7	6.2	6.8
SE	0.6	0.4	0.2	0.3	0.3	0.4	0.4	0.5	0.2	0.1	0.2	0.2	0.5	0.3

*Total number of seminiferous tubules examined was 9 672; one testicular cross section per rat.

Morphometric Studies on Rat Seminiferous Tubules, TUNG-YANG WING AND A. KENT CHRISTENSEN, THE AMERICAN JOURNAL OF ANATOMY 165:13-25 (1982)

Morphometry

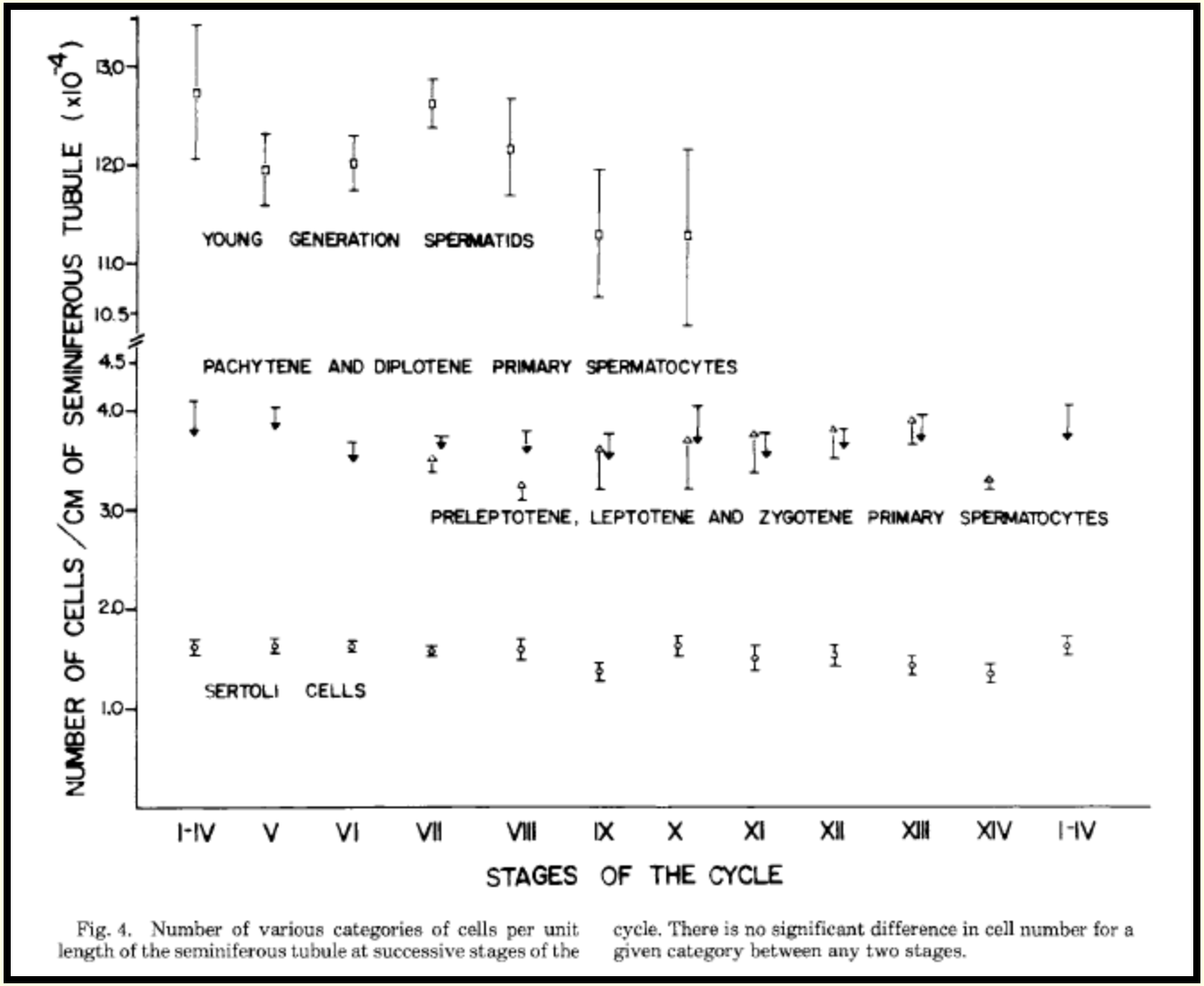
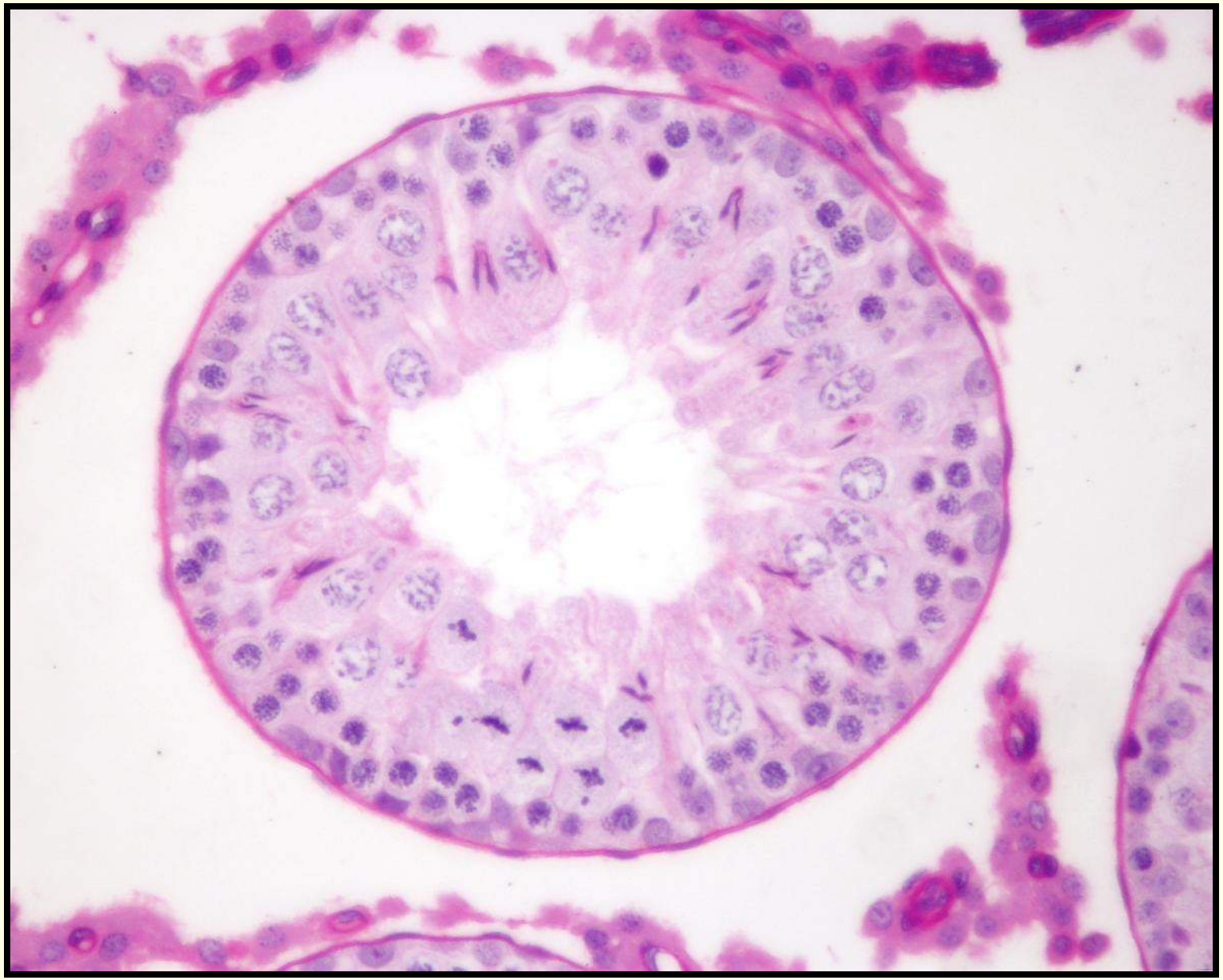


Fig. 4. Number of various categories of cells per unit length of the seminiferous tubule at successive stages of the cycle. There is no significant difference in cell number for a given category between any two stages.

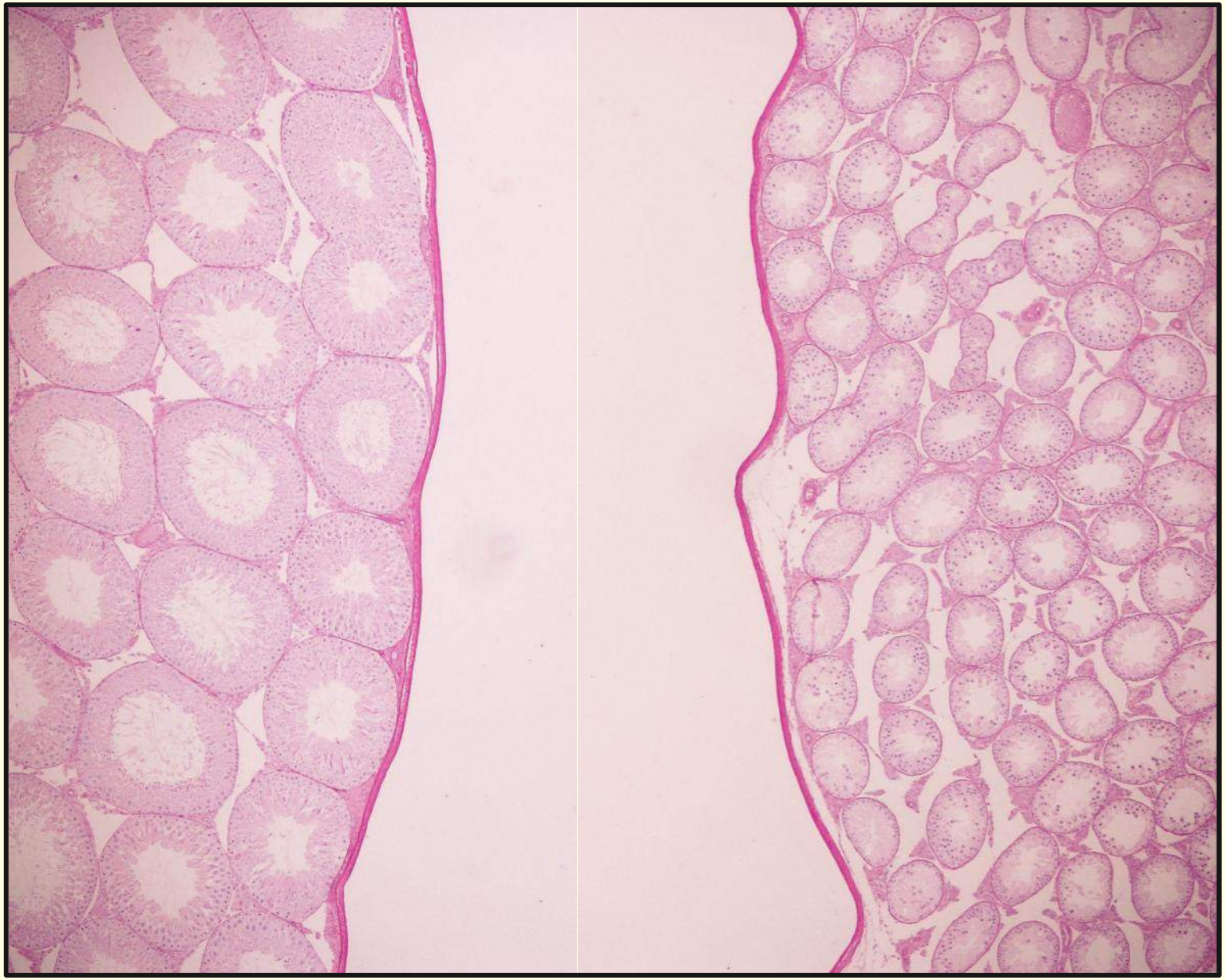
Morphometric Studies on Rat Seminiferous Tubules, TUNG-YANG WING AND A. KENT CHRISTENSEN, THE AMERICAN JOURNAL OF ANATOMY 165:13-25 (1982)

Normal Stage XIV

Tubular contraction- Stage XIV

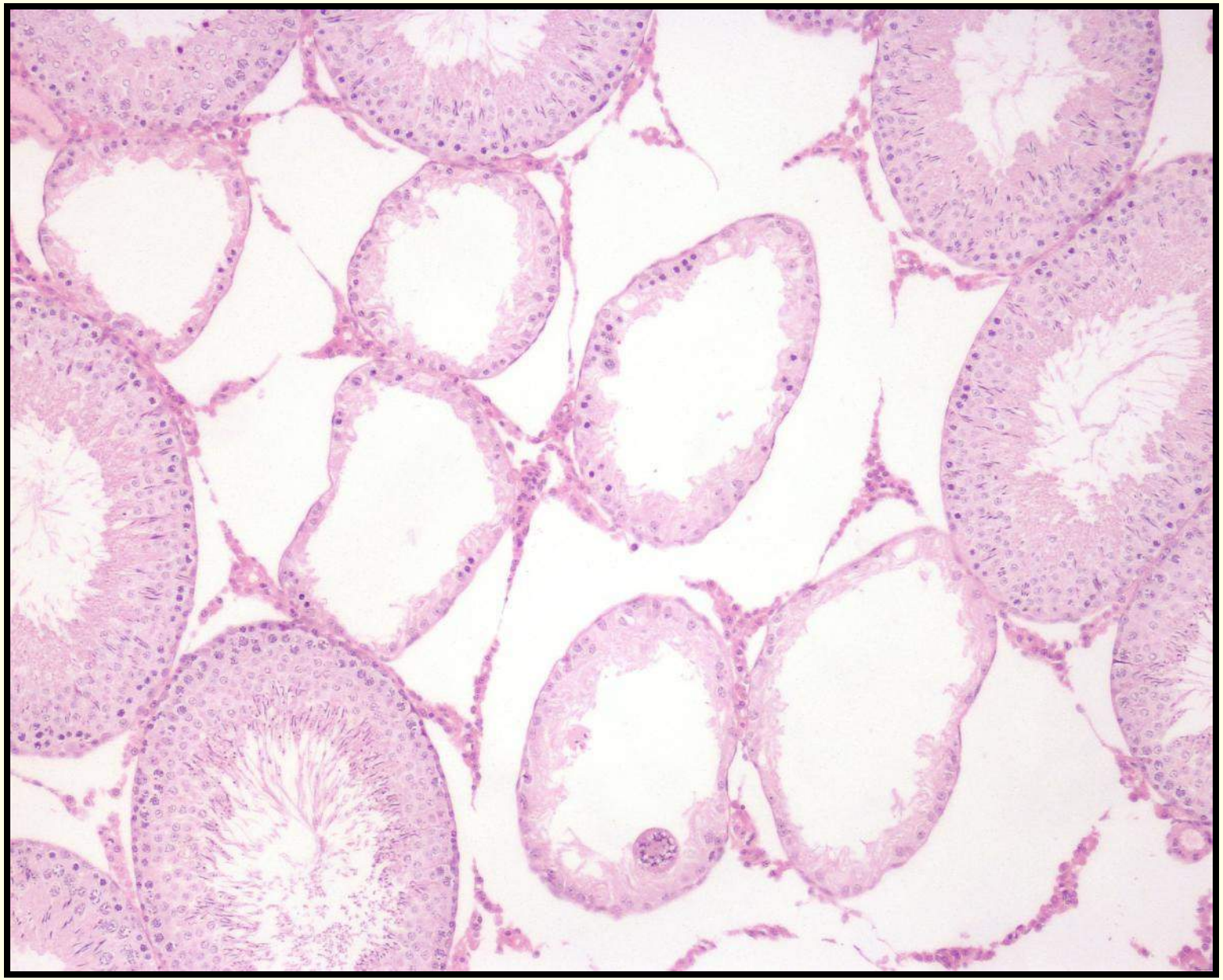


Testicular Atrophy

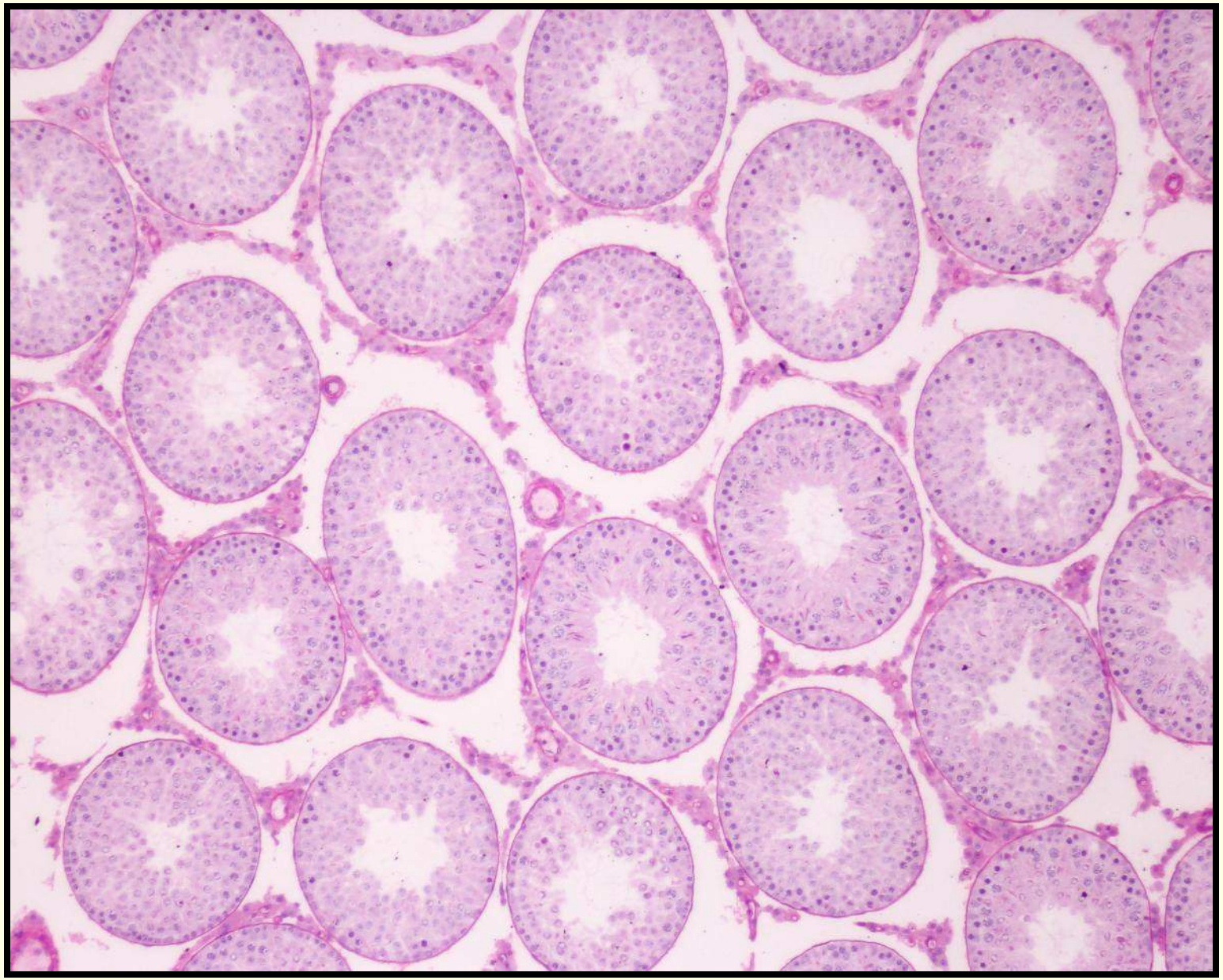


Sertoli only tubules- Agenesis

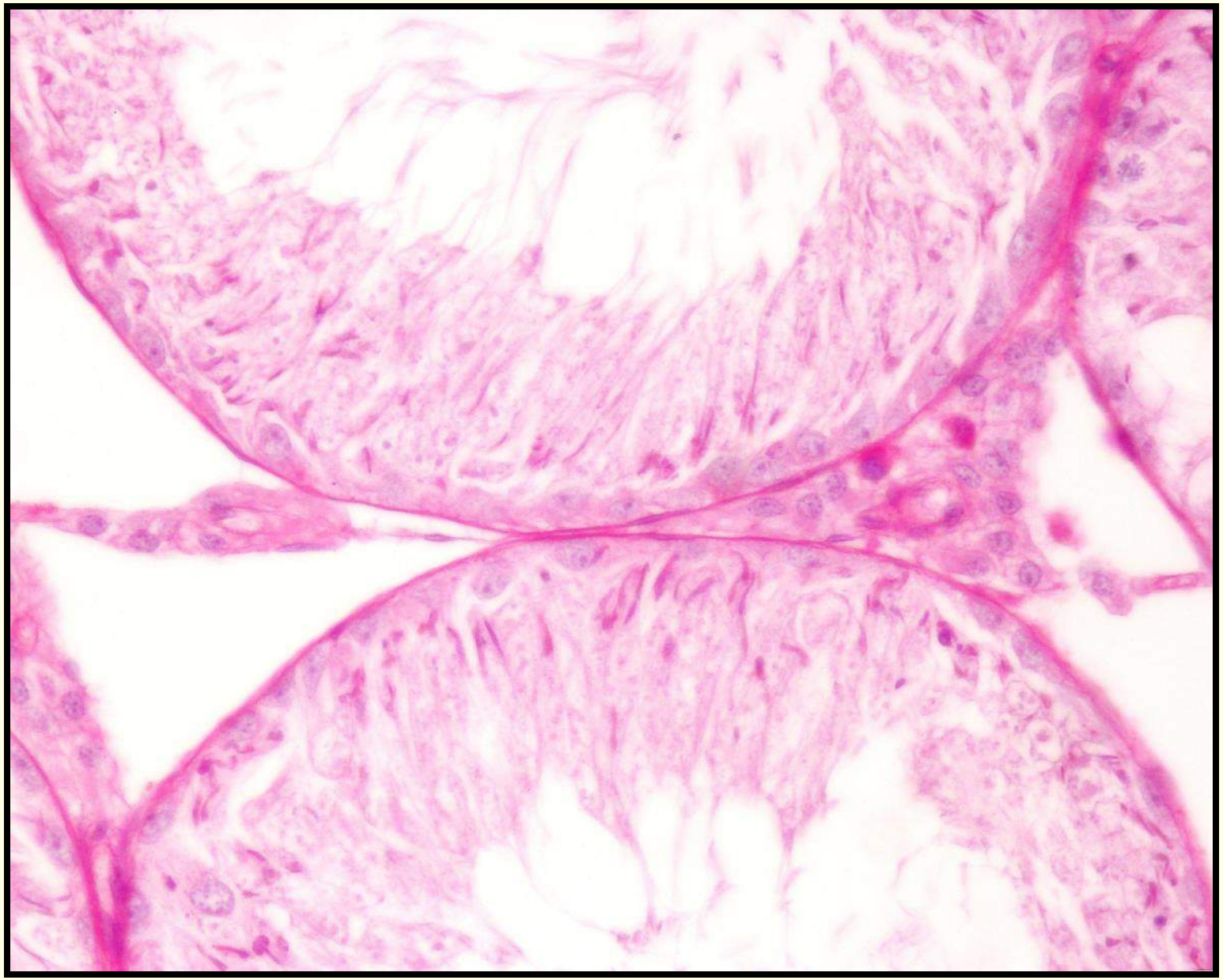
Focal Tubular Atrophy



Testis - Oligospemia



Sertoli only tubules



Gamma Irradiation

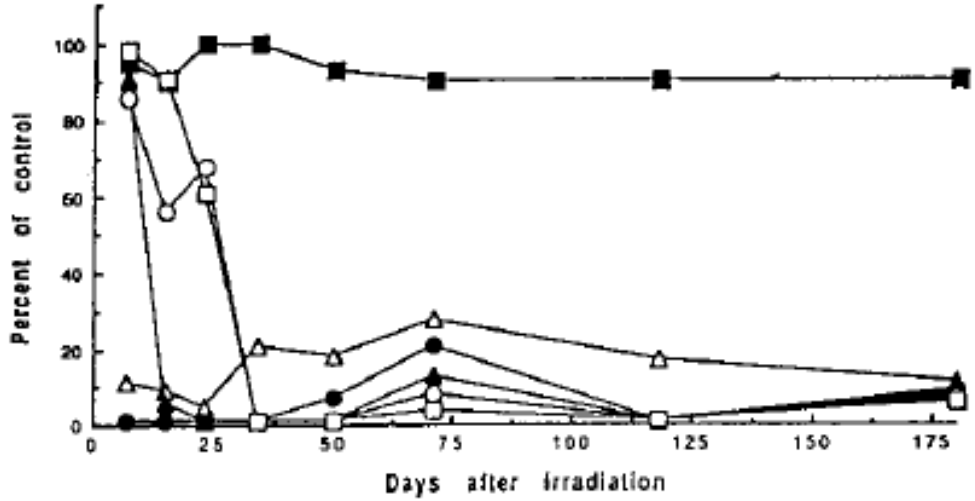
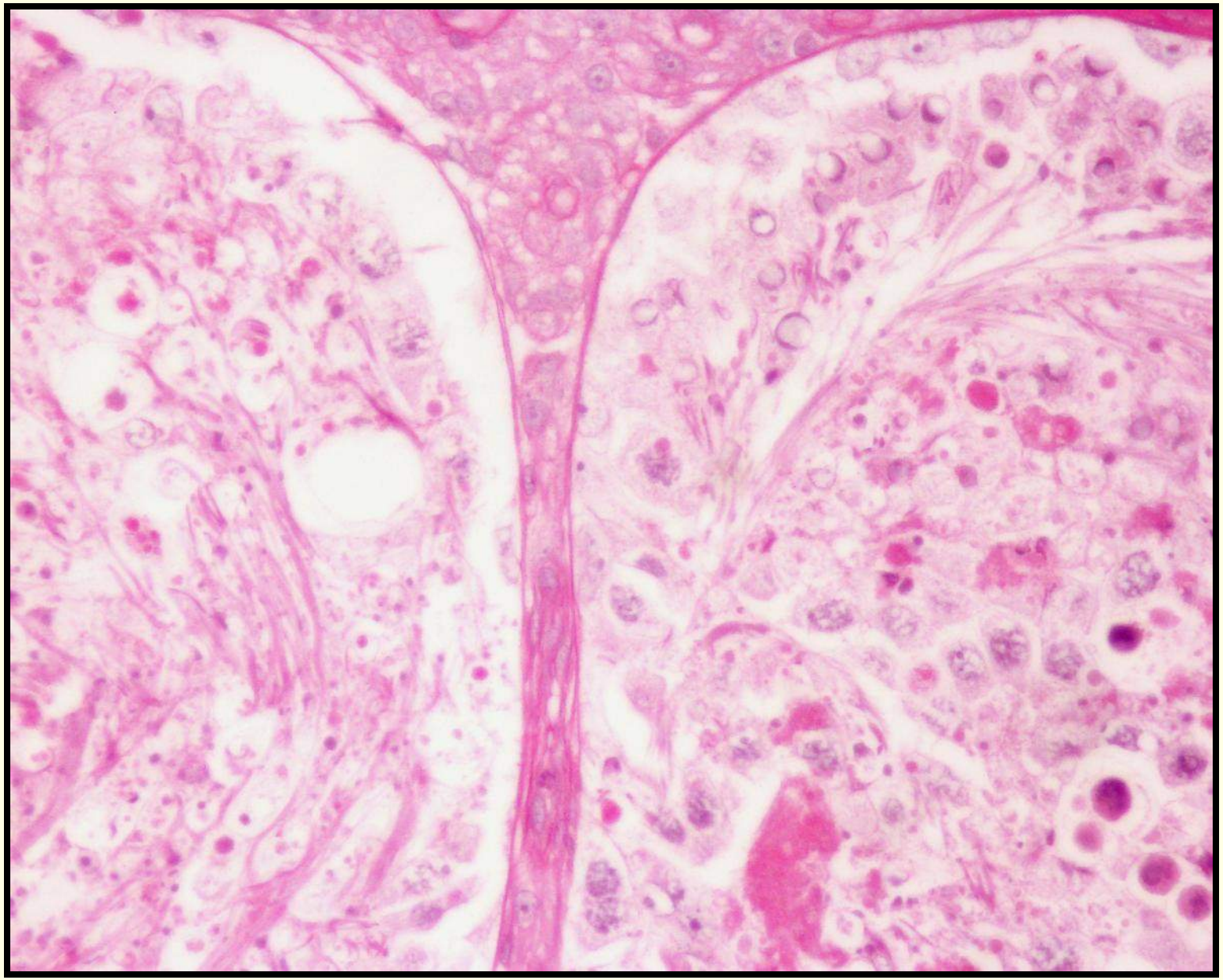


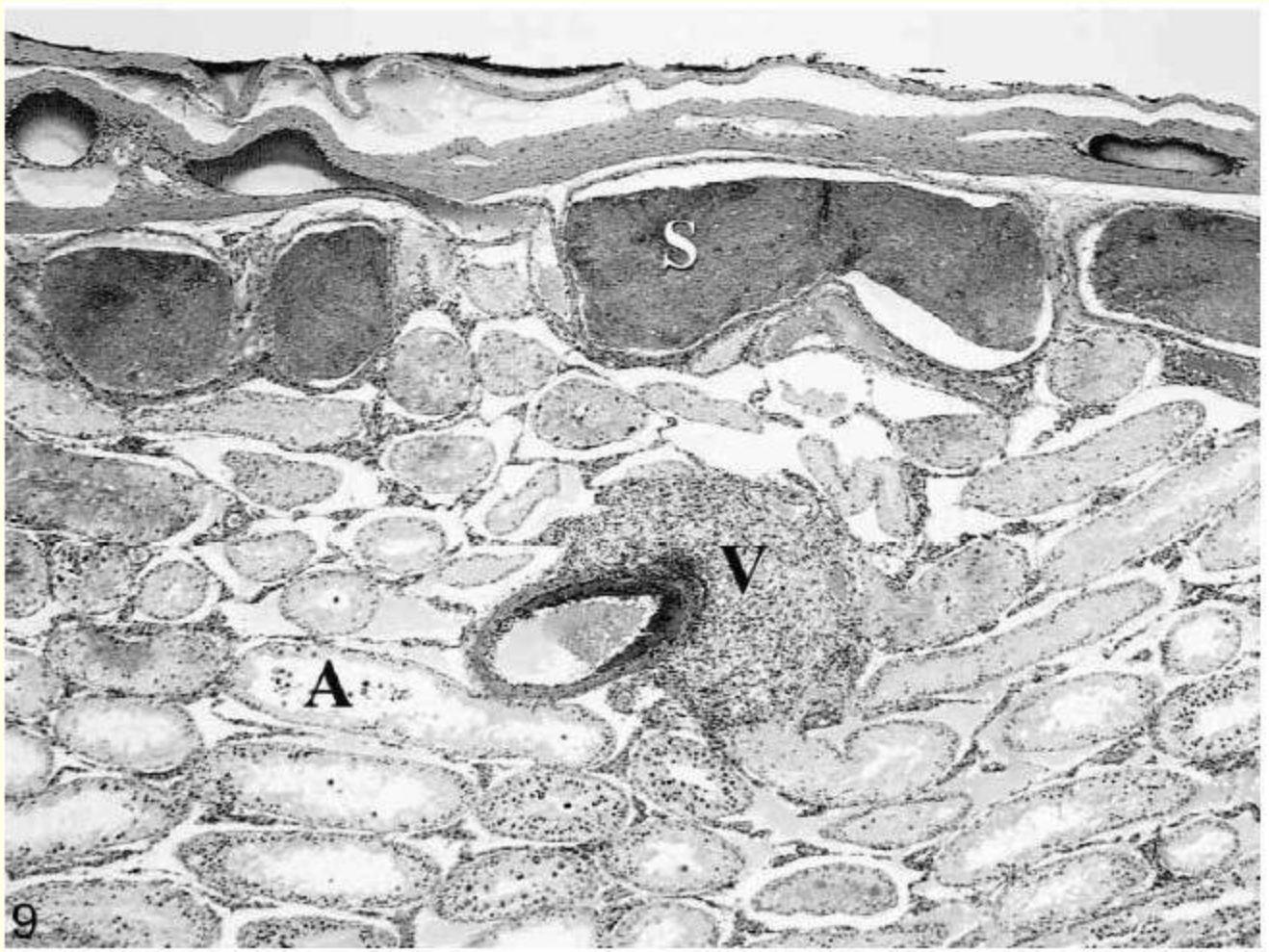
Fig 1. Temporal changes in the number of Sertoli and germ cells at stage (VII) after acute testicular γ-irradiation of rats of 2 months of age. —Δ—: spermatogonia (A_1 + A_0); —●—: preleptotene spermatocytes; —▲—: pachytene spermatocytes; —O— round spermatids; —□—: elongated spermatids: and —■—: Sertoli cells. Error bars were omitted for clarity.

Effect of an acute exposure of rat testes to gamma rays on germ cells and on Sertoli and Leydig cell functions G Pinon-Lataillade, MC Viguier-Martinez AM, Touzalin J, Maas B Jégou, Reprod Nutr Dev (1991) 31, 617

Rete Testis – Cellular debris



Dilated Rete



Theophylline-treated rat testis with sperm stasis (S) within the rete testis
The tubular atrophy (A) Vascular inflammation (V) of testicular vessels

Overview of Male Reproductive Pathology, George L. Foley, *Toxicol Pathol 2001; 29; 49*

Semineferous tubule

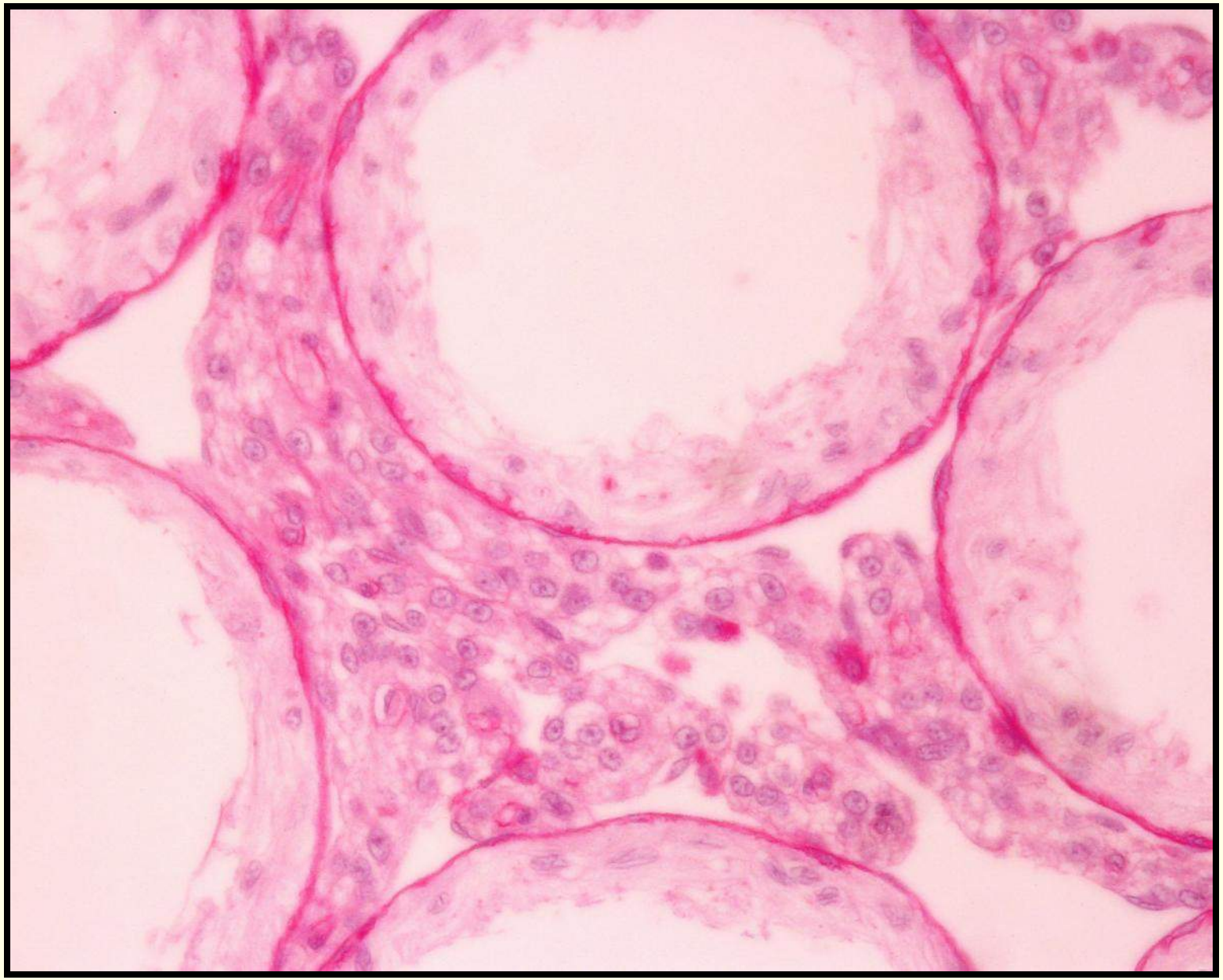
- Atrophy/contraction
- Reduction in overall diameter of tubule
  - Germ cell depletion
  - Reduced secretion of seminiferous tubule fluid- Sertoli cell (1 µL /hour)
  - Varies with stage of spermatogenesis- Testosterone dependant
  - Fluid secretion - Presence of elongating and elongated spermatids
- Tubular dilation
- Increase in overall diameter of tubule
  - Increased secretion by sertoli cell?
  - Reduced expulsion of fluid from tubule- contractile peritubular cells
  - Reduced reabsorption of fluid by the epithelial cells of rete and efferent ducts
  - Obstruction of outflow
- Inspissated sperm granulomatous inflammation

Semineferous tubule

- Vacuolation
- Focal tubular atrophy
- Oligospermia
- Mineralisation
- Sertoli only tubule
  - Cadmium, Ischemia, Serotonin, histamine
- Necrosis involves leydig cells and peritubular cells

- Dilated rete
  - Obstruction
  - Cellular debris

Leydig cell Hypertrohy/plasia



Leydig cells

- Atrophy
  - Reduced LH secretion
- Hypertrophy/Hyperplasia
  - increased stimulation by LH
  - Qualitative
  - Only in marked changes
- Degeneration and necrosis is rare
  - Ethane dimethase sulphonate, Lansaprazole
  - Regeneration thro fetal type Leydig cells
- Tissue resident macrophages- 20% of interstitial space

Cell specific toxicants

TABLE 2.—Cell-specific toxicants of the male reproductive tract.

Target cell	Toxicant	Effect
Leydig cell	Ethanedimethane sulfonate	Leydig cell necrosis with secondary germ cell death and depletion and atrophy of secondary sex organs
	Lansoprazole	Inhibition of testosterone synthesis with secondary Leydig cell tumor induction
Sertoli cell	Phthalate esters, 2,5-hexanedione	Sertoli cell vacuoles with secondary germ cell death and exfoliation
Spermatogonia	Busulfan, bleomycin	Spermatogonial death with secondary depletion of postspermatogonial germ cells
Spermatocytes	2-methoxyethanol, dinitropyrroles	Spermatocyte death with secondary depletion of postspermatocyte germ cells
Round spermatids	Ethylmethane sulfonate, methyl chloride	Spermatid death with secondary depletion of postspermatid germ cells
Elongated spermatids	Boric acid, dibromoacetic acid	Retention and phagocytosis of step 19 spermatids, abnormalities in released sperm
Testicular blood vessels	Cadmium chloride	Endothelial necrosis with secondary ischemic necrosis of all cell types
	5-hydroxytryptamine, histamine	Reduced blood flow, with secondary anoxic damage ranging from oncotic necrosis of the seminiferous epithelium to germ cell apoptosis and depletion
Epididymal epithelium	α-chlorohydrin (high doses)	Inhibits fluid resorption and causes edema of the caput resulting in sperm granulomas
	Methyl chloride	Epithelial necrosis resulting in sperm granulomas
	Carbendazim	Efferent duct necrosis resulting in sperm granulomas
Epididymal sperm	α-chlorohydrin (low doses), deoxychloroglucose	Inhibition of glycolysis resulting in sperm immotility
Vas deferens	Guanethidine	Inhibition of ejaculation due to adrenergic ganglion blockade resulting in rupture of vas-epididymal junction and sperm granulomas
Prostate and seminal vesicles	Flutamide	Androgen receptor blockade resulting in secretory inhibition and atrophy
	Finasteride	Inhibition of dihydrotestosterone production from testosterone resulting in secretory inhibition and atrophy

Dianne M. Creasy, Pathogenesis of Male Reproductive Toxicity, Toxicol Pathol 2001; 29; 64

Reversibility

- Spermatogenesis: Generally reversible as spermatogonial cell population is relatively resistant- Dormant for long period of time
- Leydig cells damaged- irreversible
- Sertoli cell injury- mild- fully reversible
- Sertoli cell destroyed- regeneration not possible
  - Do not divide in adults
  - Markedly resistant
- Epididymis- Granuloma is not reversible
- Prostate, Seminal vesicles generally reversible
- Dependant on site and severity of insult

Spontaneous lesions

TABLE I.—Incidence of nonneoplastic lesions in control male and female Fischer-344 rats.

	Feed		Inhalation		Gavage		Combined incidence	
	n^* = 80 M (%)	n = 79 F (%)	n = 40 M (%)	n = 40 F (%)	n = 40 M (%)	n = 40 F (%)	n = 160 M (%)	n = 159 F (%)
Urogenital system								
Kidney #Ex=†	80	79	40	39	40	39	160	157
Nephropathy	77 (96.3)	17 (21.5)	29 (72.5)	4 (10.2)	39 (97.5)	9 (23.1)	145 (90.6)	30 (19.1)
Mineralization	0	72 (91.1)	0	29 (74.4)	0	20 (51.3)	0	121 (77.0)
Urinary bladder #Ex=	80	79	40	40	40	40	160	159
Mineralization, subserosal	2 (2.5)	0	0	0	0	0	2 (1.3)	0
Ovary #Ex=	0	79	0	40	0	40	0	159
Cyst, bursa	—	9 (11.4)	—	3 (7.5)	—	0	—	12 (7.5)
Cyst, follicle	—	1 (1.3)	—	0	—	0	—	1 (0.6)
Uterus #Ex=	0	79	0	40	0	40	0	159
Dilation, horn	—	7 (8.9)	—	8 (20)	—	7 (17.5)	—	22 (13.8)
Decidual reaction	—	2 (2.5)	—	0	—	0	—	2 (1.3)
Clitoral gland #Ex=	0	79	0	38	0	38	0	155
Inflammation, mononuclear cell	—	26 (32.9)	—	1 (2.6)	—	7 (18.4)	—	34 (21.9)
Preputial gland #Ex=	80	0	40	0	32	0	152	0
Inflammation, mononuclear cell	25 (31.3)	—	1 (2.5)	—	10 (31.3)	—	36 (23.7)	—
Granuloma	0	—	1 (2.5)	—	0	—	1 (0.7)	—
Prostate gland #Ex=	80	0	40	0	40	0	160	0
Inflammation, suppurative	1 (1.2)	—	0	—	0	—	1 (0.6)	—
Inflammation, mononuclear cell	2 (2.5)	—	0	—	0	—	2 (1.3)	—
Testis #Ex=	80	0	30	0	40	0	150	0
Atrophy, seminiferous tubule	2 (2.5)	—	1 (3.3)	—	0	—	3 (2.0)	—
Hyperplasia, interstitial cell	1 (1.3)	—	0	—	0	—	1 (0.7)	—

Incidence of Nonneoplastic Lesions in Historical Control Male and Female Fischer-344 Rats from 90-Day Toxicity Studies, Darlene Dixon, Katharina Heider and Michael R. Elwell, *Toxicol Pathol 1995; 23; 338*

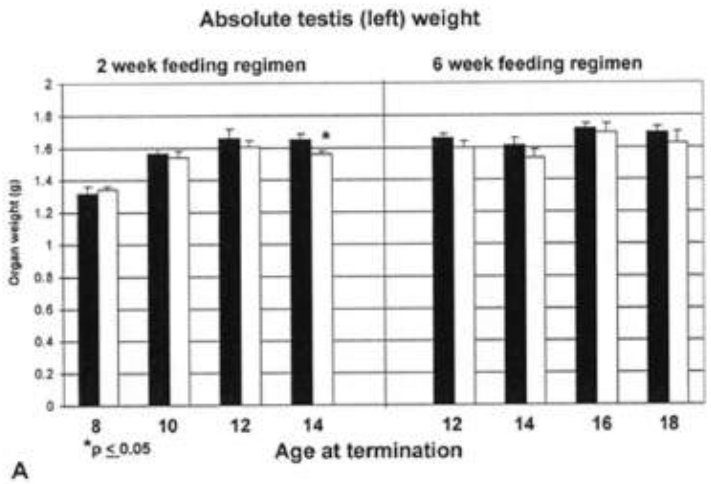
Inhalation studies

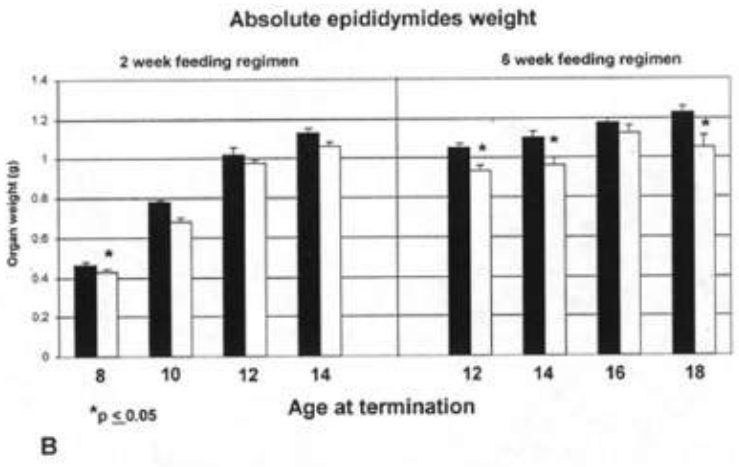
TABLE II.—Summary of testicular lesions in control male rats used for subchronic inhalation or oral toxicity studies in 1990.

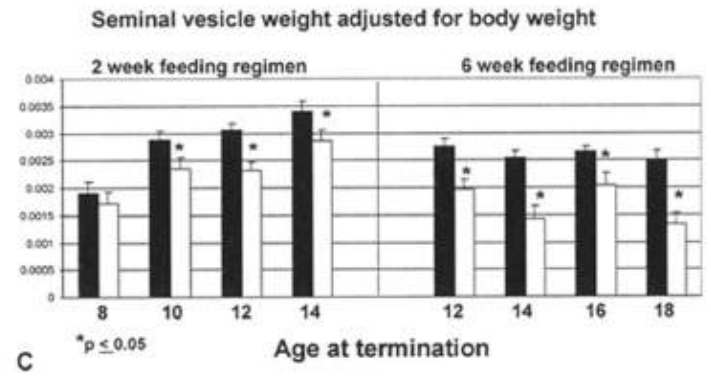
	Inhalation						Oral
Number of study:	1	2	3	4	5	6	1
Number of rats:	10	10	10	10	10	10	10
Number of rats with testicular atrophy:	3/10	3/10	2/10	4/10	1/10	1/10	1/10
Testicular lesions							
Early minimal changes							
EGB/degeneration, mature spermatids, stages I–VIII	—	3/3	2/2	2/4	1/1	1/1	—
EGB/degeneration, elongated spermatids, stages IX–XIV	—	3/3	2/2	2/4	1/1	—	—
Mature spermatid retention, stages IX–XIV	—	3/3	2/2	2/4	1/1	—	—
Early moderate changes							
Depletion, mature spermatids, stages I–VIII	2/3	1/3	1/2	2/4	1/1	—	—
Depletion, round spermatids, stages I–VIII	2/3	1/3	—	3/4	1/1	1/1	1/1
Depletion, elongated spermatids, stages IX–XIV	—	—	—	3/4	—	1/1	1/1
Advanced changes							
Degeneration, spermatocytes, stages IX–XIV	—	1/3	—	2/4	—	1/1	1/1
Degeneration, meiotic spermatocytes, stage XIV	—	1/3	—	—	—	—	—
Spermatid giant cell formation	1/3	—	—	—	—	—	—
Sertoli cell only, 1–10 seminiferous tubules	1/3	—	1/2	1/4	1/1	—	1/1
Round cell only, stages I–VIII	1/3	—	1/2	—	—	—	—
Unilateral testicular atrophy	1/3	—	—	—	—	—	—
Epididymal lesions							
Exfoliated degenerative germ cells, epididymides	2/10	7/10	3/10	3/1	3/10	2/10	1/1
Oligospermia (decreased sperm density), epididymides	2/10	4/10	2/10	—	—	—	1/1
Spermatic granuloma, epididymides	1/10	—	—	1/10	—	1/10	—

Testicular Degeneration and Spermatid Retention in Young Male Rats, Ki-Poong Lee, Steven R. Frame, Greg P. Sykes and Rudolph Valentine, *Toxicol Pathol 1993; 21; 292*

Food restriction







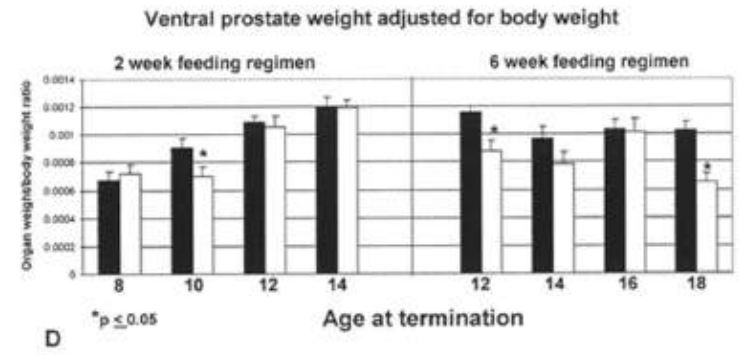


FIGURE 2.—Absolute organ weights for left testis (A) and epididymides (B) and relative organ weights for seminal vesicles (C) and ventral prostate (D) in rats fed AL or FR for two or six weeks. Solid bars are ad libitum feeding, open bars are food restricted; error bars = standard error of the mean.

Effects of Food Restriction on Testis and Accessory Sex Glands in Maturing Rats, SABINE REHM, TACEY E. WHITE, EIAS A. ZAHALKA, DINESH J. STANISLAUS, ROGELY W. BOYCE, AND PATRICK J. WIER, *Toxicologic Pathology, 36: 687-694, 2008*

Food Restriction

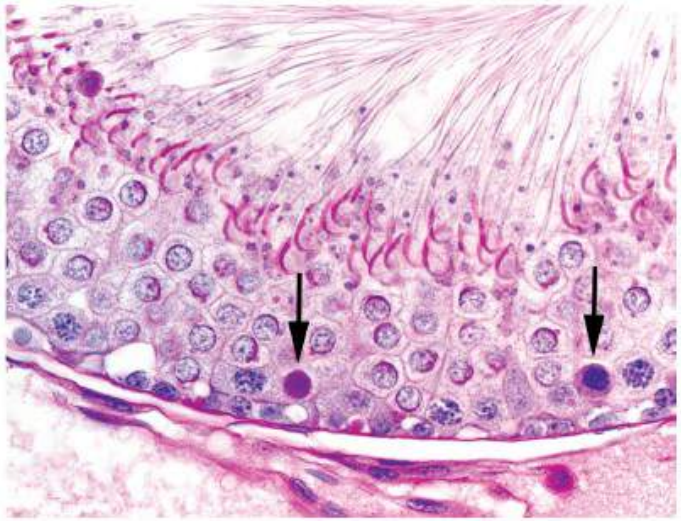


FIGURE 3.—Degeneration of pachytene spermatocytes (arrows) in stage VII of the rat spermatogenic cycle. PAS reaction.

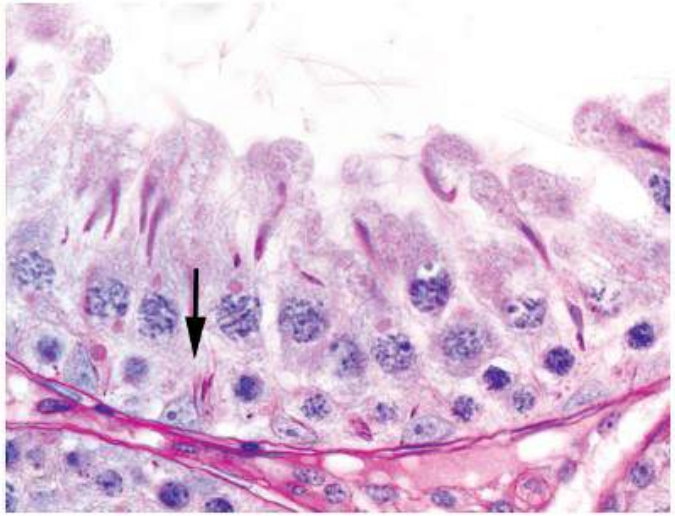


FIGURE 4.—Step 19 spermatid retention and phagocytosis by Sertoli cells (arrow), as shown in stage XI of the rat spermatogenic cycle. PAS reaction.

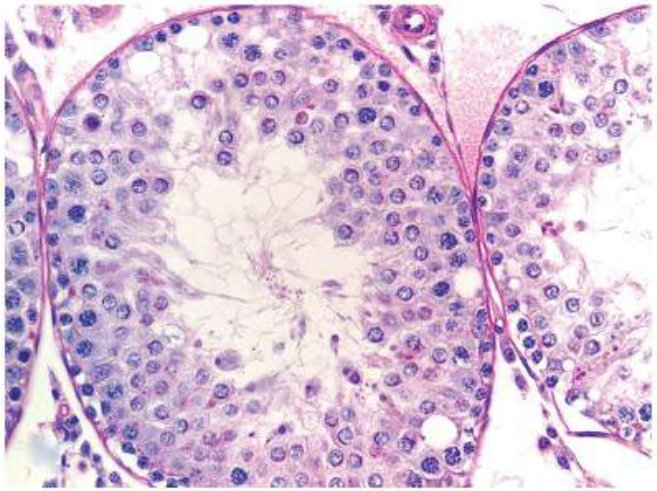
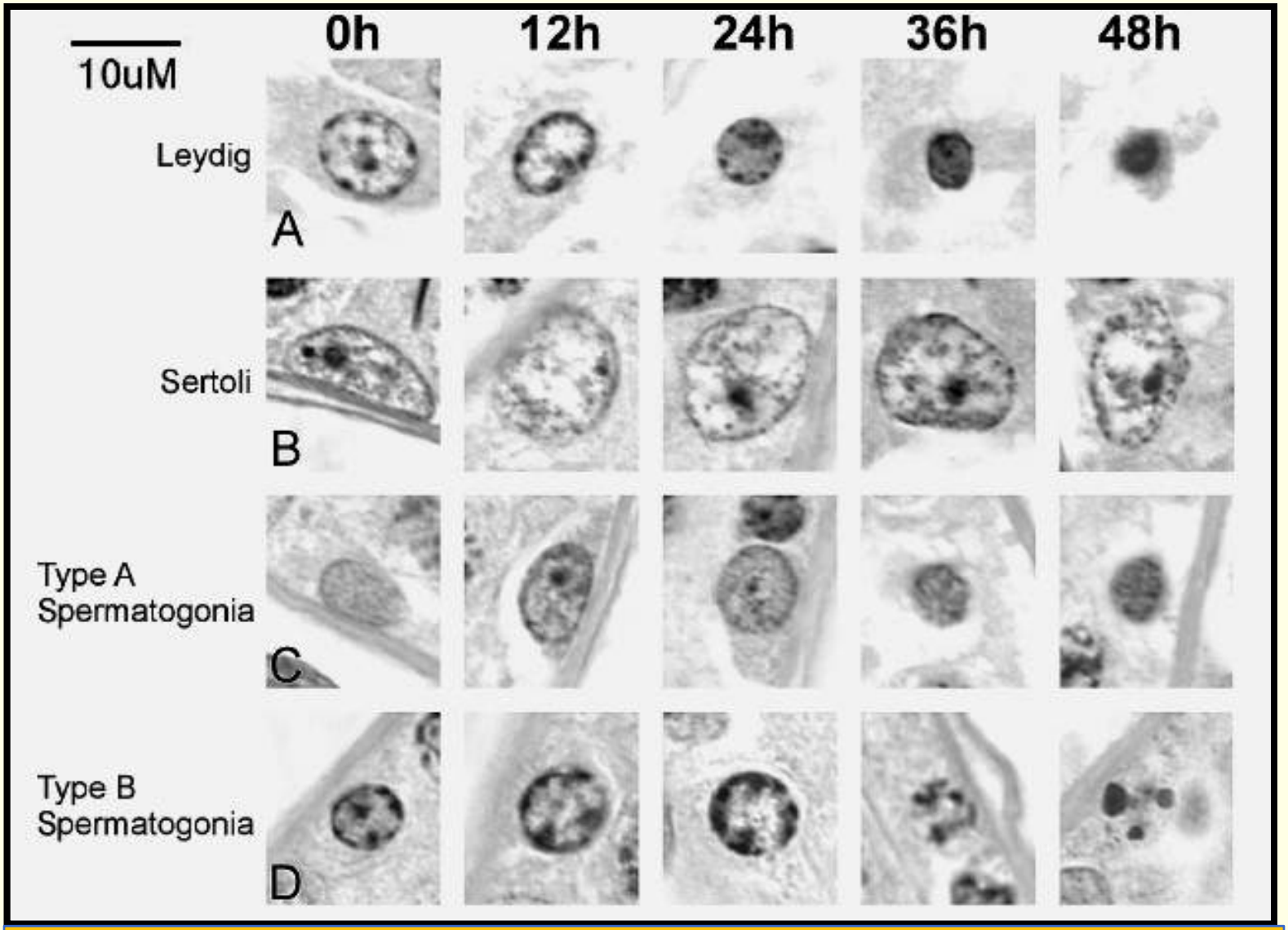


FIGURE 5.—Degeneration and loss of germinal epithelium, bilateral in a rat FR from twelve to eighteen weeks of age. PAS reaction.

Effects of Tw o Weeks of Feed Restriction on Some Common Toxicologic Parameters in Sprague-Dawley Rats*
STUARTL EVIN,D' AVIDSE MLERA,'N D ZADOKR UBEN, *Toxicol Pathol 1993; 21; 1*

Effects of Food Restriction on Testis and Accessory Sex Glands in Maturing Rats, SABINE REHM, TACEY E. WHITE, EIAS A. ZAHALKA, DINESH J. STANISLAUS, ROGELY W. BOYCE, AND PATRICK J. WIER, *Toxicologic Pathology, 36: 687-694, 2008*

Post Mortem changes

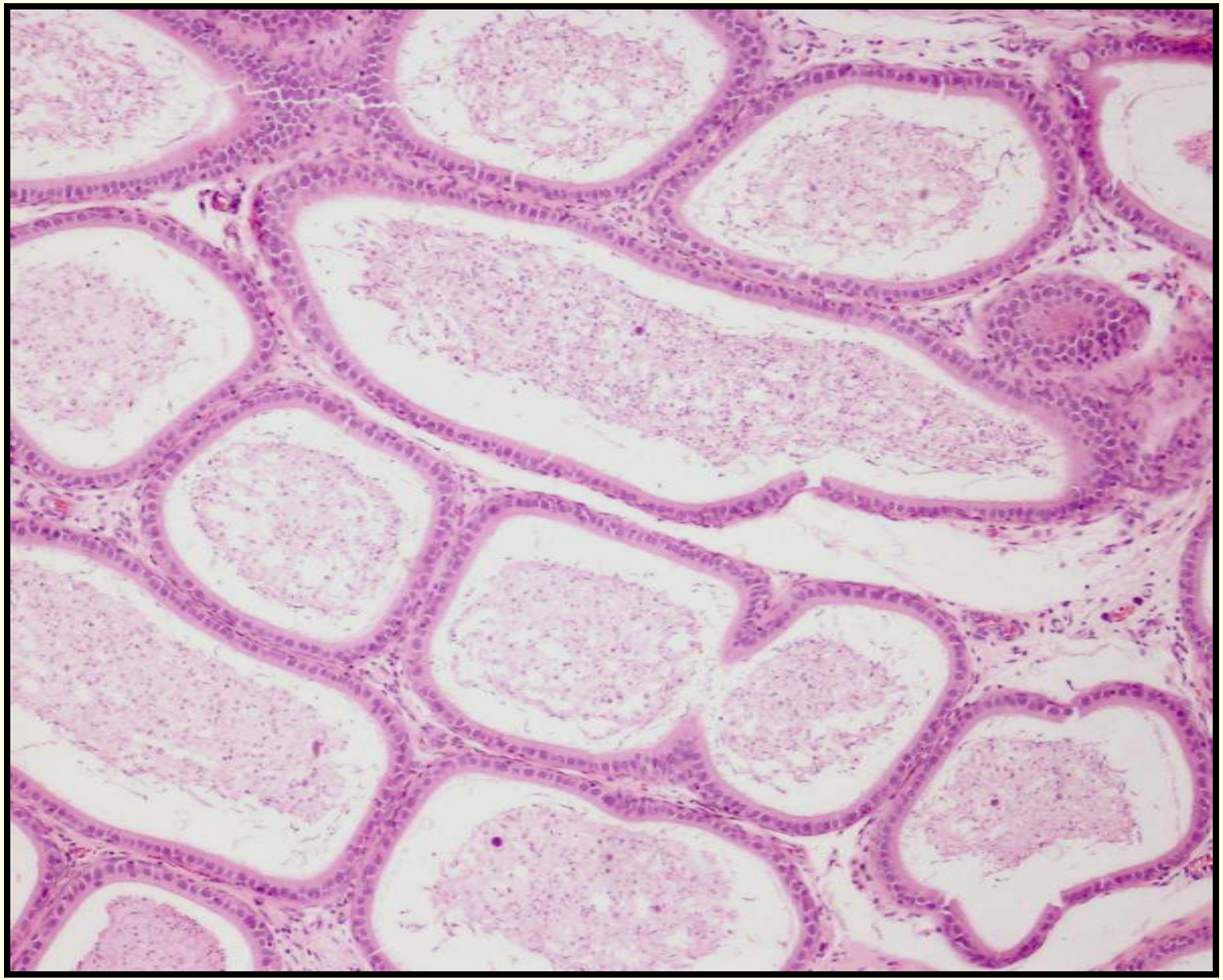


Time-Dependent Changes in Post-Mortem Testis Histopathology in the Rat, Bronwyn H. Bryant and Kim Boekelheide, *Toxicol Pathol 2007; 35; 665*

Backgroud lesions

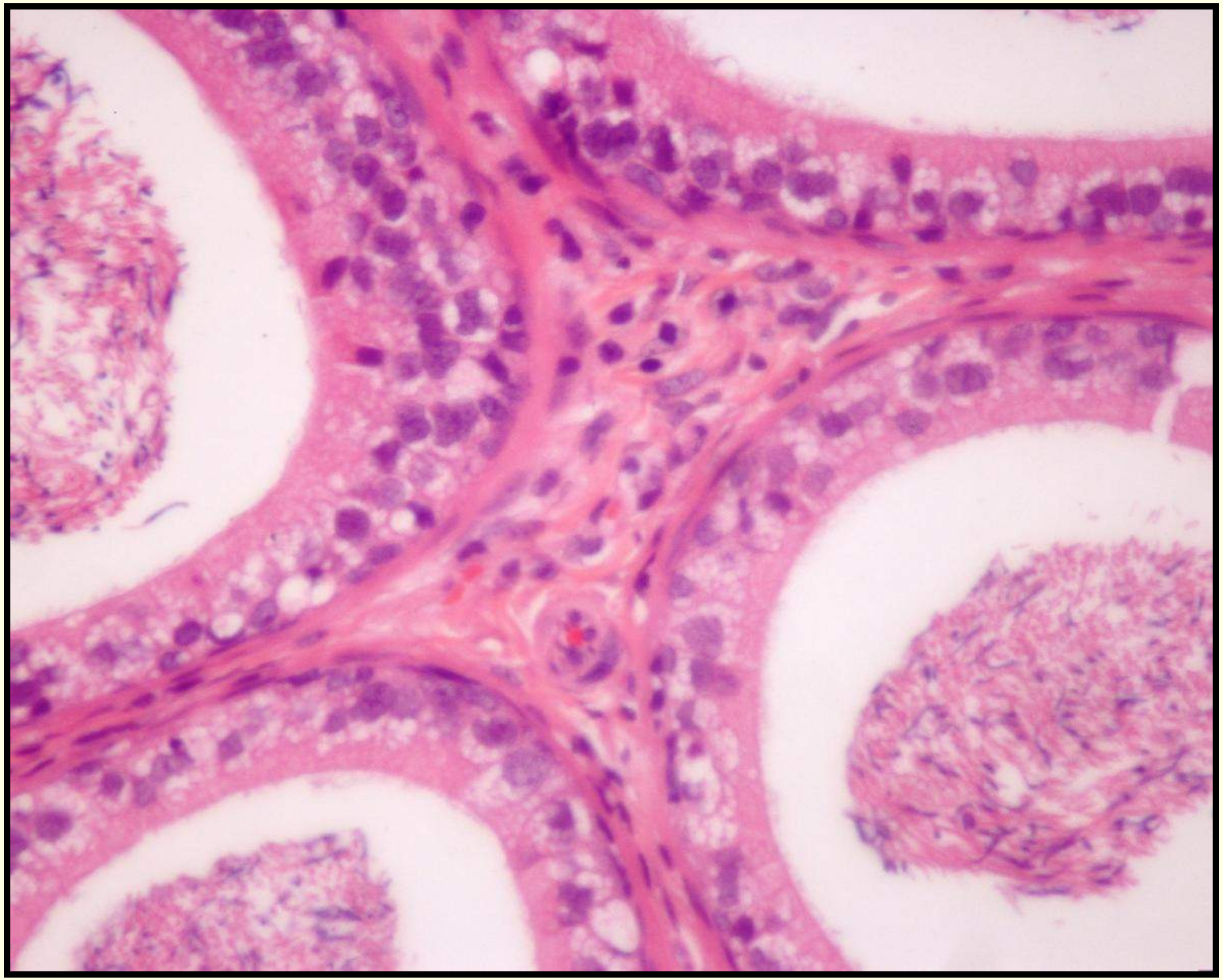
- Fixation and tissue preparation artifacts very common
- Altered temperature of the testis
- Trauma
- Testis forced in to inguinal canal and scrotum- restraint
- Stress
- Food restriction
- Common lesions
  - Atropic tubules – 1-3 contracted tubules having only sertoli cells
  - Tubular vacuolation
  - Degeneration of germ cells
  - Syncitial germ cells
  - Spermatid retention/delayed spermiation
  - Diffuse germ cell degeneration/depletion or total tubular atrophy affecting one or both testis

Epididymis - Oligospermia

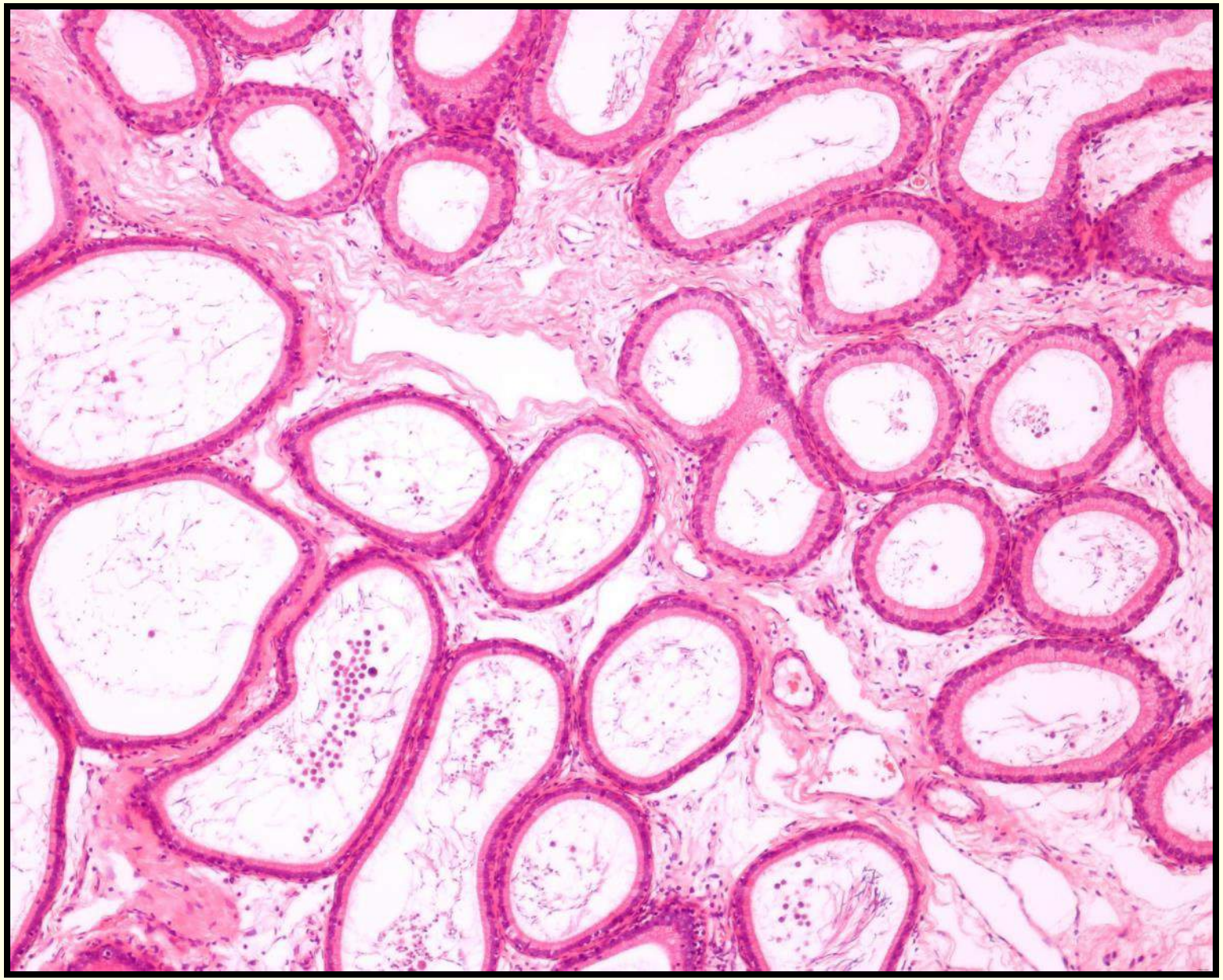


Epithelial Vacuolation

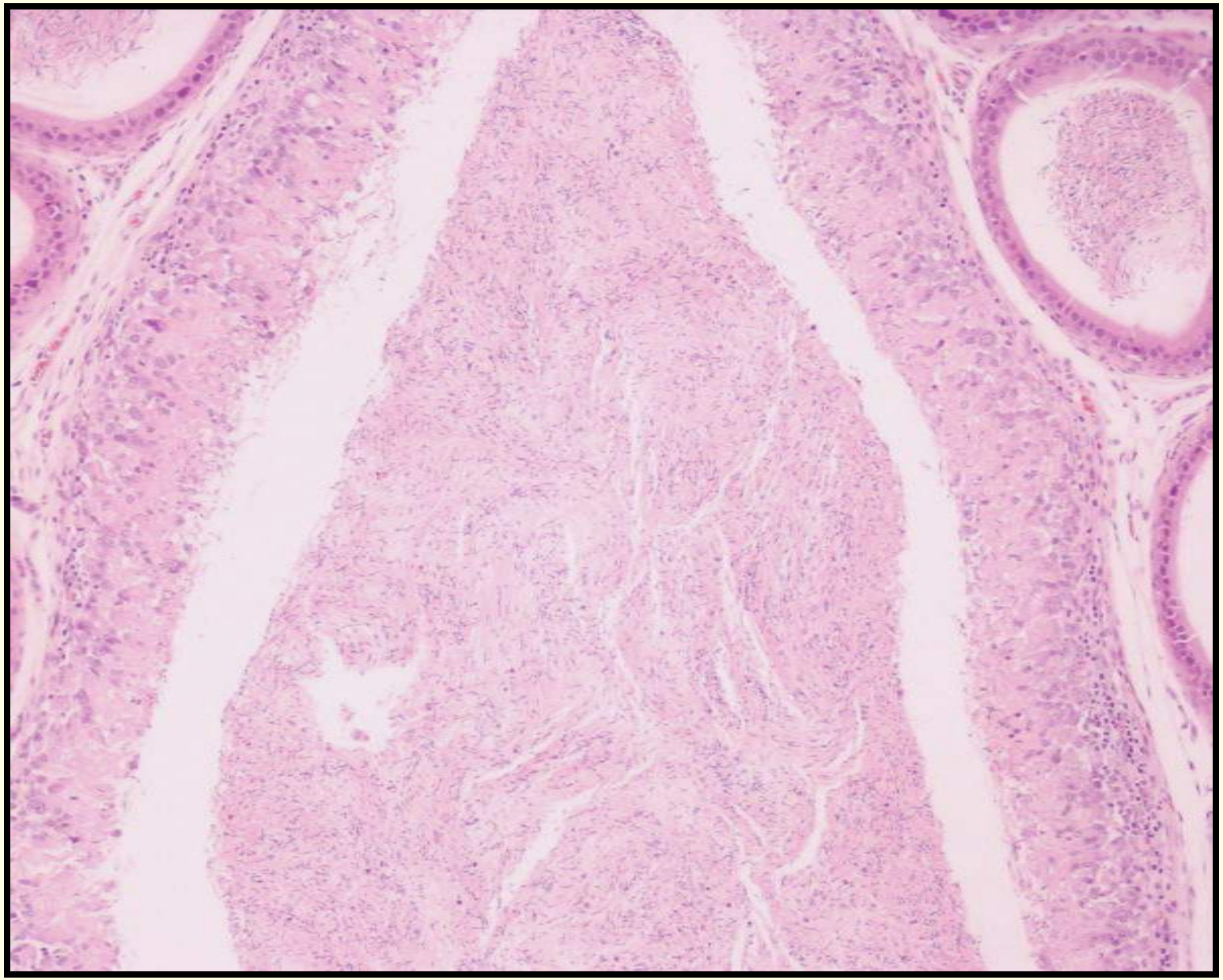
Epithelial Vacuolation



Atrophy



Sperm Granuloma

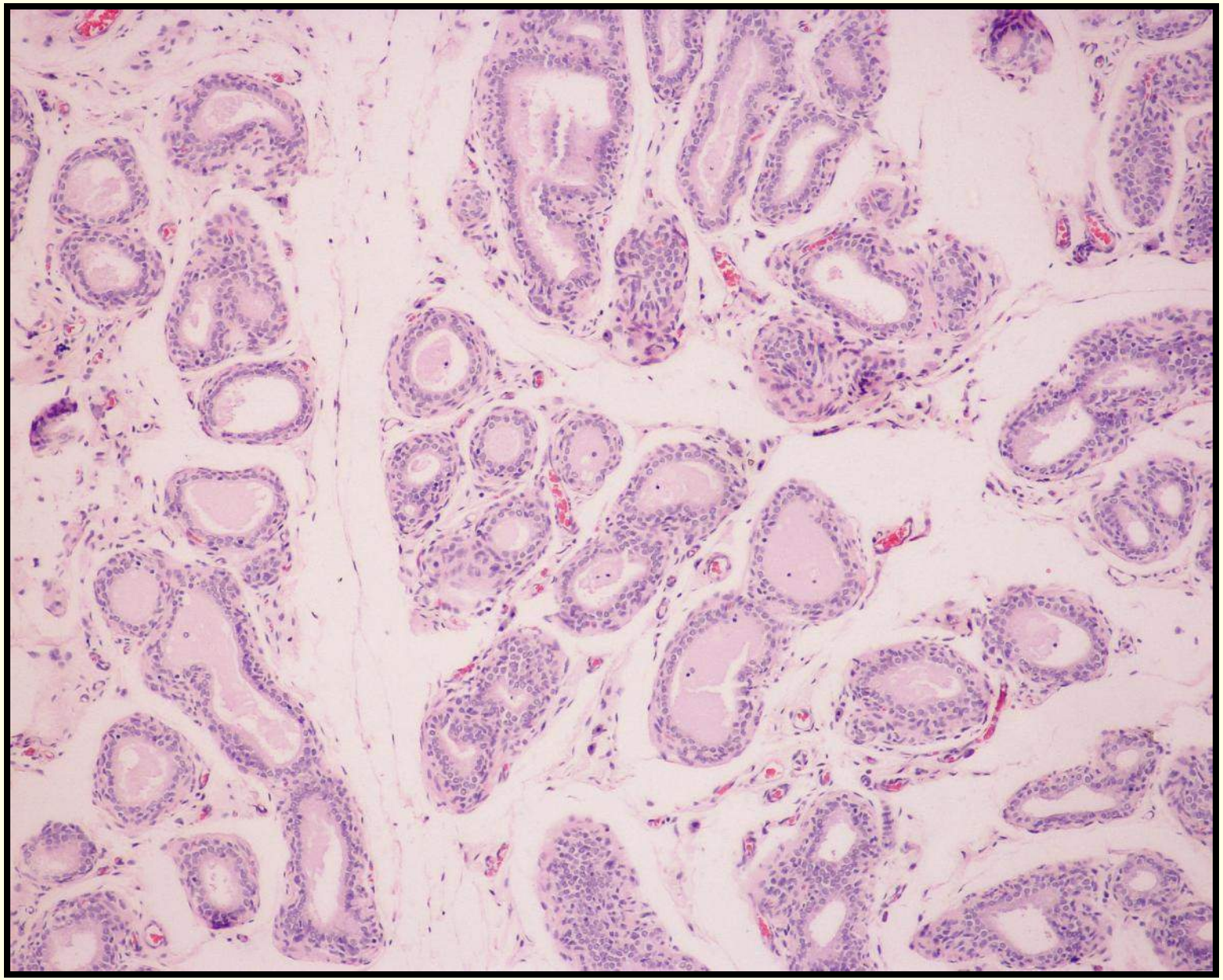


Epididymis

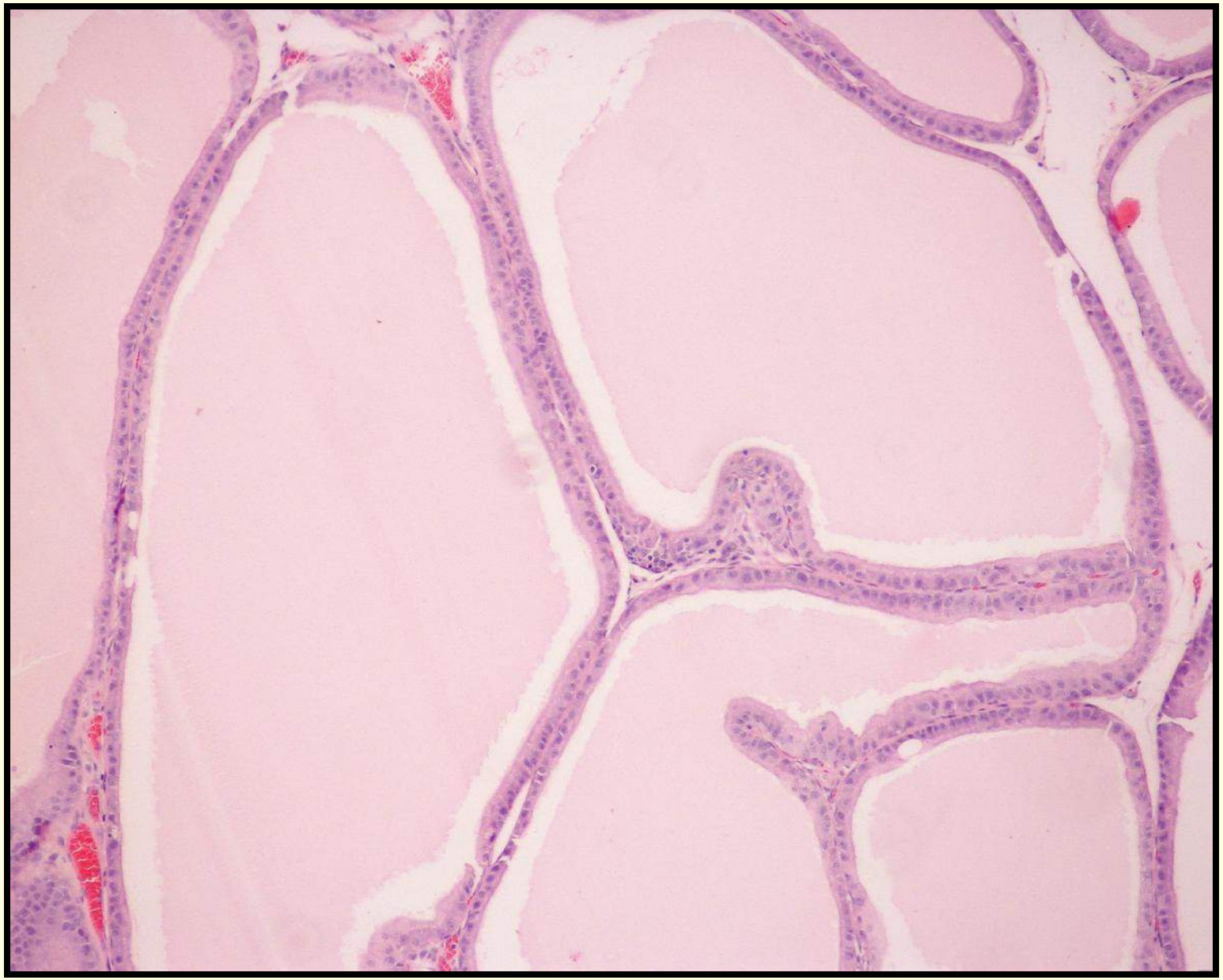
- Blood epididymal barrier not as strong as blood testis barrier
- Weight is a very sensitive method of detection of alterations
- Atrophy, Oligospermia, Cellular debris
  - Degenerating cells and residual bodies
- Sperm granuloma
  - More common in epididymis than testes
- Epithelial changes
  - Vacuolation-direct toxic effect - α Chlorohydrin, Methyl Chloride or secondary to reduced androgenic stimulation
  - Inflammation
- Disruption of sperm maturation-TCDD, - α Chlorohydrin
  - Only when toxicant has direct effect on sperm in epididymis
  - Reduced sperm motility
  - Reduced motile sperms
  - Increase in morphologically abnormal sperms

Normal Prostate

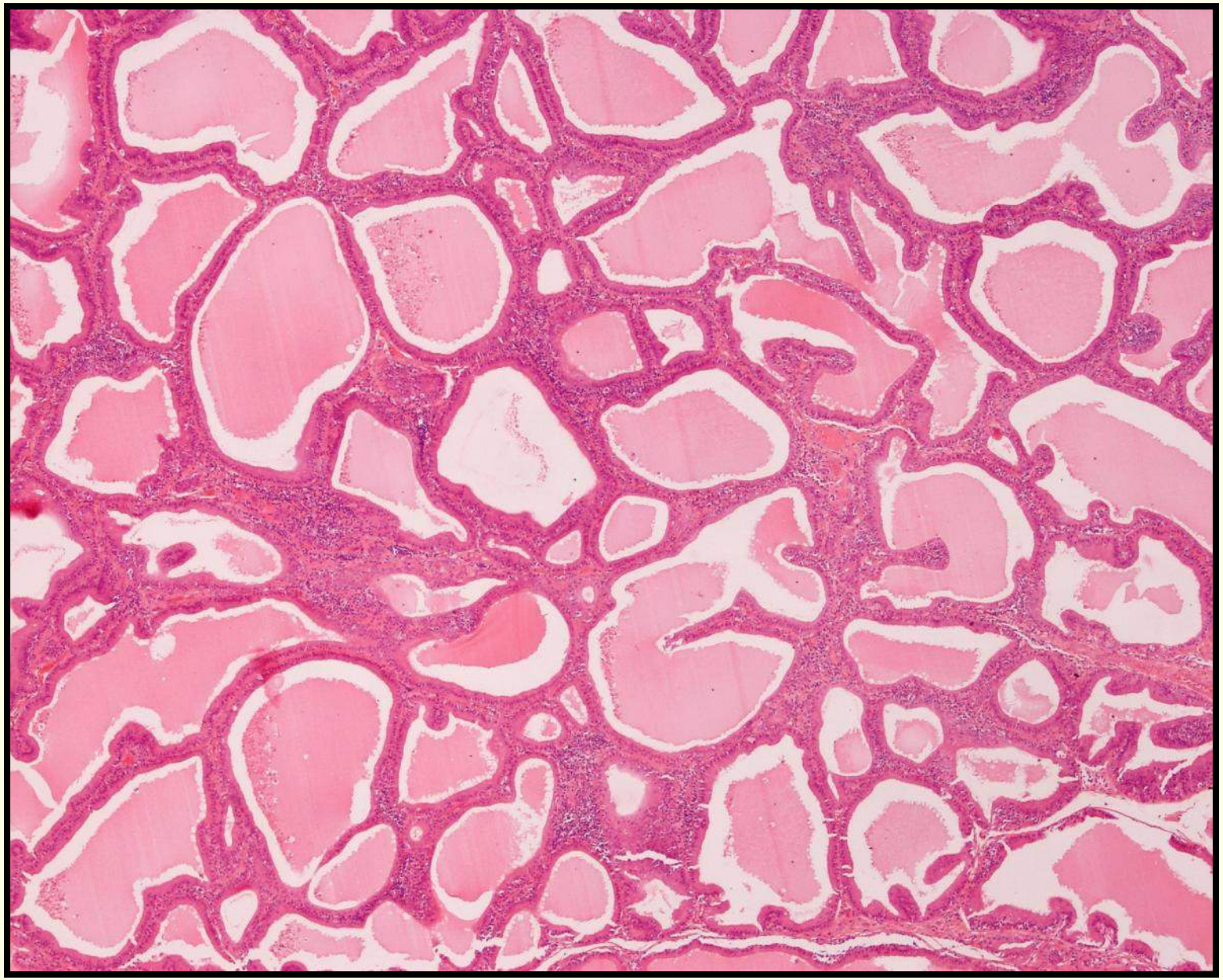
Prostate - Atrophy



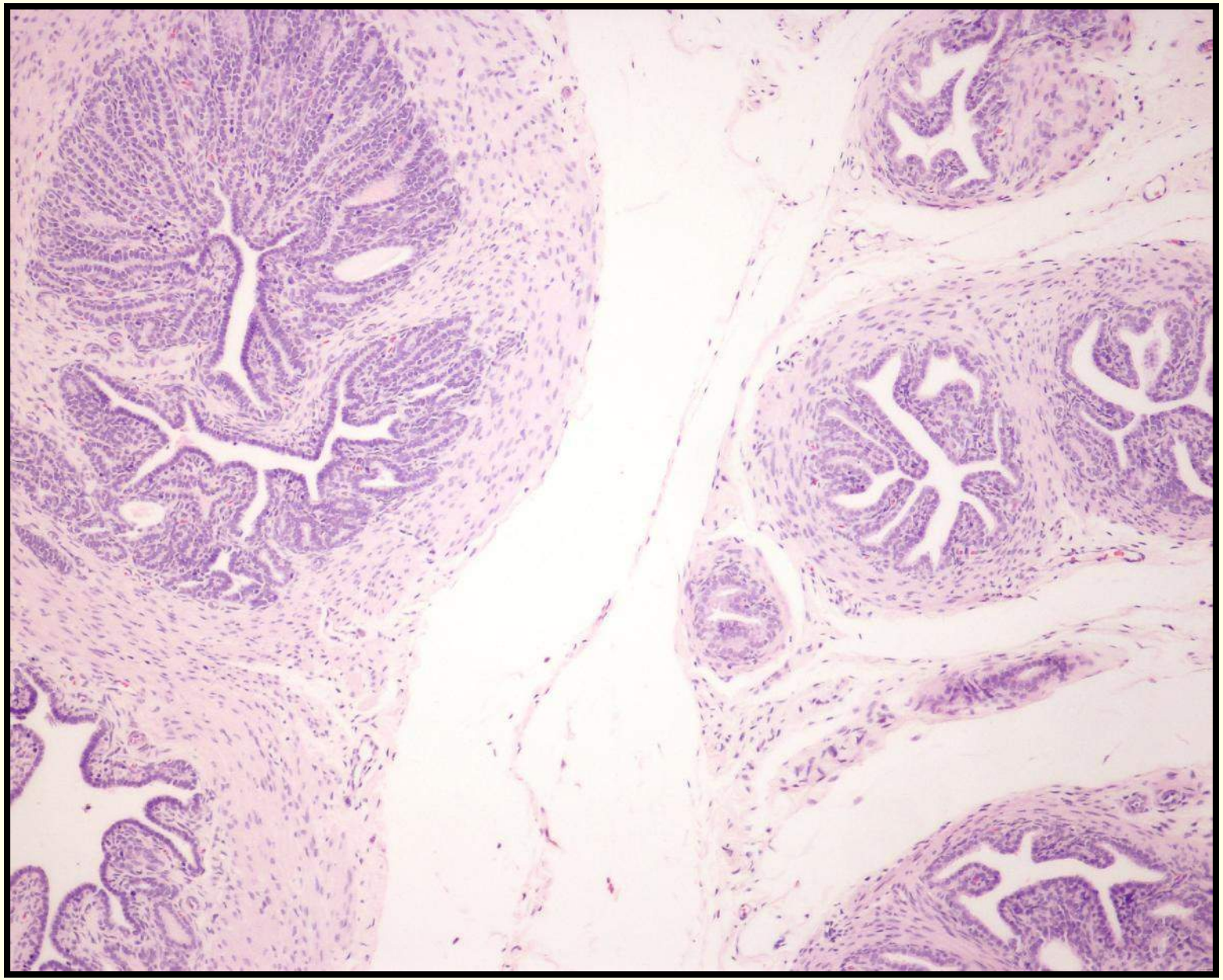
Prostate - Hypertrophy



Prostate – MNC inflitration



Seminal Vesicles & Coagulation gland



Prostate and Seminal Vesicles

- Highly androgen dependant
- Weight is more sensitive than histology
  - Atrophy
  - Hypertrophy
  - Inflammation
  - Flutamide, Finasteride, 17-20 Lyase inhibitors

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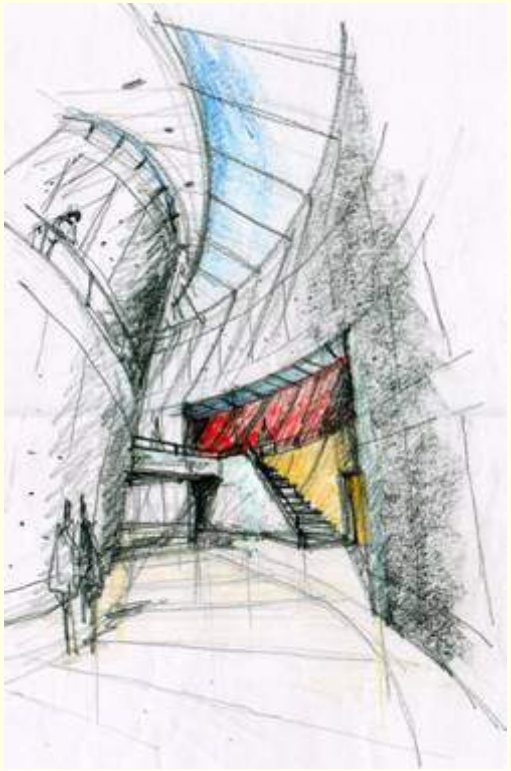

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Picture of the Atrium. *The building has a curved hallway and circular atrium designed to maximize the use of wind tunnels in the area. The use of natural wind and light are consistent with our support for the environment*