Toxicologic pathology of the male reproductive system Practice

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As far as not mentioned otherwise, some pictures, diagrams, drawings, etc. were made during the preparation of the following book and were partly published therein:

Histological and histopathological evaluation of the testis

Lonnie D. Russell, Robert A. Ettlin, Amiya P. Sinha Hikim, Eric D. Clegg

Cache River Press 1990

ISBN 0-9627422-0-1

Toxicologic Pathology of the MR System

- 2 lectures
  - Part 1 Basis:
    - The male reproductive (MR) system
    - MR toxicity
  - Part 2 Practice (present lecture):
    - Recommended approaches for evaluation of MR organs (general methods)
    - Morphologic evaluation of MR organs
- Covered: mainly *rats* as well as some particularities of other species
- *Not covered:* Developmental reproductive toxicity

Lecture 2: Practice

C Recommended approaches for evaluation of MR organs (general methods)
- Guidelines
- Study design
- “Non-anatomic” parameters, especially hormones
- Organ weights
- Tissue preparation
- Histopathological evaluation
- Dealing with unexpected findings
- Conclusions

D Morphologic evaluation of MR organs
- General toxicity
- Endocrine disruption

C. Evaluation of MR organs

- Guidelines
- Study design
- “Non-anatomic” parameters, especially hormones
- Organ weights
- Tissue preparation
- Histopathological evaluation
- Dealing with unexpected findings
- Conclusions

Guidelines (selection)

- General safety guidelines
- Guidelines for assessing male and female reproductive (FR) toxicity including offspring
  - ICH S5(R2): Parent guideline 'Detection of toxicity to reproduction for medicinal products'
    Addendum to the parent guideline: 'Toxicity to male fertility'
  - EPA: Guidelines for reproductive toxicity risk assessment
  - FDA: Food additives, etc.
  - OECD:
    - Testing of chemicals (415, 416, 421, 422)
    - Endocrine disrupters
  - Etc.

Standard reproductive toxicity studies not addressed in this presentation

C. Evaluation of MR organs

- Guidelines
- Study design
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Male-specific endpoints of reproductive toxicity

Organ weights	Testes, epididymides, seminal vesicles, prostate, pituitary
Macroscopic examination and histopathology	Testes, epididymides, seminal vesicles, prostate, pituitary, mammary gland area
Sperm evaluation*	Sperm number (count) and quality (morphology, motility)
Sexual behavior*	Mounts, intromissions, ejaculations
Hormone levels* (selection)	Luteinizing hormone (LH), follicle stimulating Hormone (FSH), testosterone (T), estrogen (E), prolactin (PRL)
Developmental effects*	Number/status of offspring, in particular: Testis descent, preputial separation, sperm production, anogenital distance, external genitalia, other malformations
* Can be obtained or estimated relatively easily in humans	

Sensitivity to detect effects on MR parameters

Parameters	Detection Rate (%)
Epididymal sperm count	90
Histopathology	89
Testicular sperm count	81
Sperm motility	76
Accessory gland weights	76
Sperm morphology	73
Epididymal weight	73
Testis weight	71

Detection of Effects on Male Reproduction - A Literature Survey
Beate Ulbrich and Anthony K. Palmer. Int J Toxicol 1995 vol. 14 no. 4 293-327

Sensitivity in combination

Parameters	Detection Rate (%)
Histopathology alone	89
+ Organ weights	94
Sperm motility alone (Percent motility + motility parameters)	76
+ Histopathology + Organ weights	100
Sperm analysis alone (Sperm counts + sp. motility + sp. morphology)	97
+ Histopathology	100

Detection of Effects on Male Reproduction - A Literature Survey
Beate Ulbrich and Anthony K. Palmer. Int J Toxicol 1995 vol. 14 no. 4 293-327

Study types and MR parameters

- General (4 or) 13 week toxicity studies often most appropriate, because various endpoints of relevance to MR can be assessed:
  - Organ weights
  - Morphology (macro/microscopic)
  - Clinical chemistry parameters and, if appropriate, hormone levels
- Dedicated studies are need to assess function (not a sensitive parameter) and genotoxicity
- Tailor-made studies designed on a case-by-case basis may be needed for trouble-shooting in case on unexpected preclinical MR findings

Protocol for detailed investigation of MR toxicity

Current Protocols in Toxicology - Wiley Online Library

In Vivo Models for Male Reproductive Toxicology - Rochelle W. Tyl

Center of Life Sciences and Toxicology Research Triangle Institute, North Carolina, USA

<table>
<tr><th colspan="5">Live animals</th><th rowspan="2">Necropsy</th></tr>
<tr><th rowspan="2">Electro-ejaculation</th><th rowspan="2">Blood sampling</th><th colspan="3">Unilateral orchidectomy</th></tr>
<tr><th>Culture</th><th>Homogenization resistant spt/sp</th><th>Histo-pathology</th></tr>
<tr><td rowspan="4">Cauda sperm
- Number
- Motility
- Morphology</td><td>FSH, LH, DHT</td><td rowspan="4">T, DHT
Inhibin
In culture and in the whole testis

Morphology

Other parameters</td><td rowspan="4">Sperm production</td><td rowspan="4">Staging</td><td>Organ weights</td></tr>
<tr><td>If normal:
Repeat after GnRH stimulation</td><td>Testis:
- Histopathology including staging
- Sperm production
- Culture</td></tr>
<tr><td>Epididymis:
- Histopathology
- Cauda sperm</td></tr>
<tr><td>CNS including pituitary
Adrenals, liver, mammary gland area</td></tr>
<tr><td colspan="6">Routine parameters are marked in brown</td></tr>
</table>

Species selection

- Generally no one species better
- Expect differences in susceptibility to toxins
  Reasons for these differences mostly unknown

See also Parker and Tyl, 2003, EPA White Paper

Time

- 4 week toxicity studies often sufficient, but *13 week studies* are more reliable
- For tailor-made studies
  - *Recovery period*: to cover at least the duration of the full spermatogenic process
    - Mouse ~ 35 days
    - Rat ~ 52 days
  - Consider *time-course* study with serial autopsies (hours to weeks apart): cell-specific toxicity is only seen at *early* time points

Animals are immature at test start

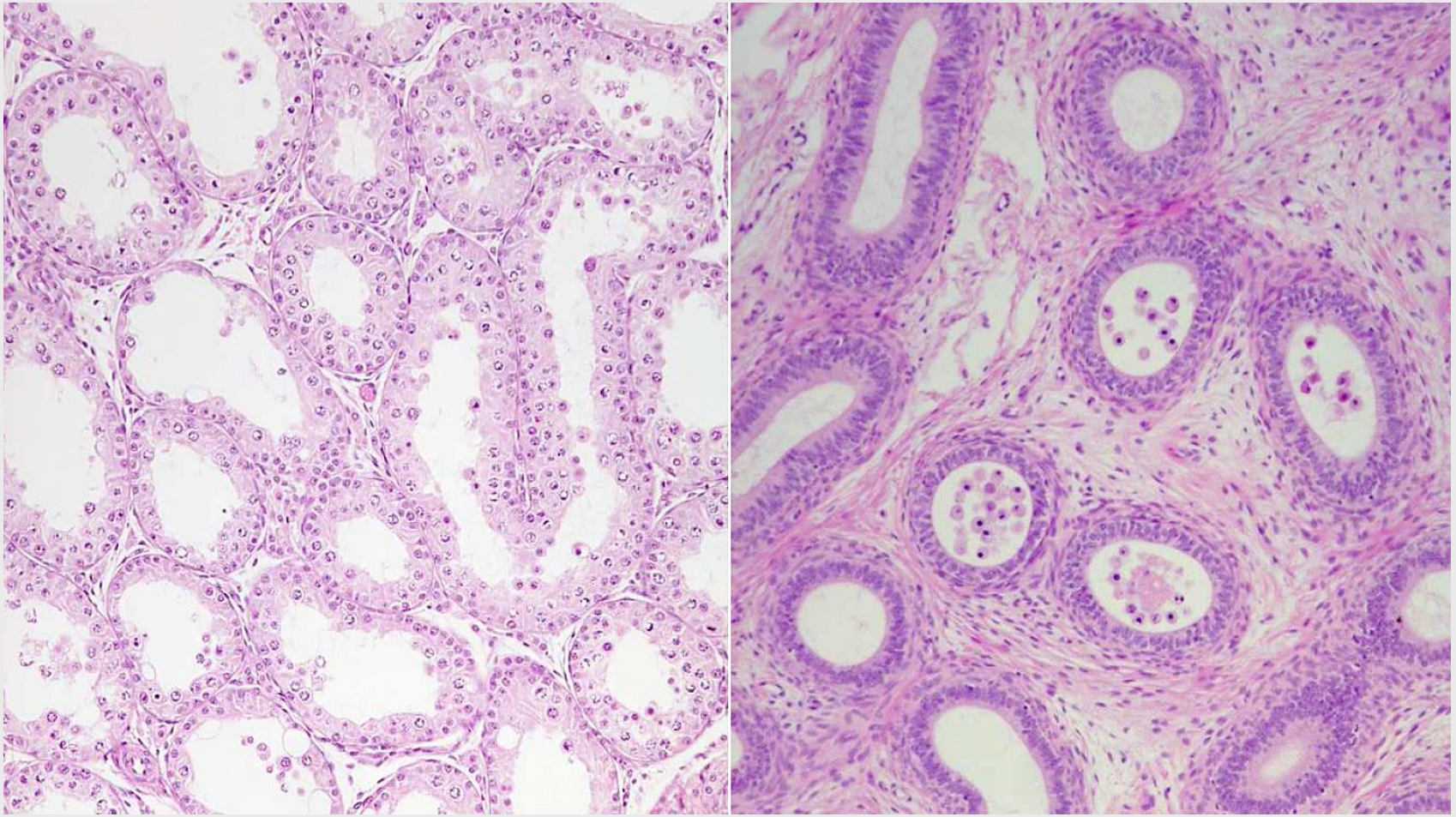
Species	*Regulatory starting age*	*Age of sexual maturity*
Rat	Soon after weaning = 6-7 weeks (after acclimation)	8 – 10 weeks
Mouse	Soon after weaning = 6-7 weeks (after acclimation)	7 – 8 weeks
Dog	4-6 months, max. 9 months	7 – 12 months
Primate	Young adults (often < 3 years)	3.5 – 4.5 years

Use mature animals for specific MR studies

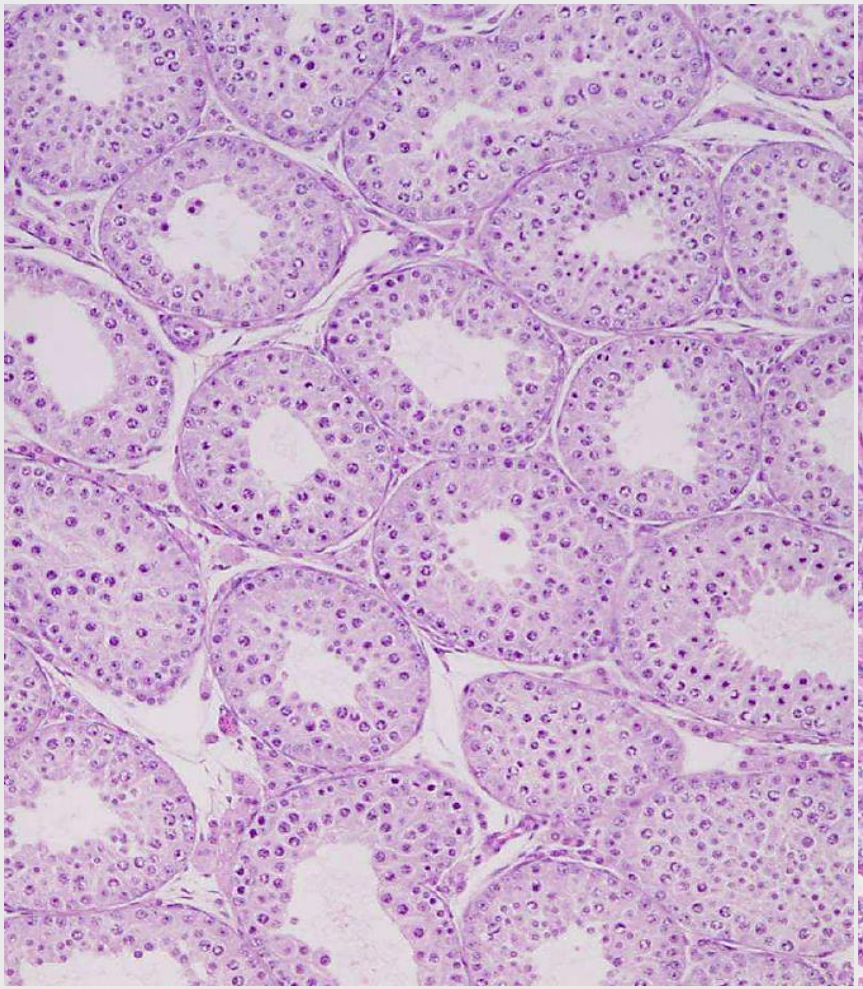
- Immature testes: spermatogenesis absent or incomplete
- Pubertal MR system
  - Testes: often degenerating and sloughing germ cells (GC), giant cells, spermatogenesis focally incomplete
  - Epididymis: sloughed GC, giant cells, reduced sperm content

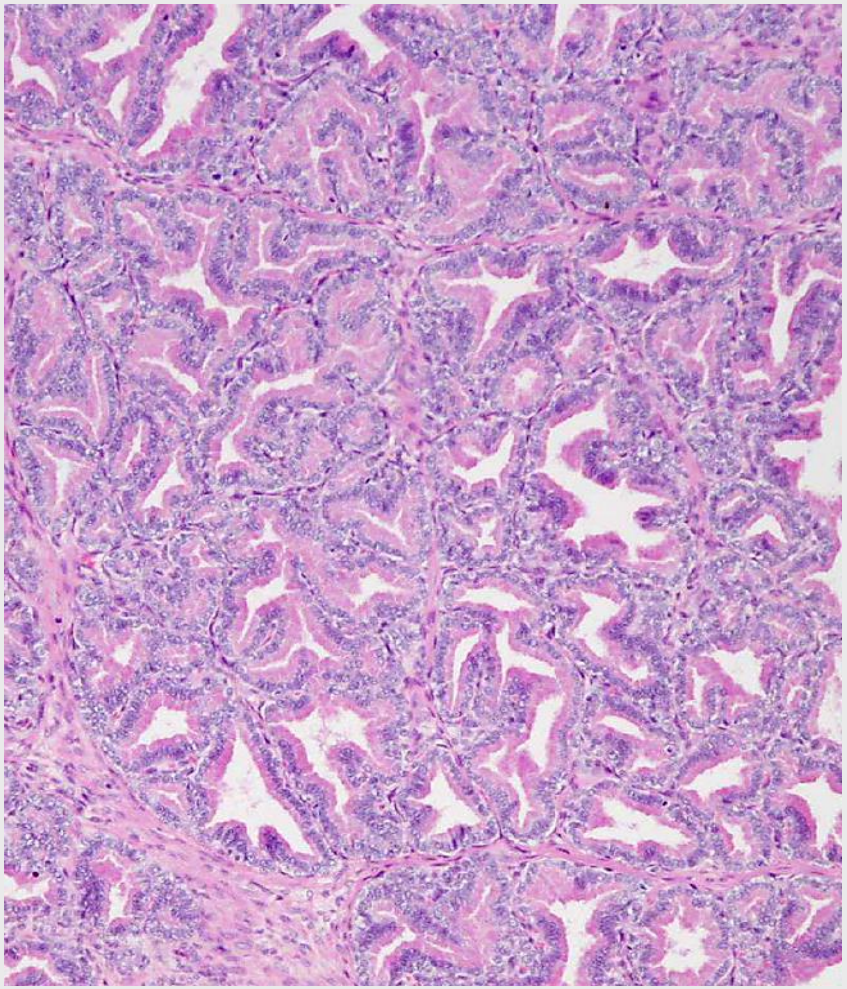
→ *Same picture in case of toxicity!*

Immature spermatogenesis 6-7 months old dog



Immature spermatogenesis
Secreting prostate





C. Evaluation of MR organs

- Guidelines
- Study design
- “Non-anatomic” parameters, especially hormones
- Organ weights
- Tissue preparation
- Histopathological evaluation
- Dealing with unexpected findings
- Conclusions

Hormone measurements

- LH, FSH, PRL, T: technically relatively easy
- *Interpretation complicated* by irregular, diurnal variation and pulsatile release of GnRH, LH and T with 1-2(+) hour intervals and no clear daily pattern
- Age-dependent
- Hormone levels do not provide information on *receptor status*
- Difficult to distinguish
  - Primary effects as relevant for pathogenesis
  - Secondary effects reactive to injury

Sperm evaluation

- Epididymal sperm
  - Obtained
    - After electroejaculation of living animals or
    - From cauda epididymidis at necropsy
  - Parameters
    - Number: Production, variability
    - Quality: Morphology
    - Function: Motility
- Testicular “sperm”
  Count of homogenization resistant spermatids (spt; mainly steps 17-19)
- Evaluation of whole spermatogenic process
  Flowcytometry of testis preparations

C. Evaluation of MR organs

- Guidelines
- Study design
- “Non-anatomic” parameters, especially hormones
- **Organ weights**
- Tissue preparation
- Histopathological evaluation
- Dealing with unexpected findings
- Conclusions

Organ weights

- Testes

  Epididymides
  - In toto
  - Plus possibly cauda (stored sperm) separately

  Accessory sex organs: seminal vesicles and prostates

  Other endocrine organs, in particular pituitary and thyroid
- Absolute (especially testis) and relative weight values
- Organ weights: sensitive indicators of hormonal balance
  In particular, accessory sex organ depending on circulating T levels
  Prerequisite: normal receptor function

C. Evaluation of MR organs

- Guidelines
- Study design
- “Non-anatomic” parameters, especially hormones
- Organ weights
- Tissue preparation
  - Sampling
  - Fixation
- Histopathological evaluation
- Dealing with unexpected findings
- Conclusions

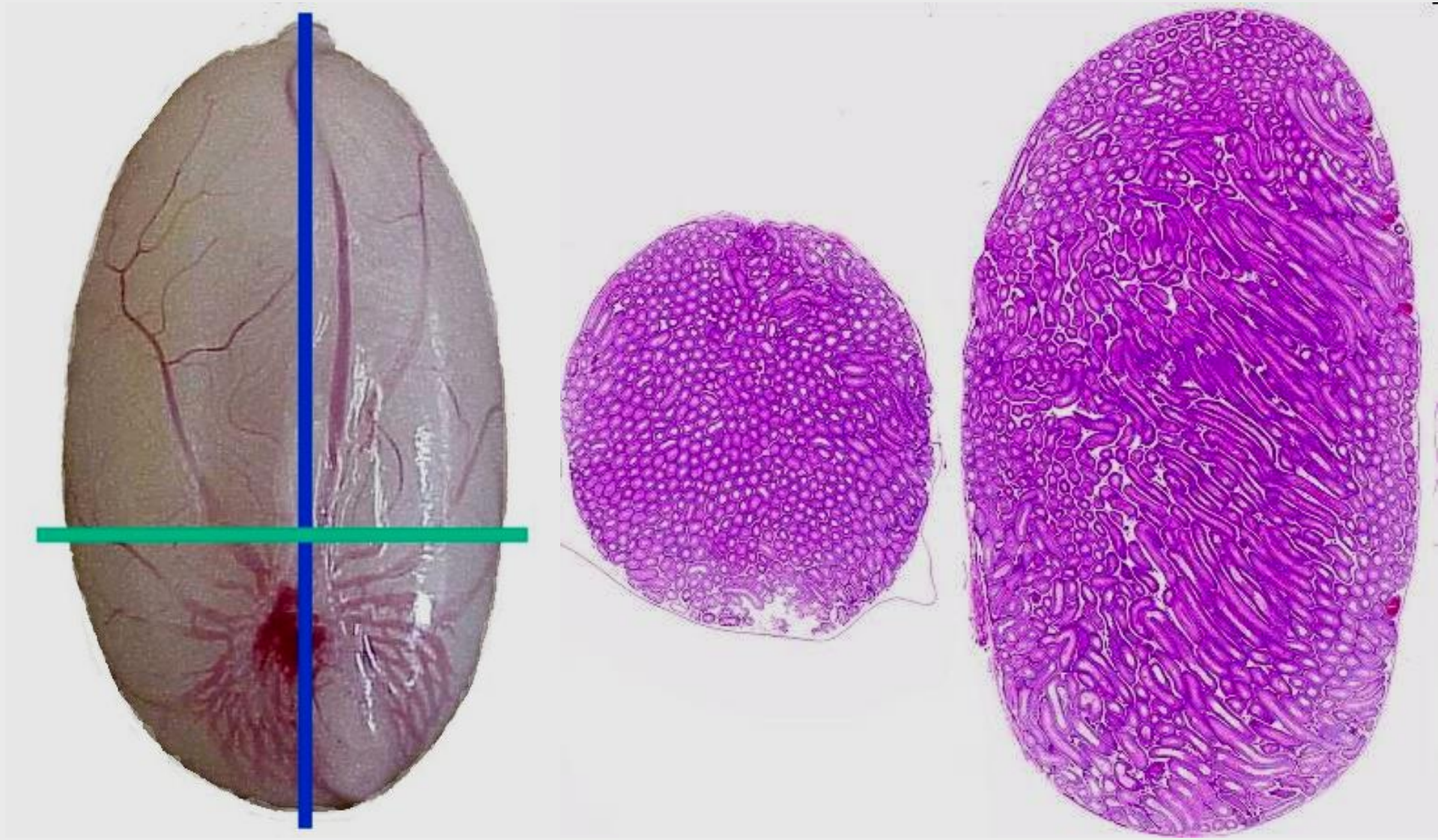
MR System: sampling & trimming

Revised guides for organ sampling and trimming in rats and mice

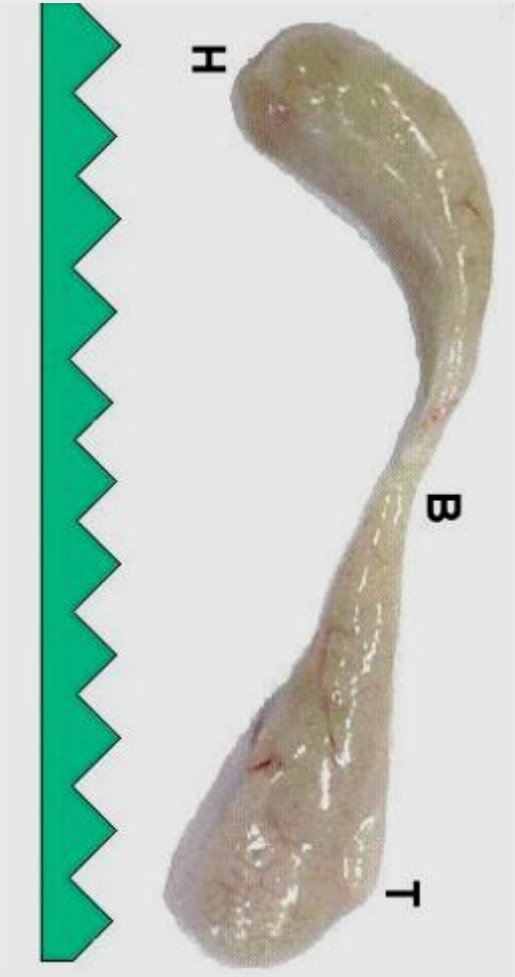
http://reni.item.fraunhofer.de/reni/trimming/
(accessed January 2021)

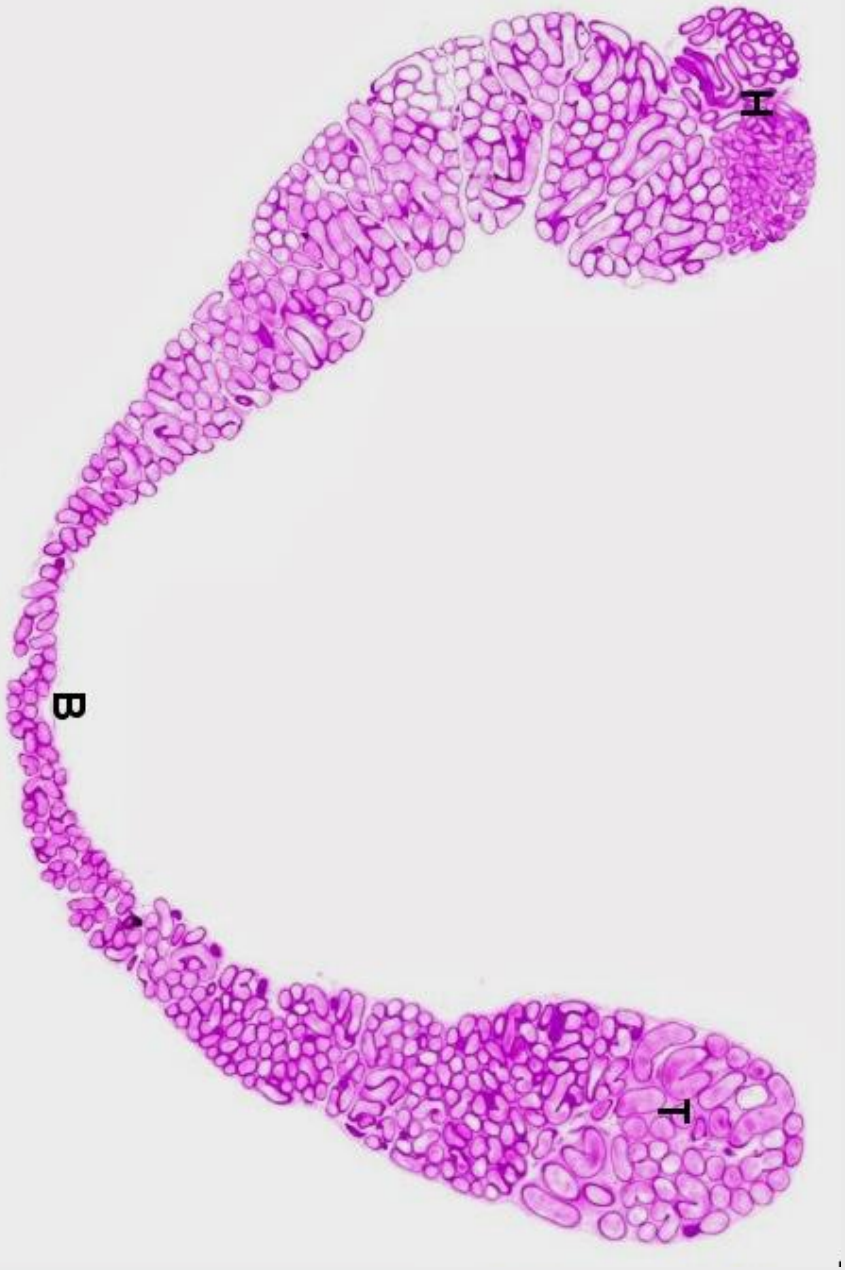
- Ruehl-Fehlert C et al (2003)
  Revised guides for organ sampling and trimming in rats and mice - Part 1.
  Exp Toxicol Pathol **55:** 91–106
- Kittel B, Ruehl-Fehlert C et al (2004) … Part 2.
  Exp Toxicol Pathol **55:** 413–431
- Morawietz G et al (2004) … Part 3.
  Exp Toxicol Pathol **55:** 433–449

Testis including rete

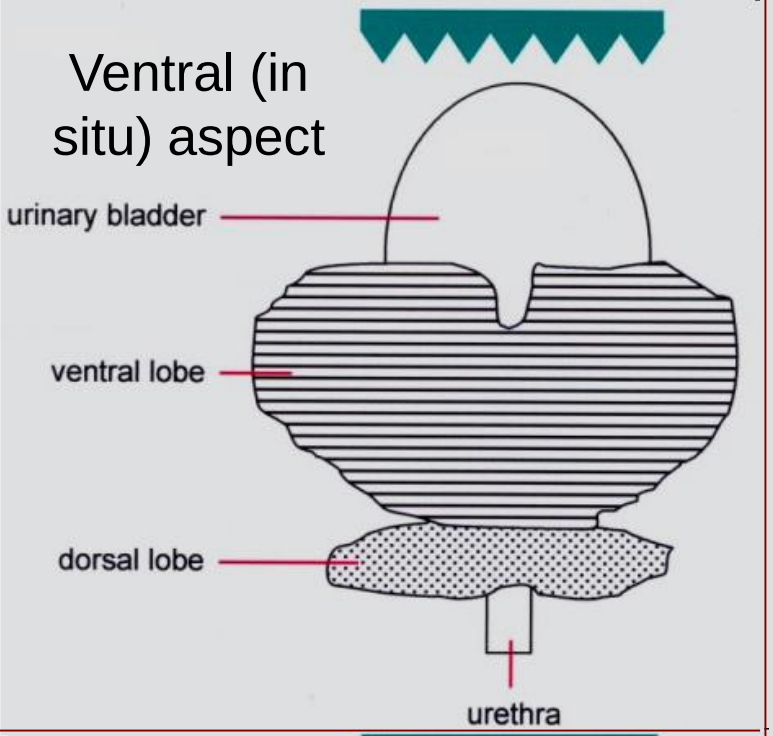


Epididymis

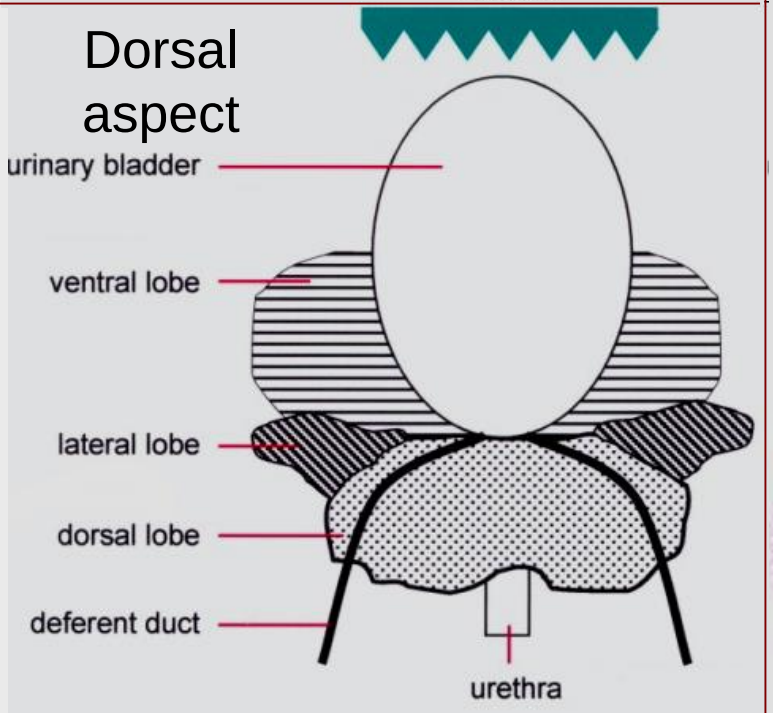




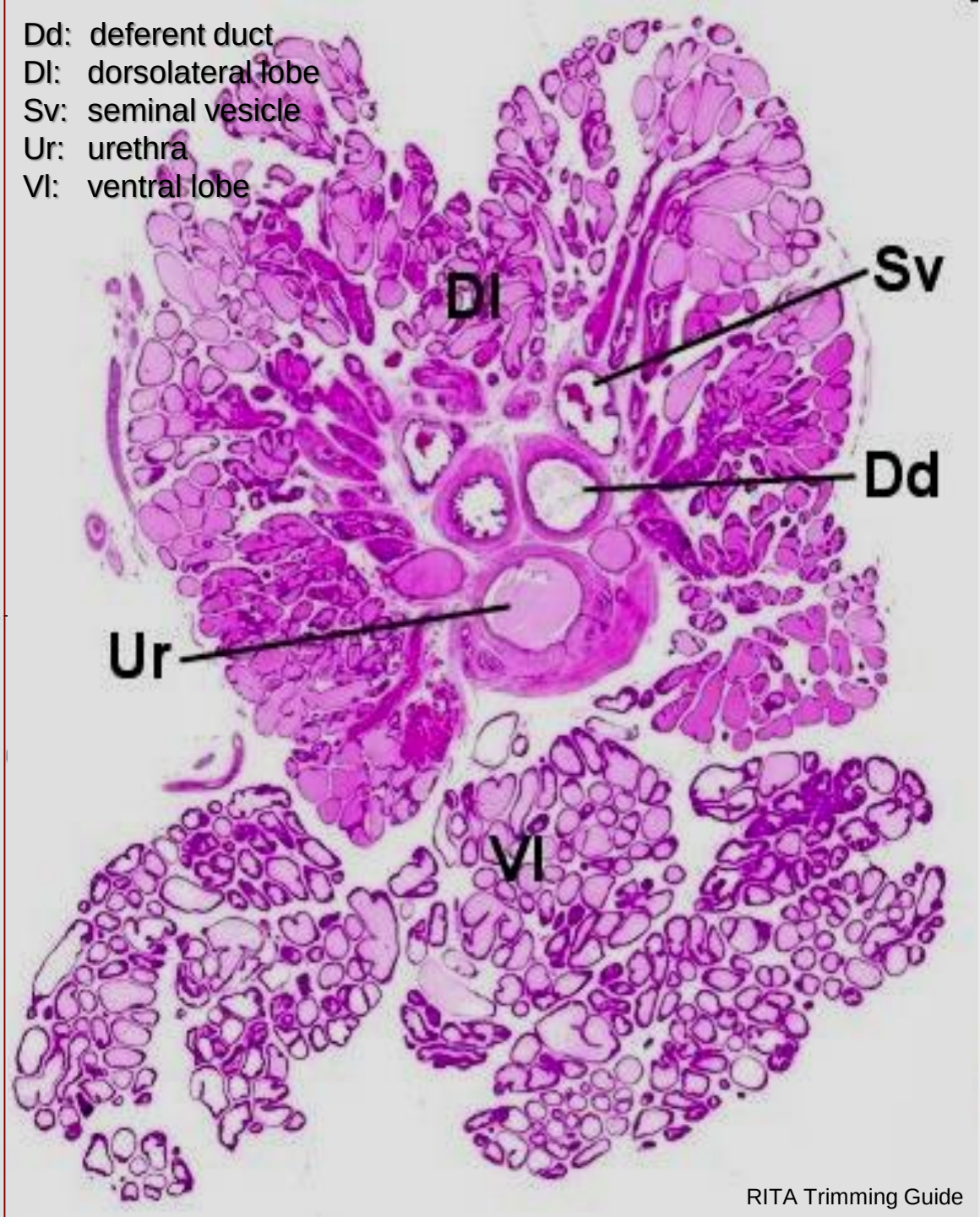
Ventral (in situ) aspect



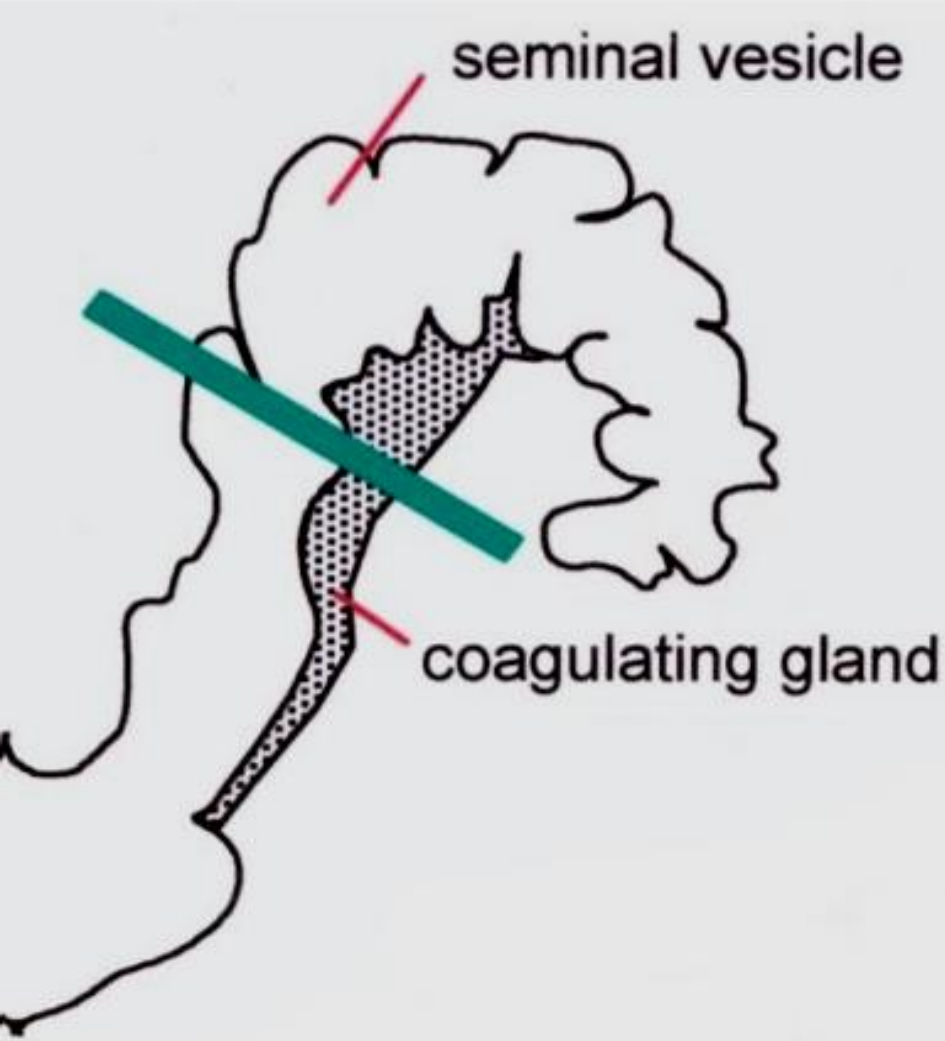
Dorsal aspect

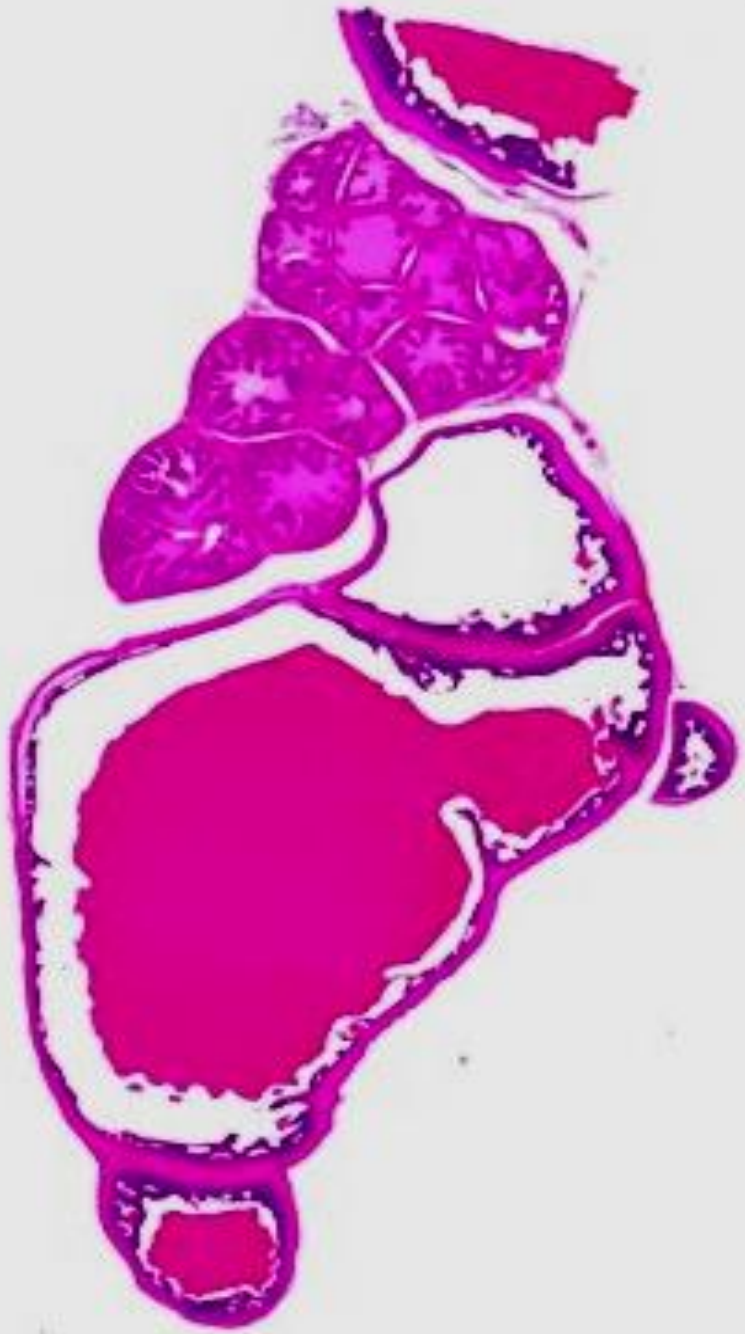


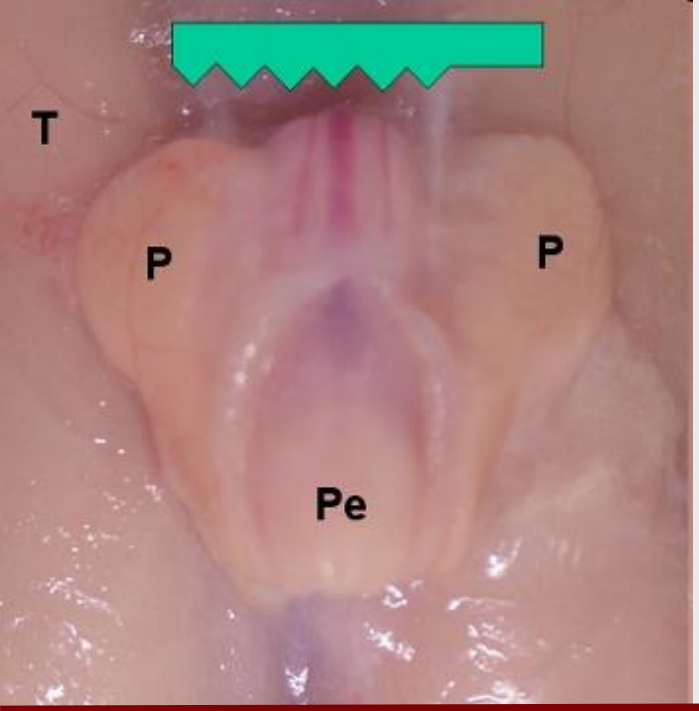
Dd: deferent duct
Dl: dorsolateral lobe
Sv: seminal vesicle
Ur: urethra
Vl: ventral lobe

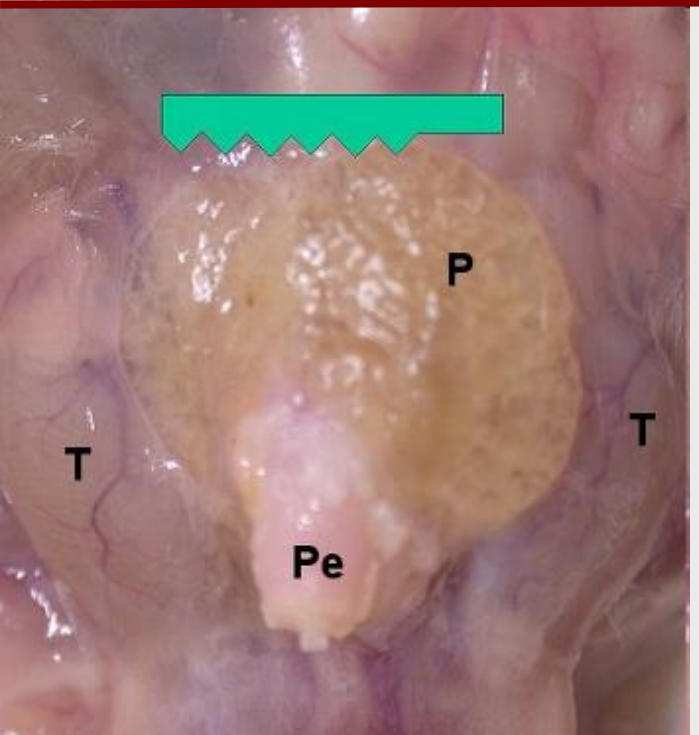


Seminal vesicle
Coagulating gland





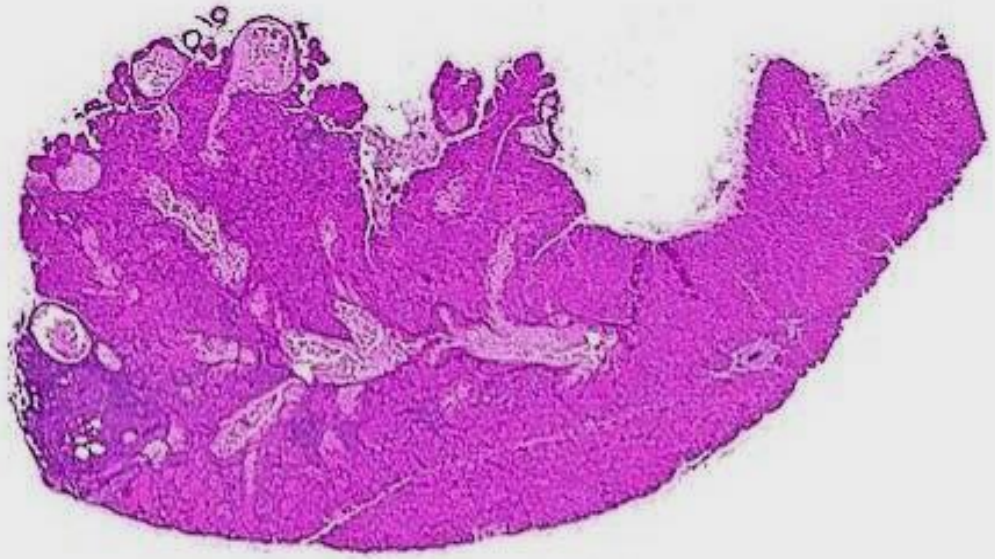


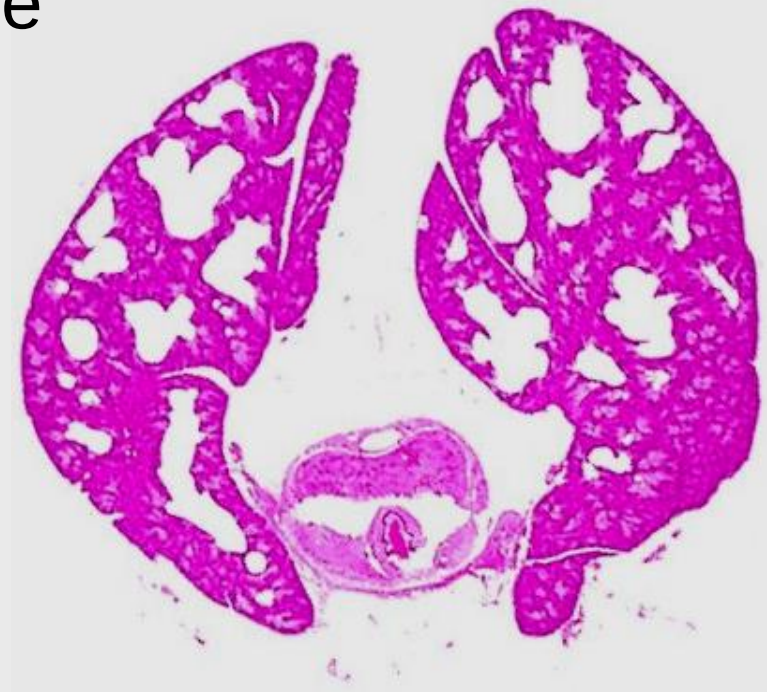


Rat

Mouse

Preputial gland



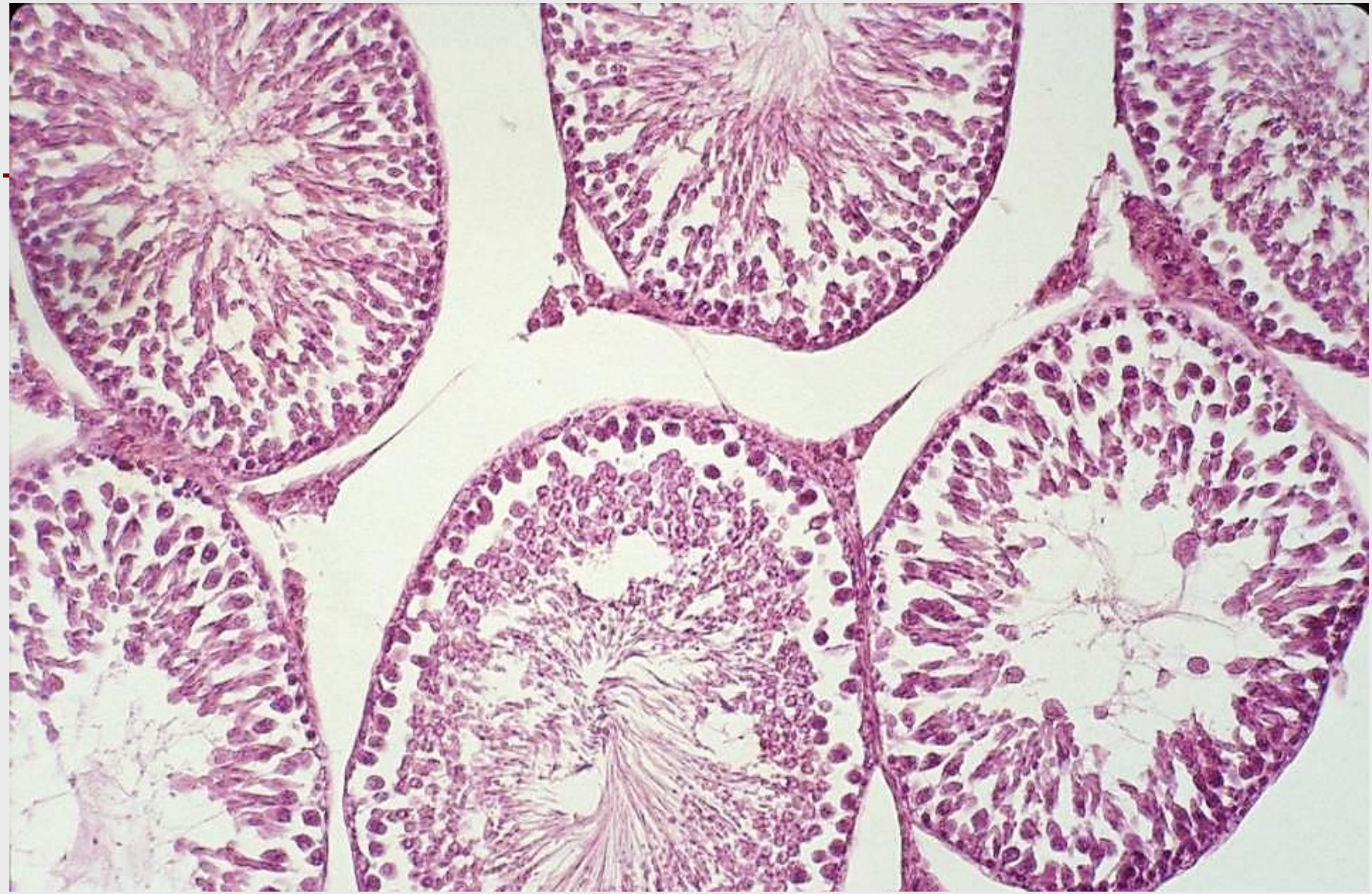


P: preputial gland
T: testis
Pe: penis

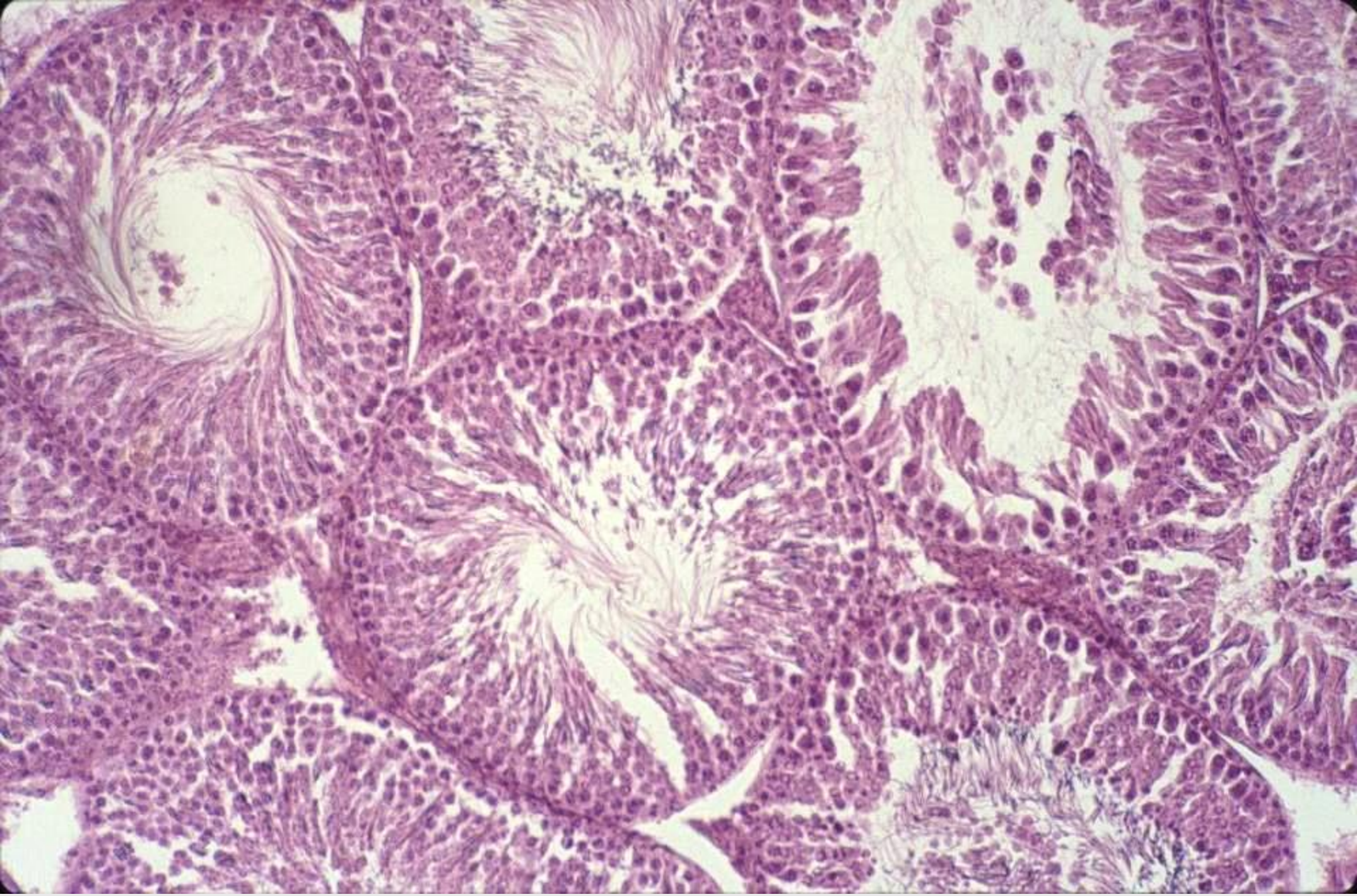
Basic histological methods for testis

Fixative		Embed-ding	Characteristics of sections				Use
Applica-tion	Type		Thick-ness µ	Size	Quality	Stain	
Immersion	Formalin	Paraffin	4-6	Cross-section	(+)	Regular	Discou-raged
	Bouin‘s*				+		Routine
		GMA	2		++	± regular	Special
Perfusion	Bouin‘s**				+++		Special Research
	Glutar-aldehyde	Epon Araldite	<< 1	15 tubules	++++	Toluidine blue***	Research

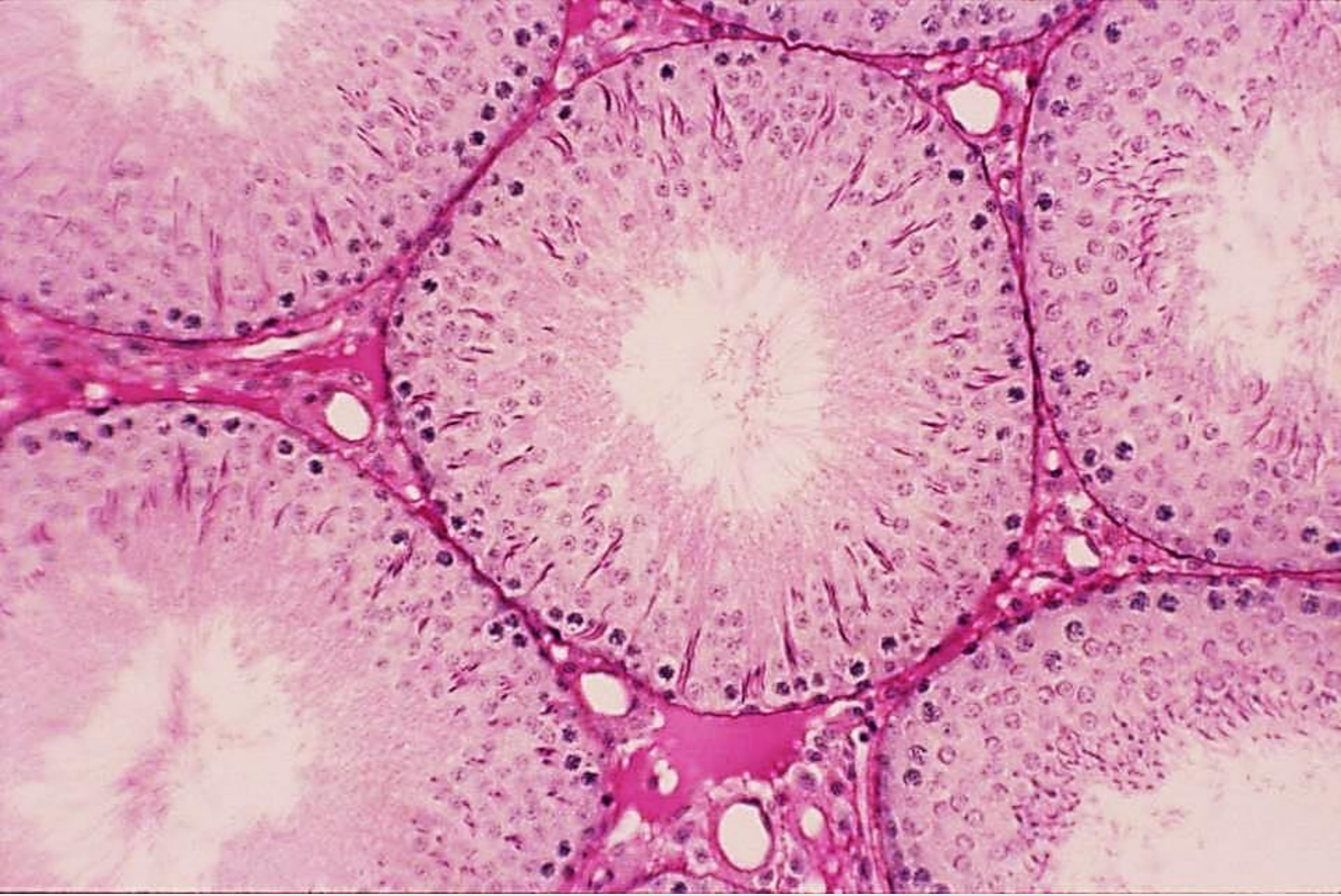
Legend	
	* or Davidson‘, Zenker‘s fluid
	** or mixture of formalin and glutaraldehyde (Karnovski‘s fluid)
	*** or methylene blue
	GMA glycol methacrylate



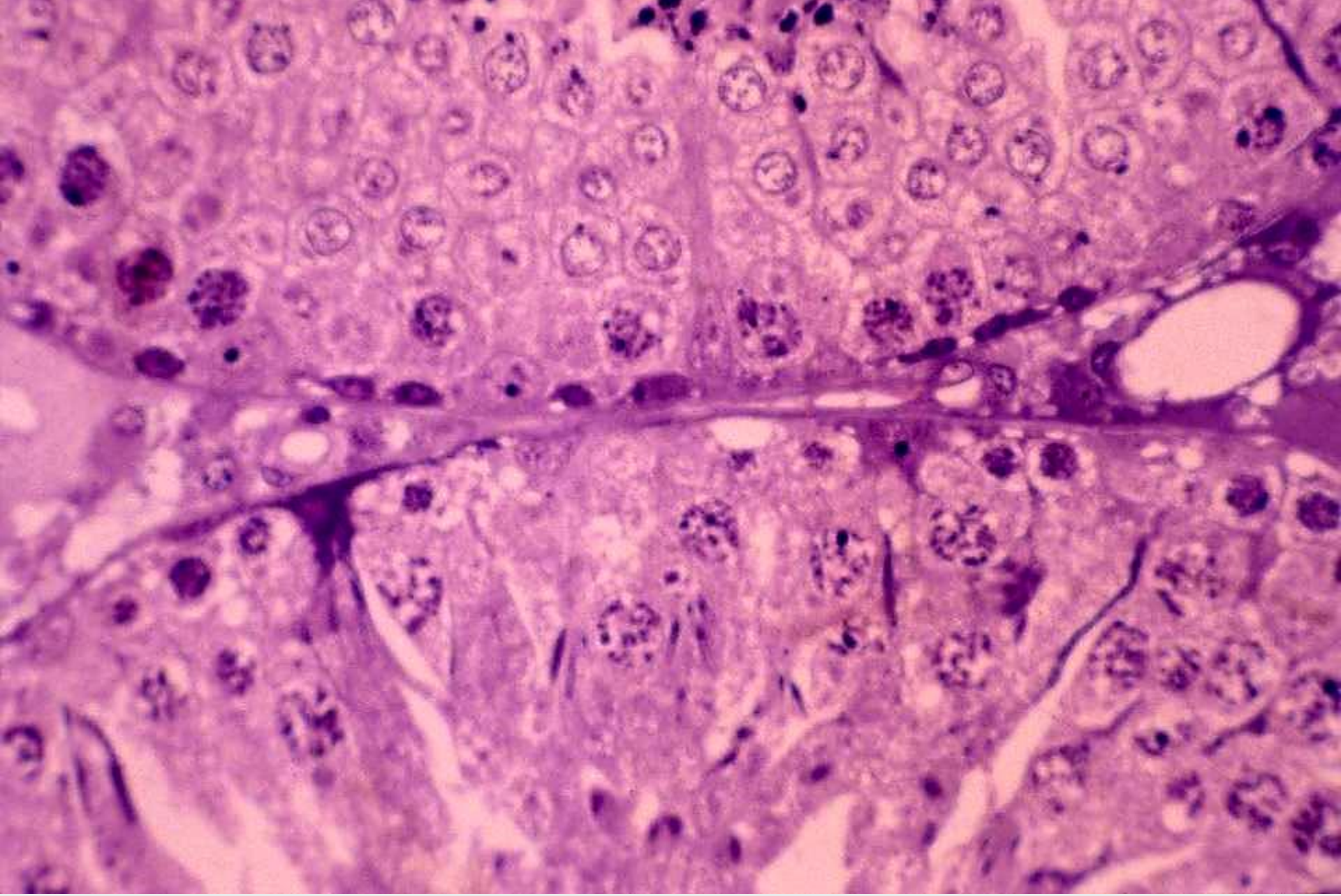
Formalin immersion and paraffin embedding



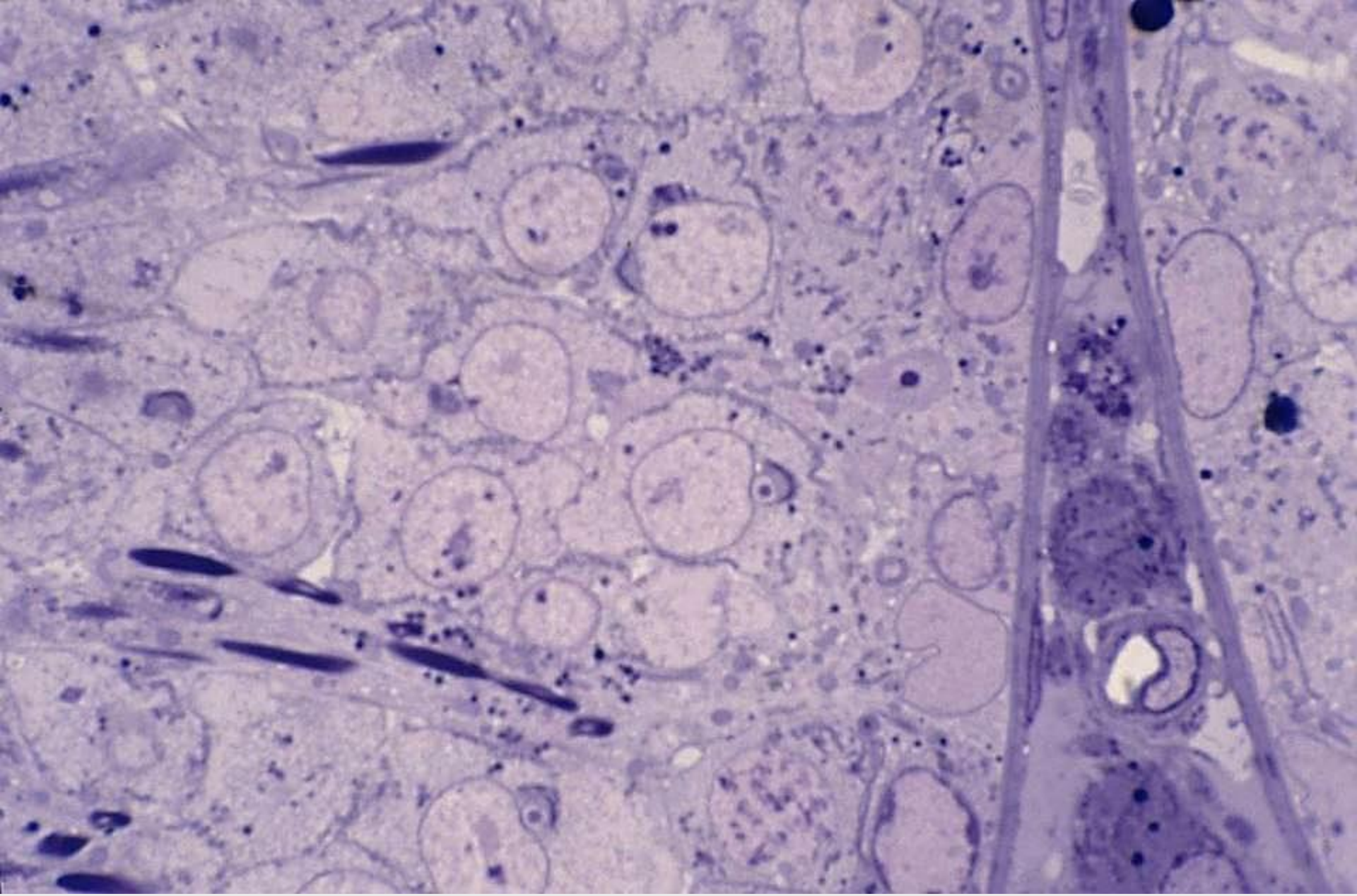
Bouin’s immersion and paraffin embedding



Bouin’s immersion and GMA embedding

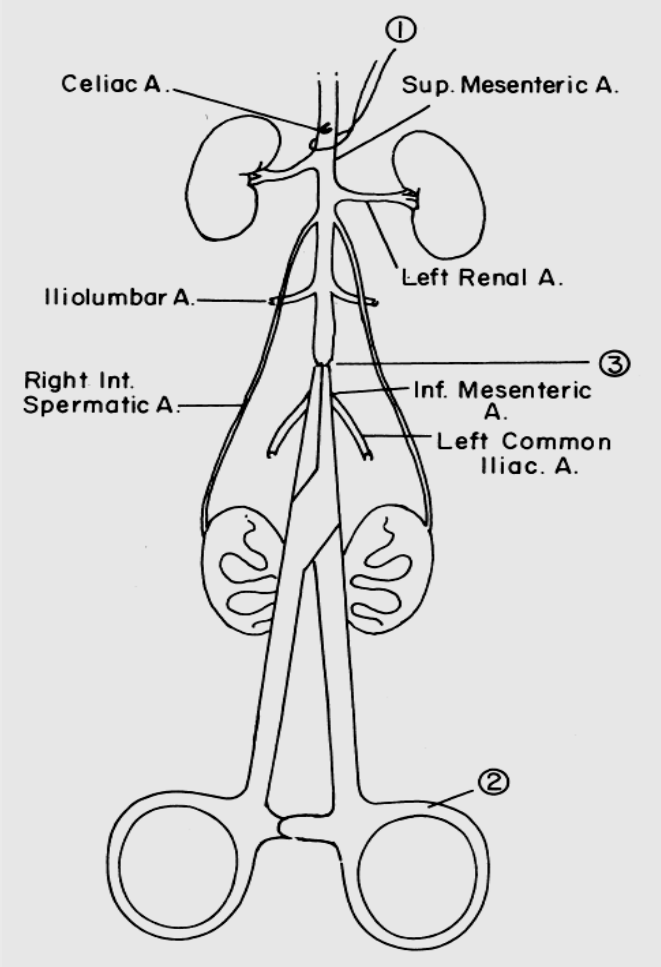


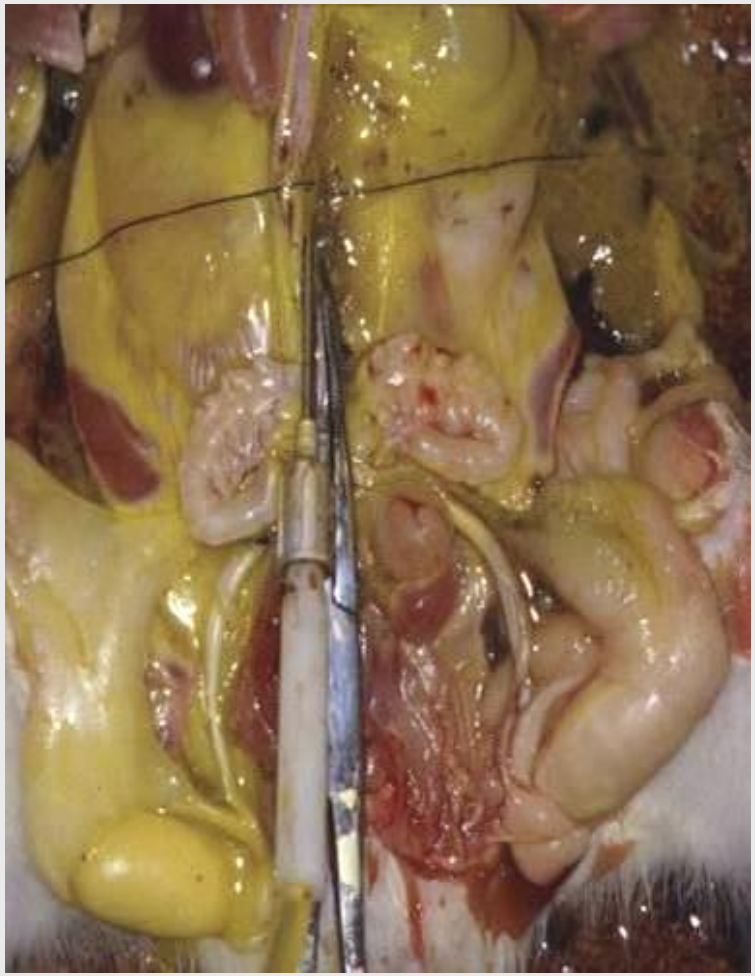
Bouin’s perfusion and GMA embedding



Glutaraldehyde perfusion and epon embedding

Part-body perfusion





C. Evaluation of MR organs

- Guidelines
- Study design
- “Non-anatomic” parameters, especially hormones
- Organ weights
- Tissue preparation
- Histopathological evaluation
  - Qualitative – (semi)quantitative - Staging
  - Primary target
- Dealing with unexpected findings
- Conclusions

Histopathological endpoints – 1

- *Organ weight* as *quantitative* measure often sufficient
- *Semi-quantitative* parameters
  - Tubular *diameter* and size of tubular lumen
  - *Height* of germinal epithelium → “Amount” of GC present
- *Qualitative* and general
  - Architecture of epithelium and interstitium
  - Location of adverse effect: focal, diffuse; partial, generalized; unilateral, bilateral

Histopathological endpoints – 2

Qualitative or semiquantitative

- *Degenerating* cells, in particular GS
- *Vacuolation* in the seminiferous epithelium, often within Sertoli cells (SC)
- Sloughing cells, a consequence of the disruption of SC-GC junctions
- Multinucleated *giant cells*, often a result of unspecific and “mild” toxicity
- Cell associations: staging (see next slides)

Qualitative staging – What for and how

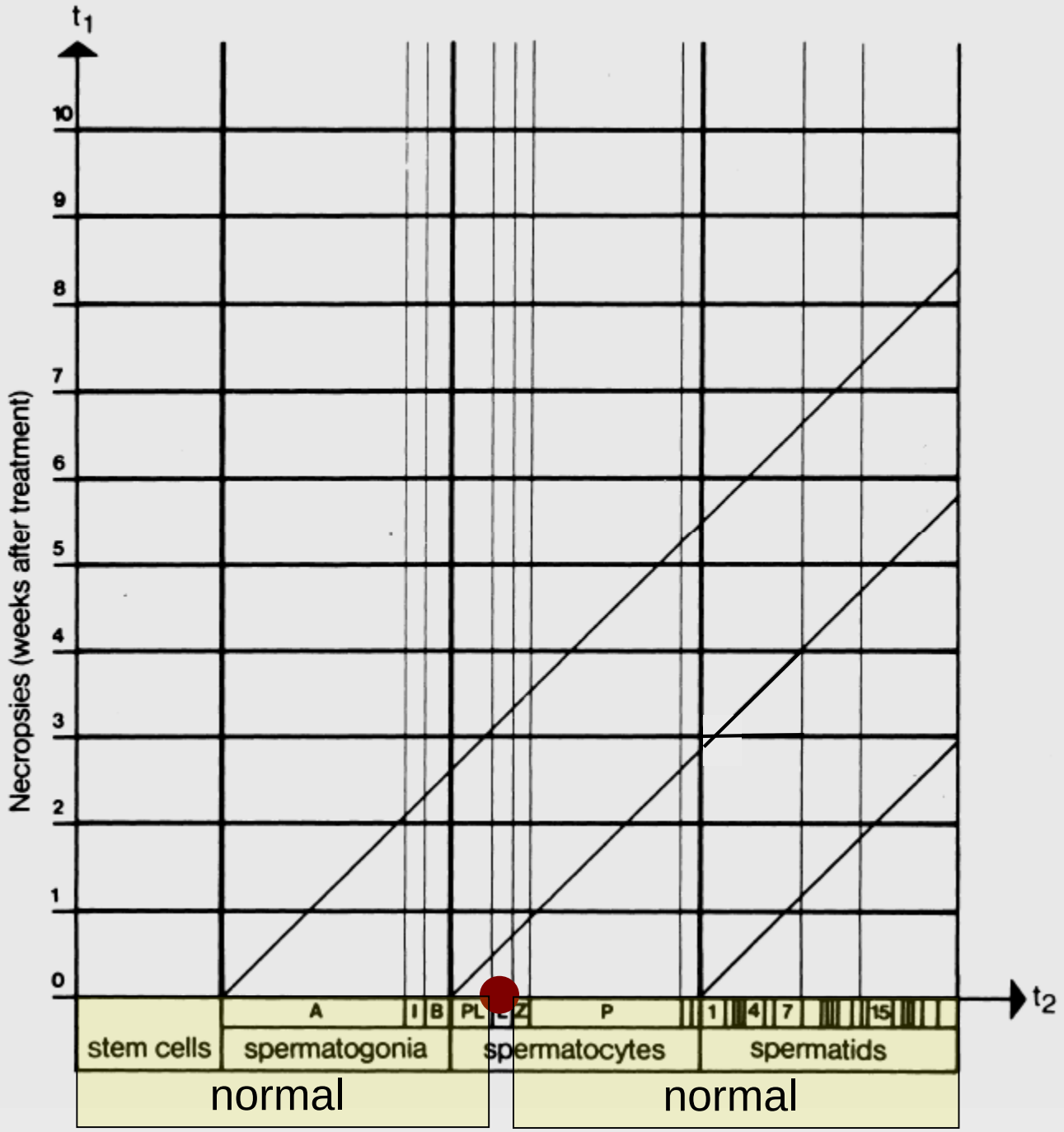
- To classify tubules for spermatogenic cycle, mainly
  - Development of spermiogenesis (spt)
    - Acrosome
    - Elongation and condensation of spt head
  - Occurrence of meiosis
- Particularly important for short studies up to 28 days
- Stain for acrosome:
  - PAS (counterstain with hematoxylin)
    Particularly for studies ≤ 4 weeks
    Dogs and non-human primates: acrosomes clearly visible only around spermiation
  - Also H&E allows approximate staging

Qualitative staging – Objectives

- *Missing* GC
- GC present inappropriately, e.g. *retained* elongated spt in stages XI – XI
- GC at *wrong location*, e.g. elongated spt e.g. in stage IX at basis of seminiferous epithelium (→ phagocytosis mainly in stage XII)
- GC with *abnormal morphology*
  - In general, e.g. malformation
  - For stage e.g. retardation of acrosome development

On day 0, treatment with compound X starts.

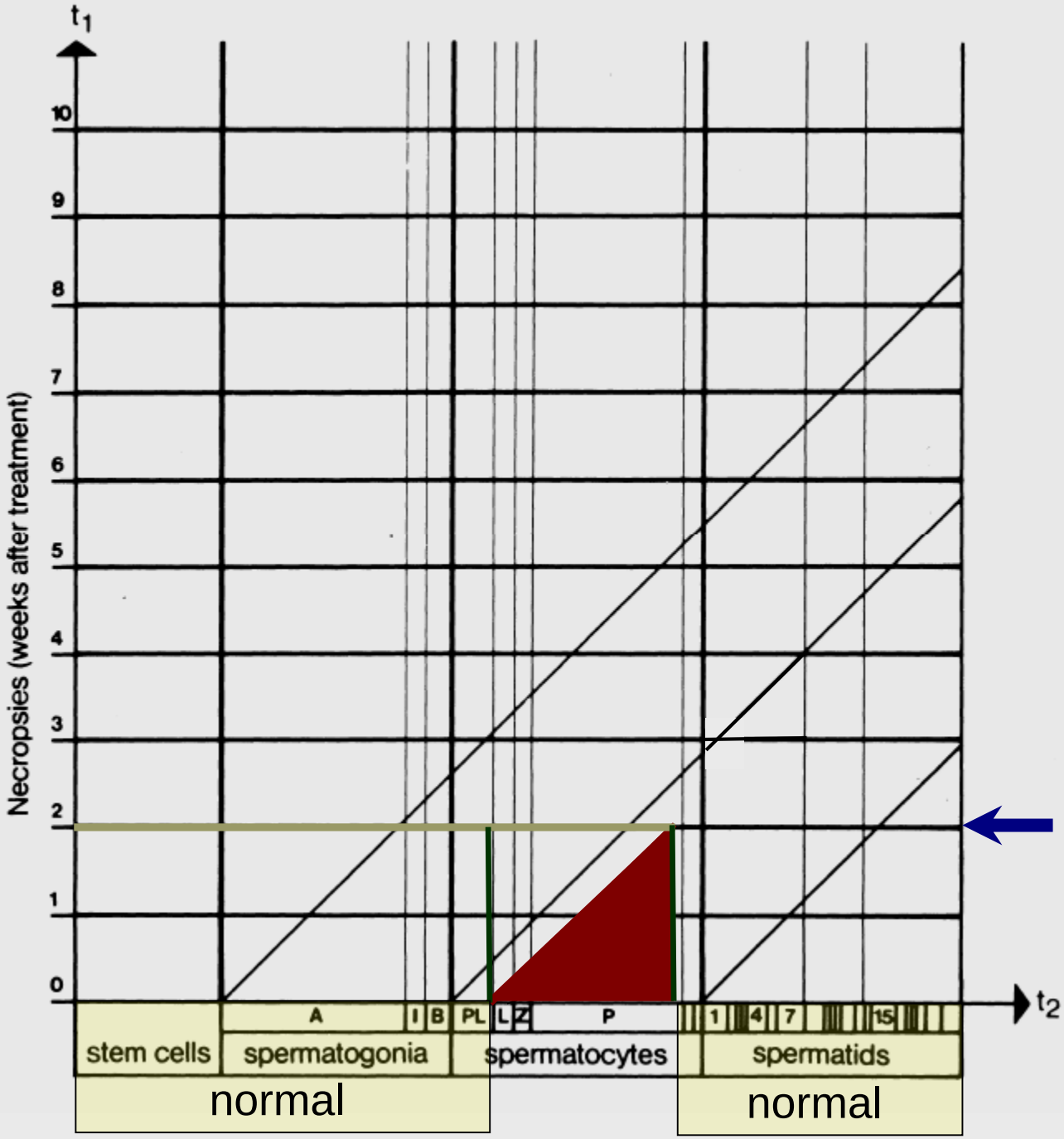
Assumption: compound X damages selectively leptotene spermatocytes (spc)



t1 and t2 axis are at same scale

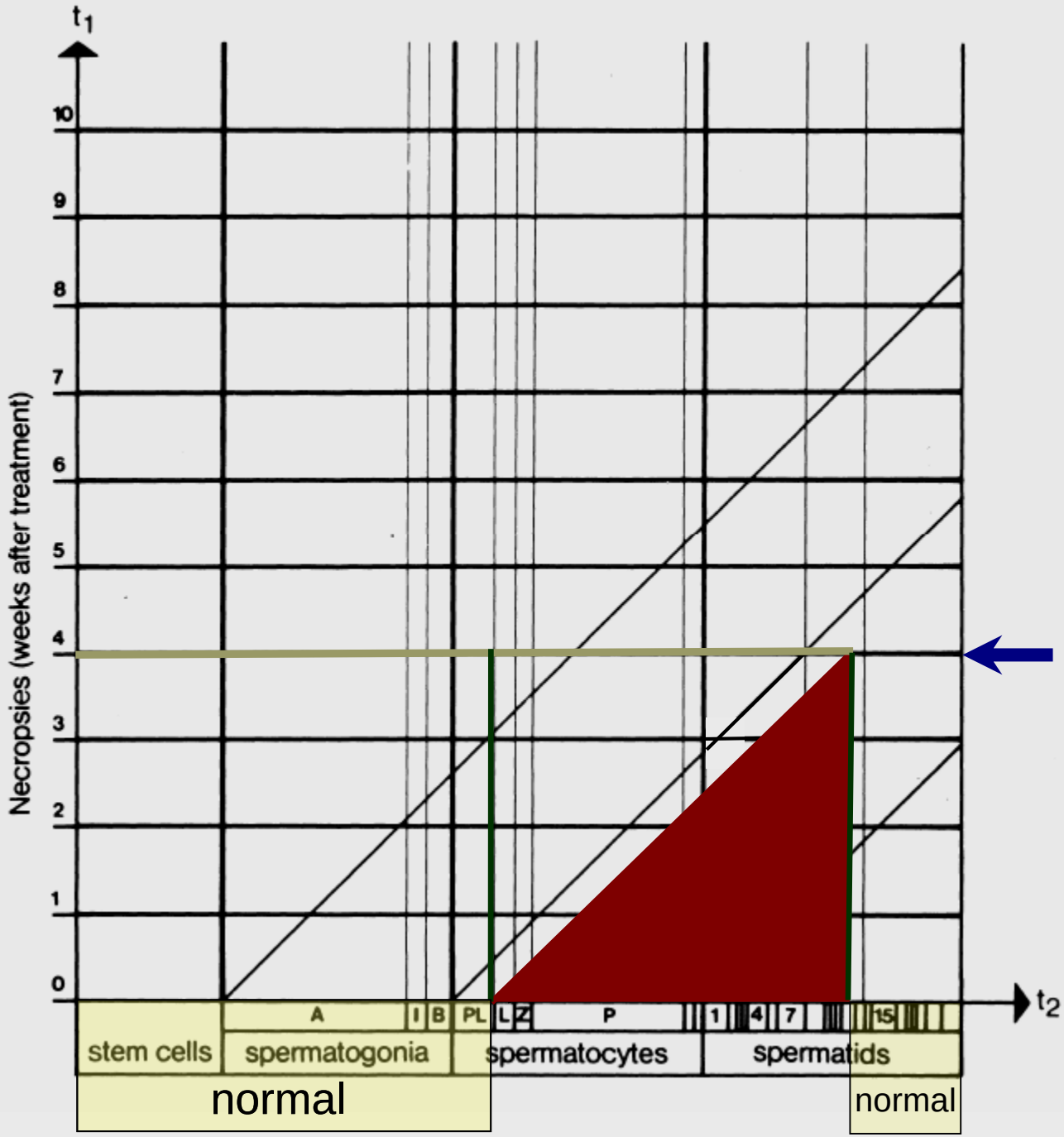
2 weeks of continuous treatment with compound X result in loss also of zygotene and most pachytene spc:

Depletion by maturation → gap



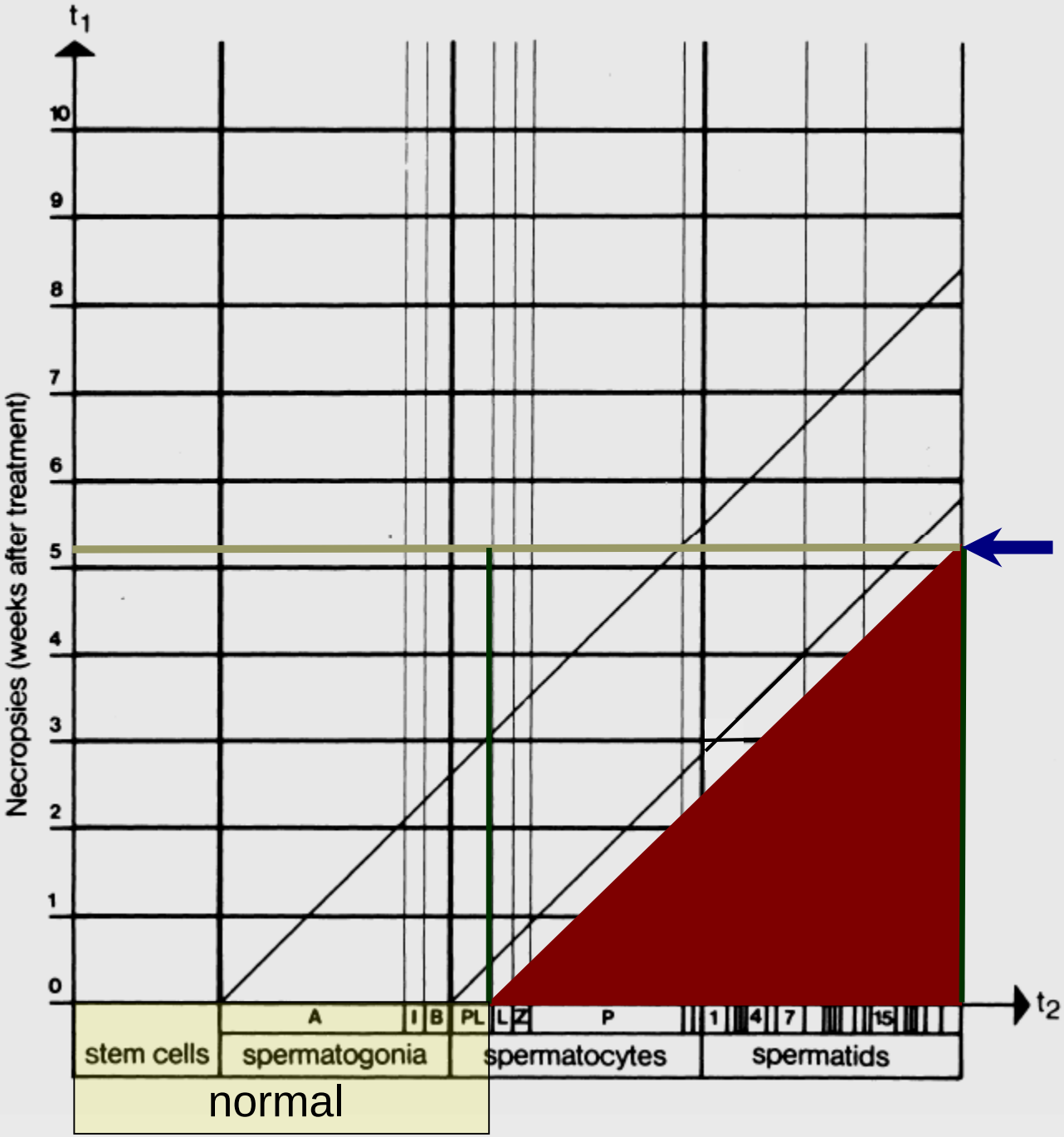
4 weeks of continuous treatment with compound X result in loss also of zygotene and most pachytene spc:

Depletion by maturation → gap



5 weeks and 2 days of continuous treatment with compound X result in loss of all GC older than pre-leptotene spc:

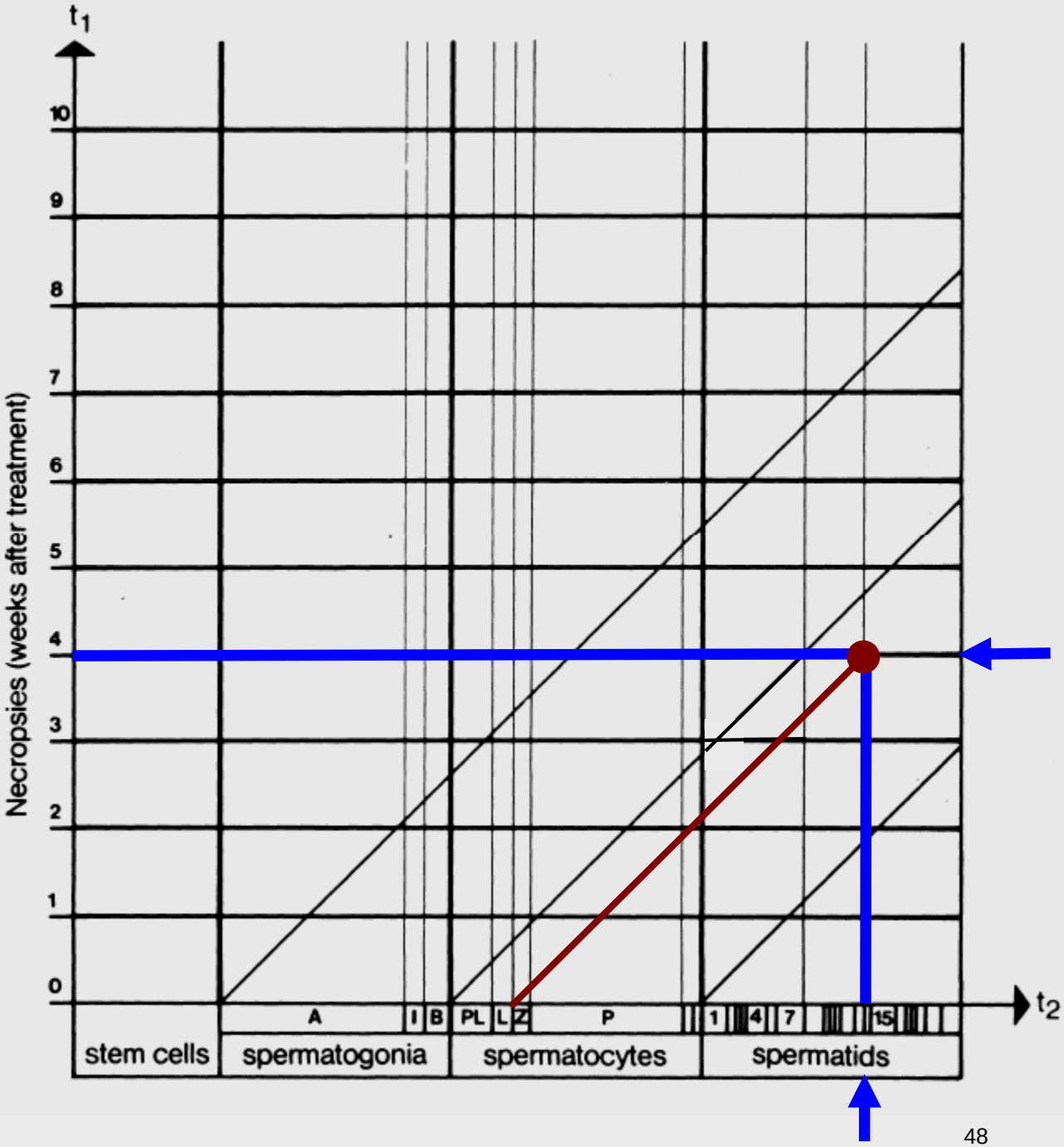
Spermatogenic "arrest"



Following single dose or short-term treatment:

Damaged or missing GC type e.g. at 4 week necropsy allows to identify the primary damaged cell by extrapolating backwards

t1 and t2 axis are at same scale



Quantitative histopathological endpoints

- Tubular or luminal *diameter* measured
- *Number* of GC per cross section (e.g. in stage I or VII/VIII)
  - Total GC
  - Specific GC type
    - Relatively easy: spc and round spt
    - Difficult: elongated spt (small diameter) and spg subpopulations (difficult to differentiate)
    - Possibly using PCNA* labeling for proliferating spg
  - Relative numbers e.g. per SC nuclei

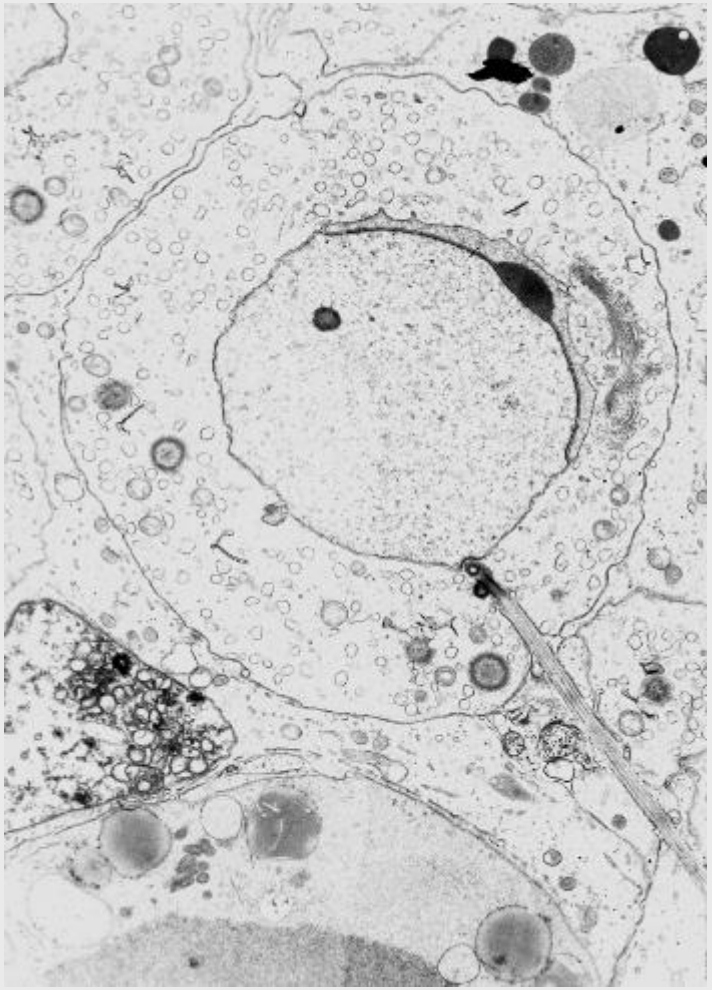
    Absolute numbers

  Standardization especially of section thickness!

* Proliferating cell nuclear antigen

Ultrastructural investigations

- Many samples / sections might be needed to find a suitable one because of the complex testicular structure and the many different elements
- Special tool to trace early changes



For an method overview see also:

Society of Toxicologic Pathology position paper

Recommended approaches for the evaluation of testicular and epididymal toxicity

Lynda L. Lancing et al

Toxicol Pathol 30/4: 507-520, 2002

C. Evaluation of MR organs

- Guidelines
- Study design
- “Non-anatomic” parameters, especially hormones
- Organ weights
- Tissue preparation
- Histopathological evaluation
- Dealing with unexpected findings
  - Review of hazard identification
  - Characterization of finding
  - Risk evaluation
  - Risk management
- Conclusions

Review articles on trouble shooting

Review: Successful Drug Development Despite Adverse Preclinical Findings

Part 1: Processes to Address Issues and Most Important Findings
Robert A. Ettlin, Junji Kuroda, Stephanie Plassmann, and David E. Prentice
*J Toxicol Pathol 2010; **23**: 189–211*

Part 2: Examples
Robert A. Ettlin, Junji Kuroda, Stephanie Plassmann, Makoto Hayashi, and David E. Prentice
*J Toxicol Pathol 2010; **23**: 213–234*
http://www.jstage.jst.go.jp/browse/tox/23/4/_contents

1. Review of hazard identification

- Is there *indeed* an adverse effect?
  "Spontaneous" alterations, particularly in non-rodents (low number of animals per test group!)
- What else is *known* about the drug in question?
- Were there *other* relevant findings?
  System approach
- Is the study technically *valid?*
  (Im)maturity of reproductive system?
- Is the *model* valid?
  Cave species with seasonal variations of spermatogenesis etc.
- Were there other *modifying* factors?
  E.g. endocrine effects

2. Characterization of finding

- *Review* of older studies for subtle changes
- *NOAEL*
- *Target* cells
- Time to toxicity and *reversibility*
- *ADME*, including e.g. accumulation in MR organs

→ Hypothesis regarding mode(s) of action (MoA)

- To (dis)prove potential MoA
  - *Additional investigations* on available material
  - Additional *tailor-made studies* (“trouble-shooting”), possibly with serial necropsies for time-course investigation (early lesions are generally more specific)

3. Risk evaluation

- Important factors
  - Species specificity
  - Safety ratio
  - Reversibility
  - Monitorable in man before permanent damage
  - Intended use, market situation
  - etc.

  → Overall weight of evidence

- In principle
  - Any preclinical MR toxicity is of concern
  - Unless proven otherwise (difficult), preclinical MR toxicity considered to be relevant for man

4. Management

- Monitoring of patients for early changes
  Limited choices of methods, e.g.
  - Sperm analysis
  - Hormonal assays
  - (Pregnancy, offspring)

  → Ultimate proof regarding human risk
- Other measures such as limitations of the use of a new drug

Conclusions Topic C: Methods – 1

- For a general assessment of the MR system *standard subacute* toxicity studies are sufficient
  (plus conventional reproductive toxicity studies for function and genotoxicity studies for genotoxicity)
  - *Holistic* approach, combining the evaluation of multiple parameters
  - Keep in mind that animals are *often sexually immature* at start of study
- For trouble shooting studies consider
  - *Time course* investigations (primary target cell)
  - *Hormone* measurements
  - A standard 4-week recovery period is generally not sufficient

Conclusions Topic C: Methods – 2

- In general terms, there is *no best species*
- *Organ weights* are important quantitative parameters
- *Tissue preparation* is particularly important:
  - Standardized sampling
  - Improved fixation (formalin is not sufficient!)
  - Paraffin sections are generally sufficient
- *Qualitative staging* is a must. PAS-H staining helps
- For the assessment of unexpected adverse findings in the MR system follow general procedures, but as always take a case-by-case approach

Lecture 2: Practice

C Recommended approaches for evaluation of MR organs (general methods)

D Morphologic evaluation of MR organs

- General toxicity
  - Objectives
  - Background lesions
  - Germ cell toxicity
  - Sertoli cell toxicity
  - Leydig cell toxicity
  - Testicular necrosis
  - Epididymal toxicity
- Endocrine disruption

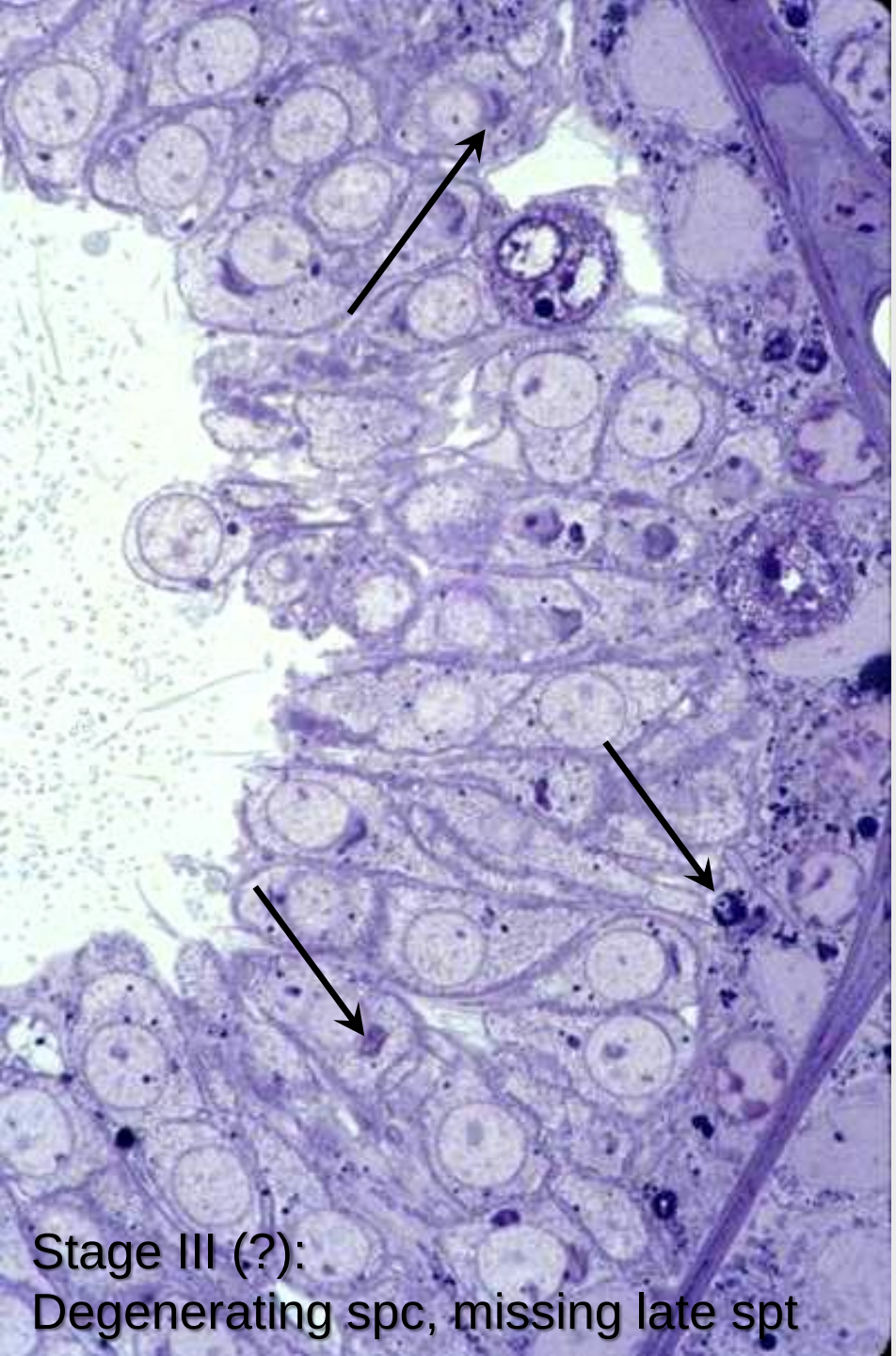
Morphologic evaluation of the testis

- *When* is lesion observed
- What *cells* are first affected
- Morphological *pattern* including other organs → *MoA*
- *Progression* and maximal response
- *Reversibility* (partial/complete) and by when

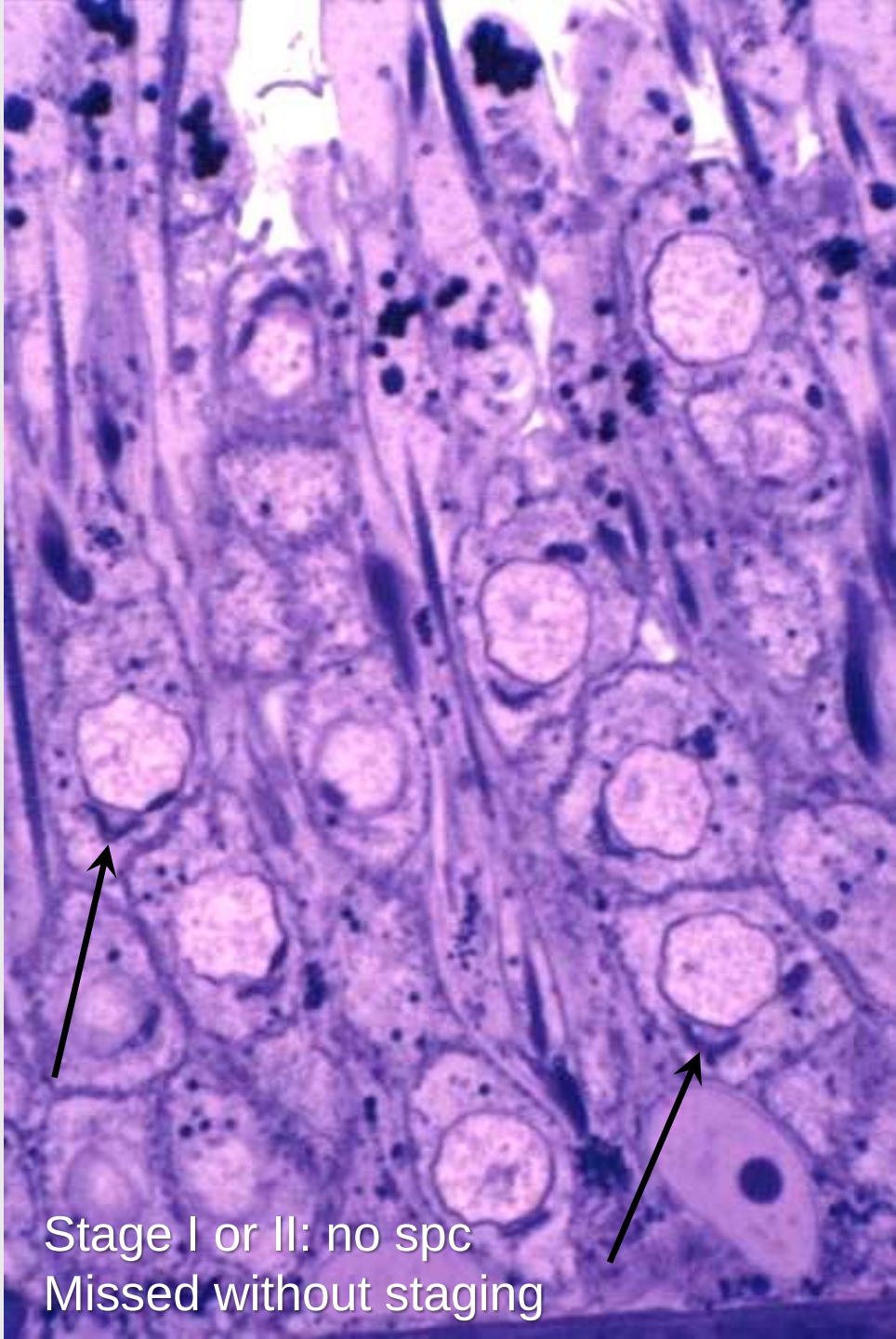
Beagle dog - Background lesions

Finding	Incidence %	Severity
Decreased spermatogenesis	30	Mild to severe (> 6 to most tubules)
Tubular atrophy or hypoplasia	30	1-2 areas per testis
Multinucleated giant cells	98	Average of 5 affected tubules
Apoptotic GC	Low	Irrespective of cell type and stage
Spt retention	12	Occasional tubules

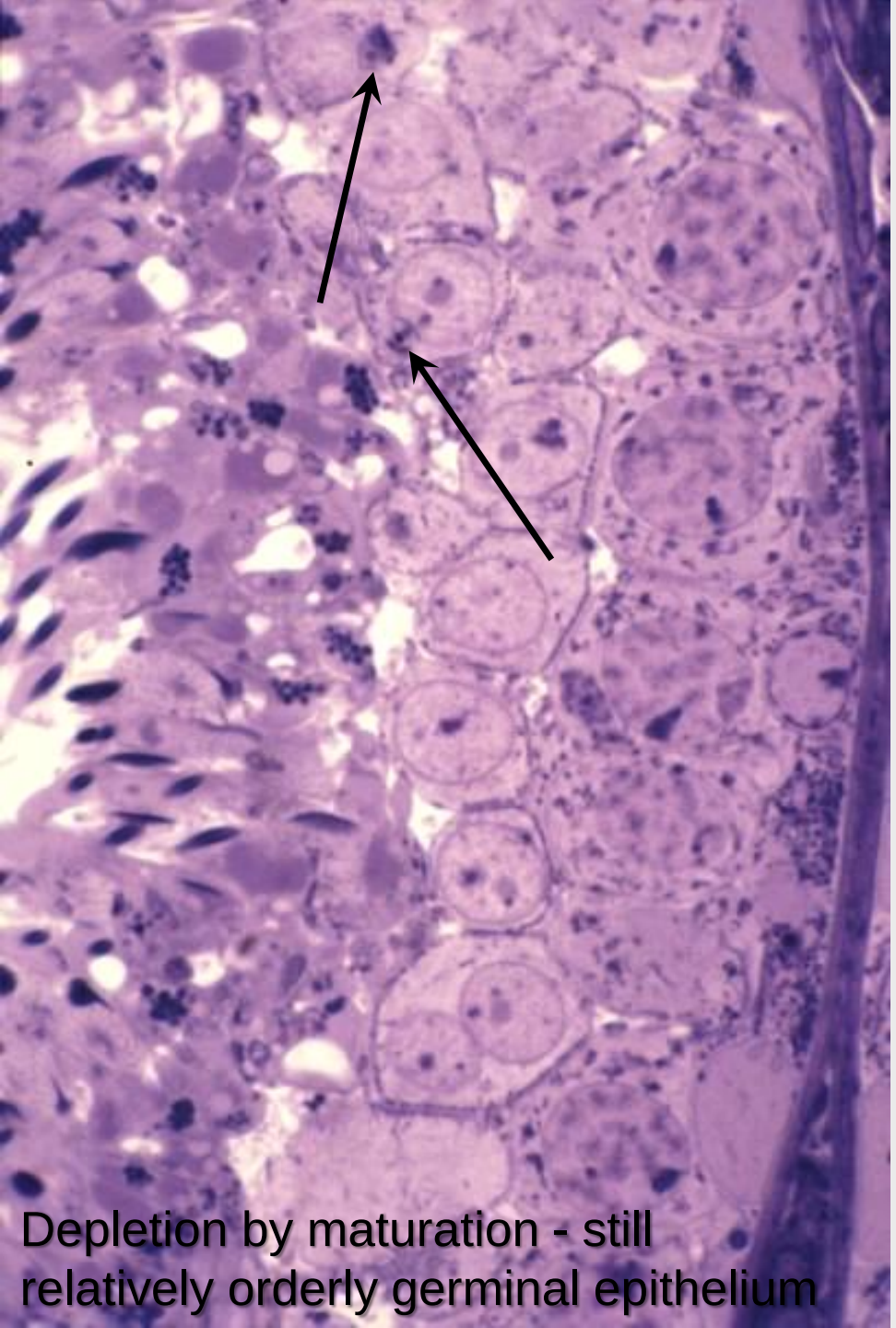
S. Rehm. Spontaneous testicular lesions in purpose-bread beagle dogs. Toxicol Pathol 28: 782-7, 2000



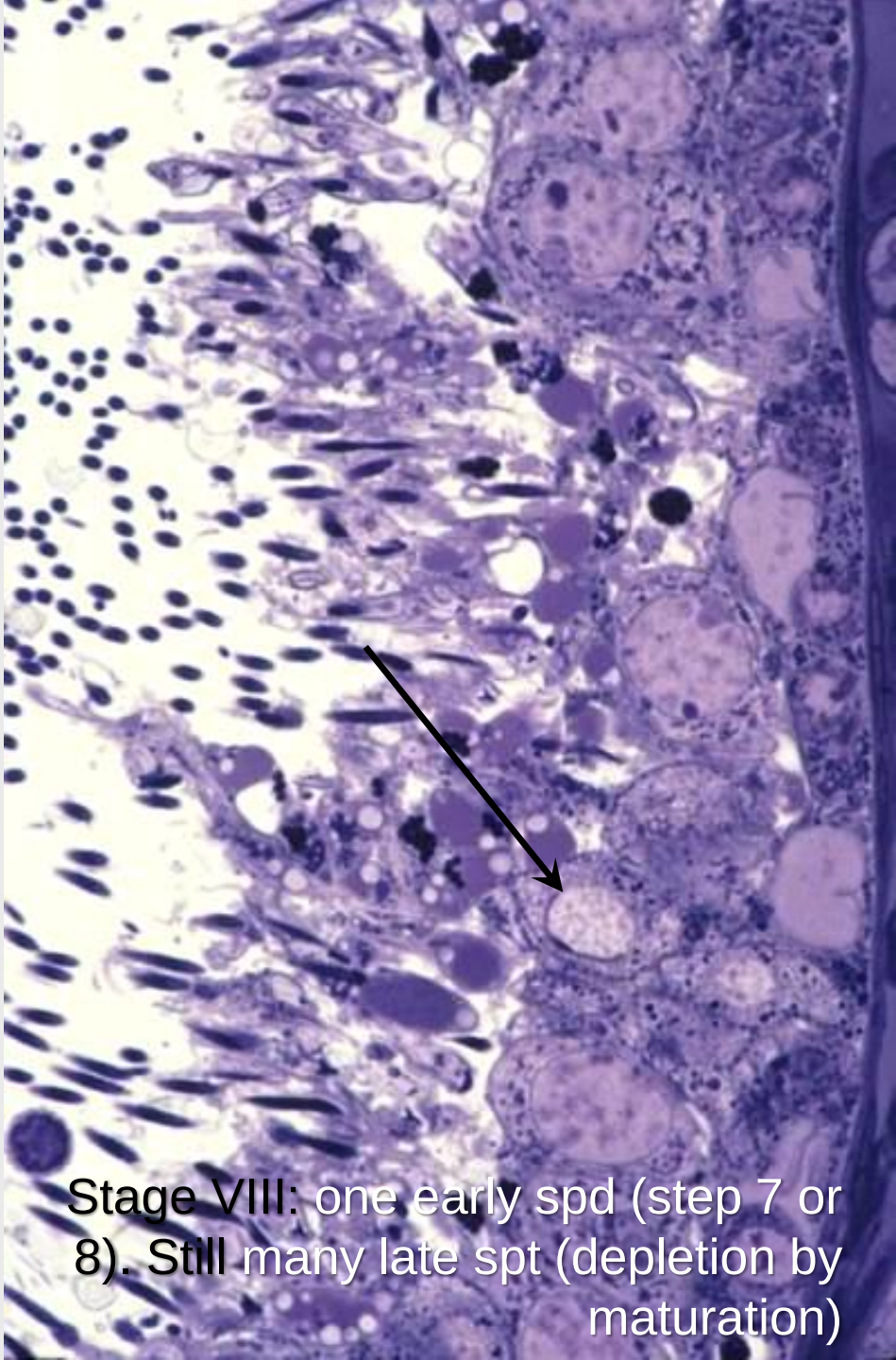
Stage III (?):
Degenerating spc, missing late spt



Stage I or II: no spc
Missed without staging



Depletion by maturation - still relatively orderly germinal epithelium



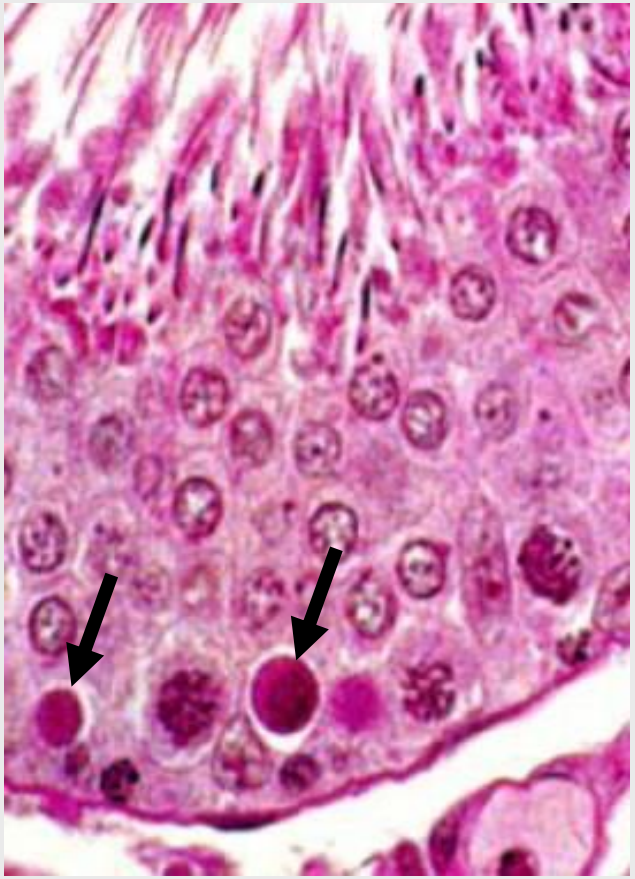
Stage VIII: one early spd (step 7 or 8). Still many late spt (depletion by maturation)

Germ cell (GC) toxicity – Early signs – 1

Within hours

- GC death, generally by apoptosis
  - Rapid
  - No inflammation
  - Rapid phagocytosis by SC

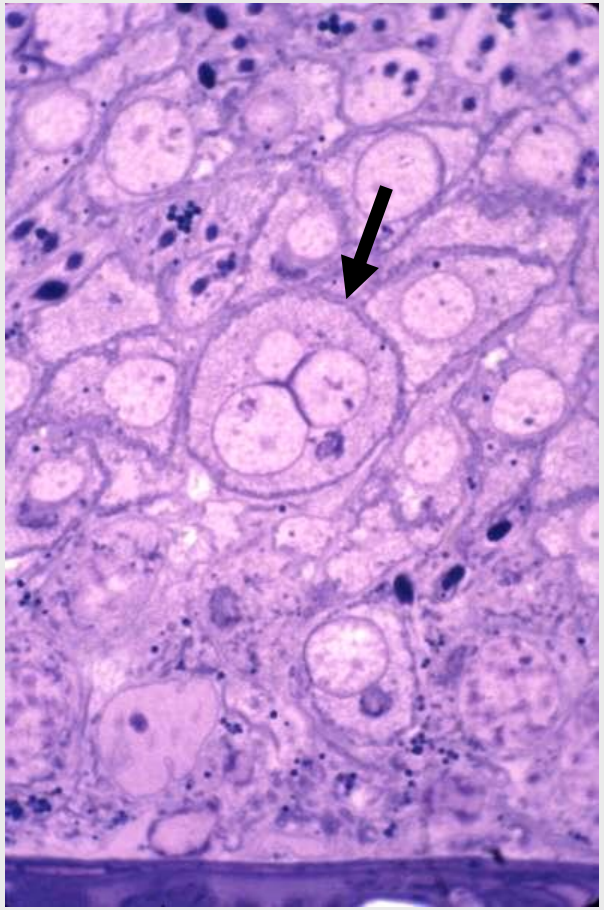
    All GC may disappear within 24-48 hours
- Most vulnerable:
  - late/early stage, such as
    - Spg A stages XI-I
    - Spc in meiosis stage XIV
  - Mid stage
    - Mid pachytene spc stage VII
    - Step 7 and 19 spt in stage VII



Germ cell (GC) toxicity – Early signs – 2

Spt

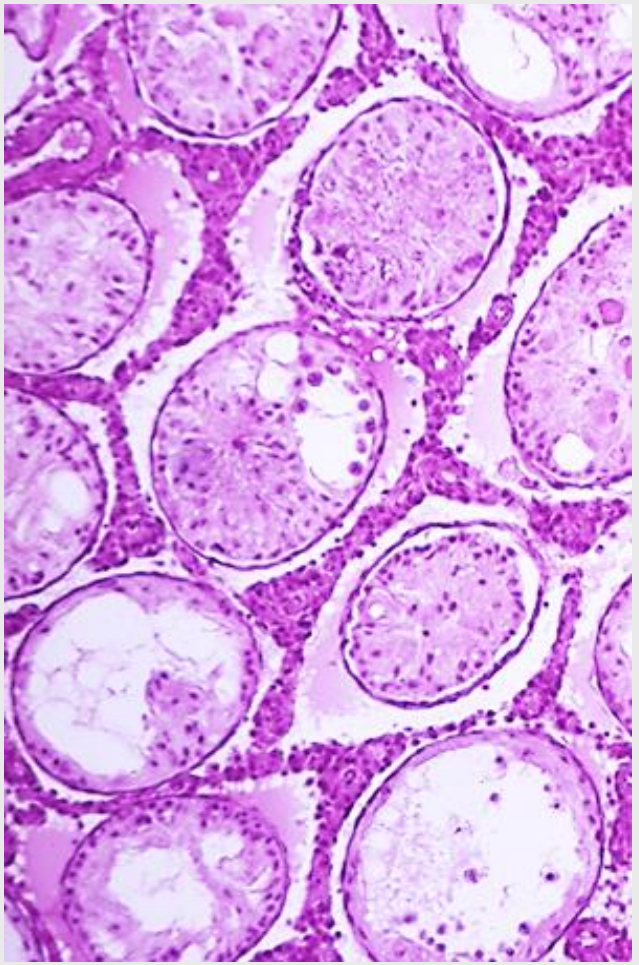
- Early spt form multinucleated *giant cells**: fusion of syncytial cell groups, often with fused nuclear acrosome
- Late spt are *not released* but move to basal portion of tubule: spermatid retention in stages VIII to XII, an early sign of testicular toxicity

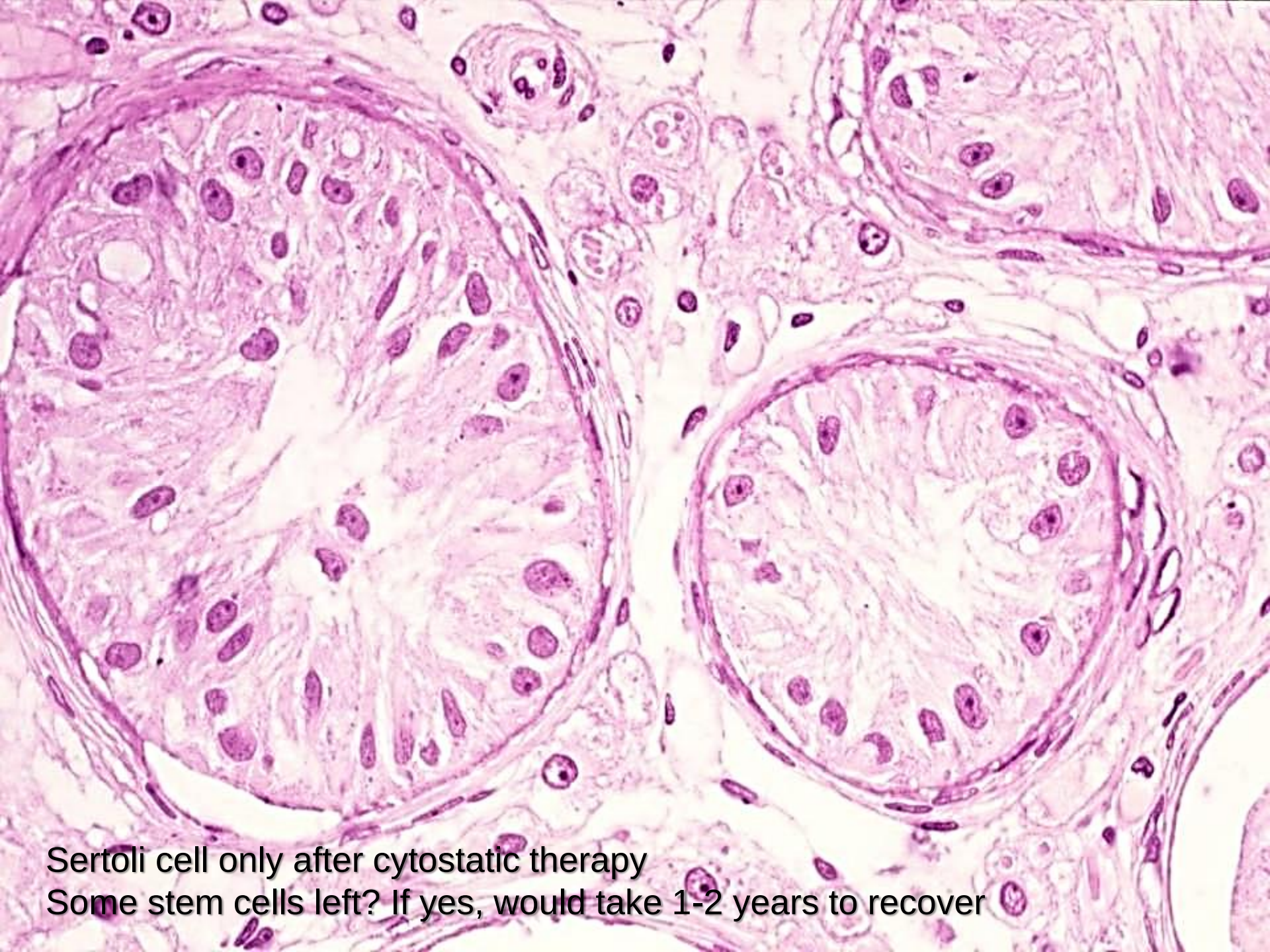


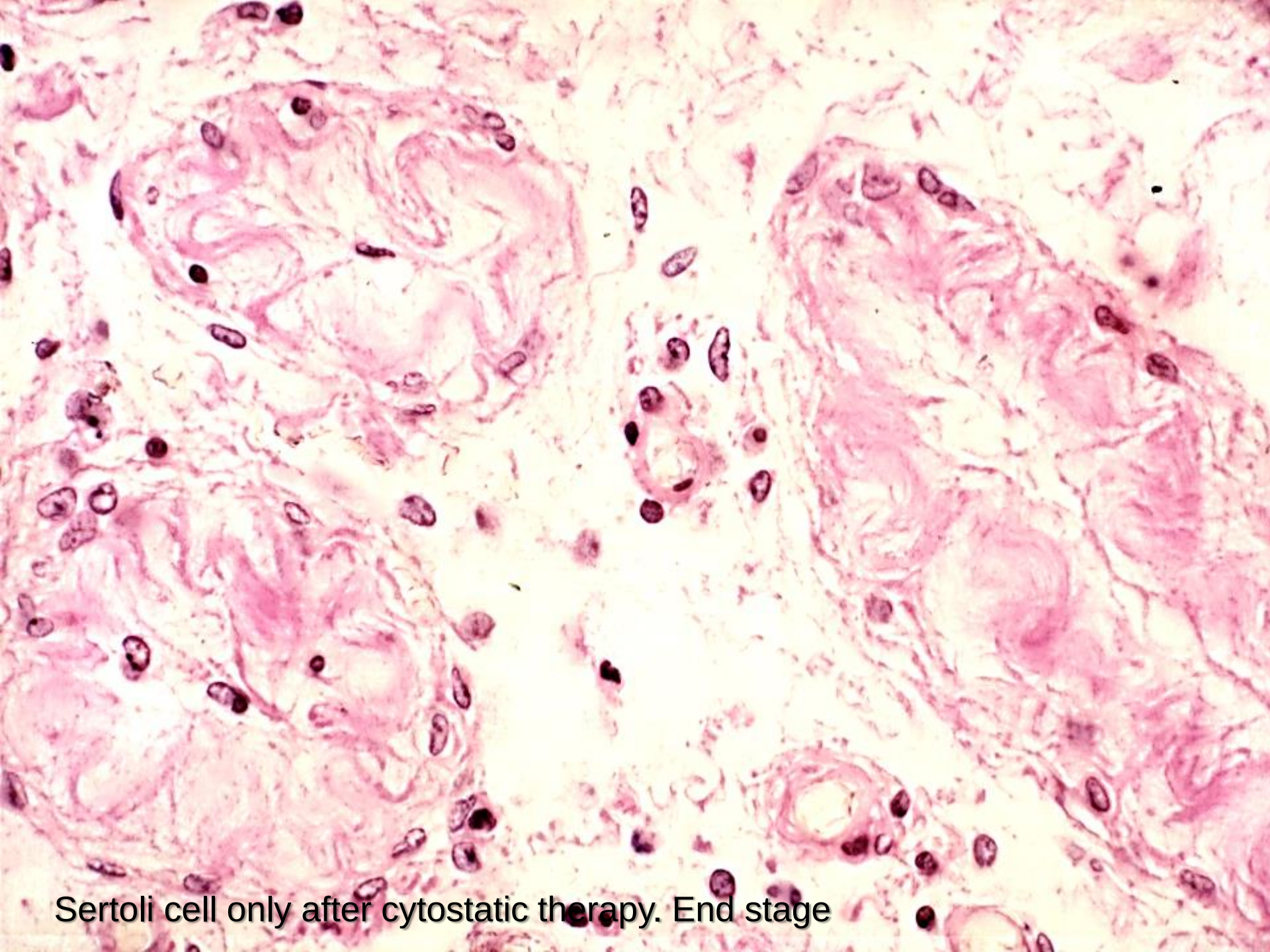
* Occasionally also arising from spc

Germ cell (GC) toxicity – mid-long term

- After “a couple” of *days*
  - Depletion of specific GC generations: small gaps
- After “a couple” of *weeks*
  - Maturation depletion of target and more mature GC: larger gaps
  - Spermatogenesis may appear “arrested” at earlier cell types
- Long-term effects
  - If also spermatogonia (spg) affected: SC-only tubules
  - Otherwise reversible: rat >(>) 56 d, man > 2 years



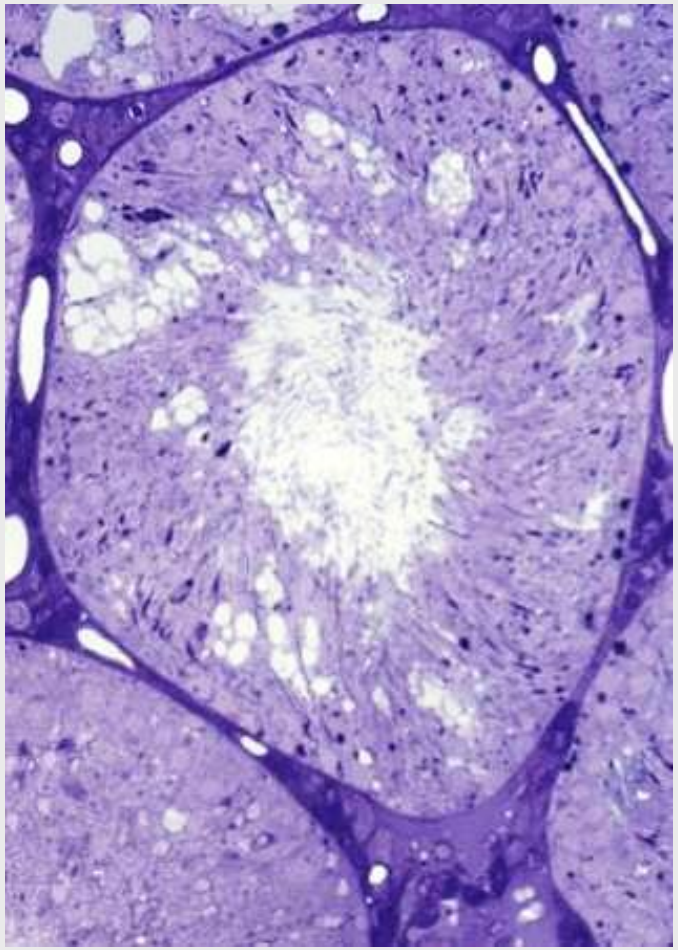

Sertoli cell only after cytostatic therapy
Some stem cells left? If yes, would take 1-2 years to recover



Sertoli cell only after cytostatic therapy. End stage

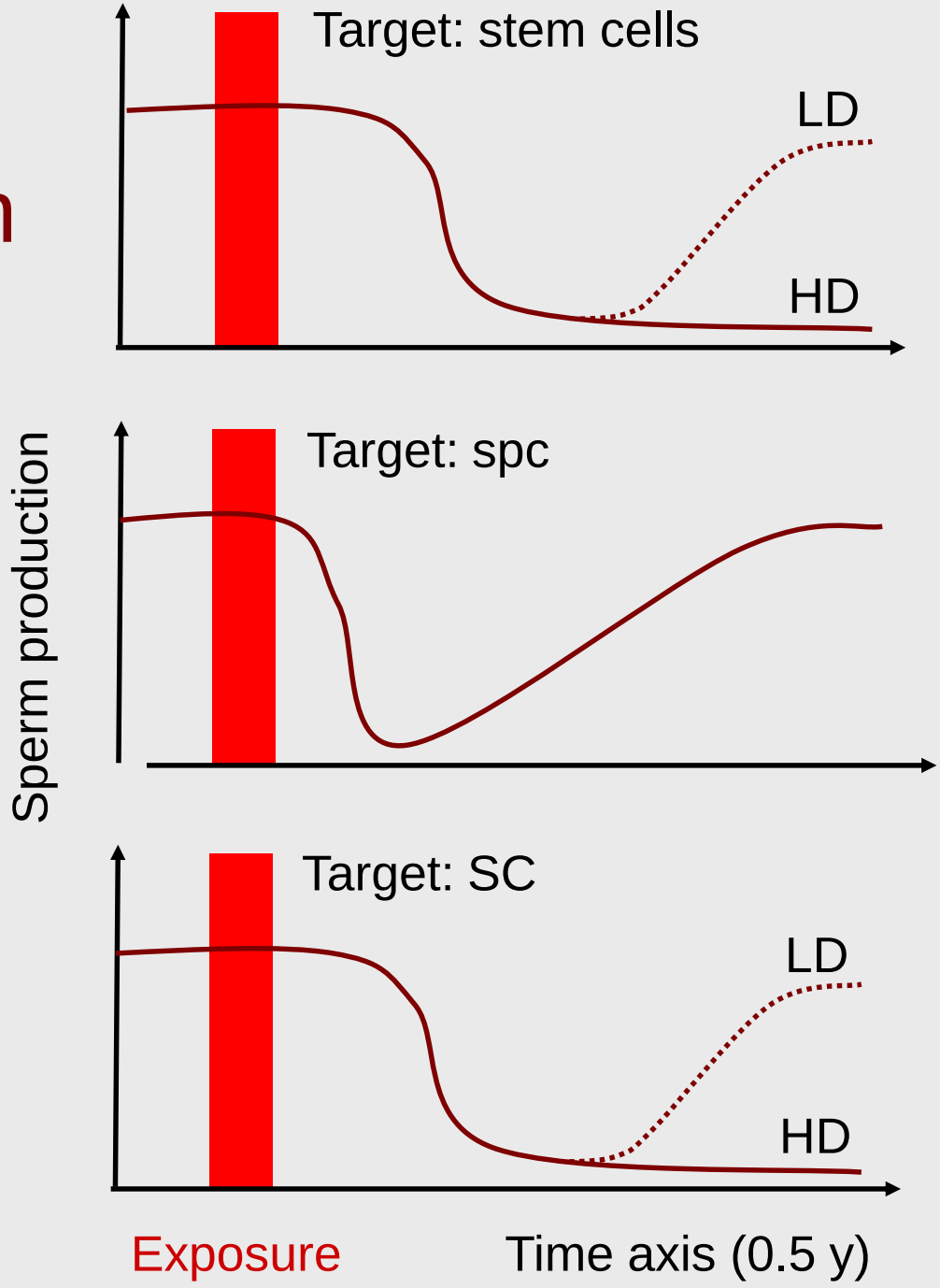
Sertoli cells (SC) – a frequent target

- Early signs
  - *Vacuoles* (often dilated ER) resulting in SC swelling
  - GC *sloughing* (epididymal lumen)
  - *Retention* of elongated spt
  - *Degenerating* GC (secondary effect)
  - Foci of missing GC
    → Disturbed architecture of germinal epithelium
- Advanced changes
  - Progressive degeneration of GC with increased sloughing
- End stage
  - *SC-only tubules*: irreversible



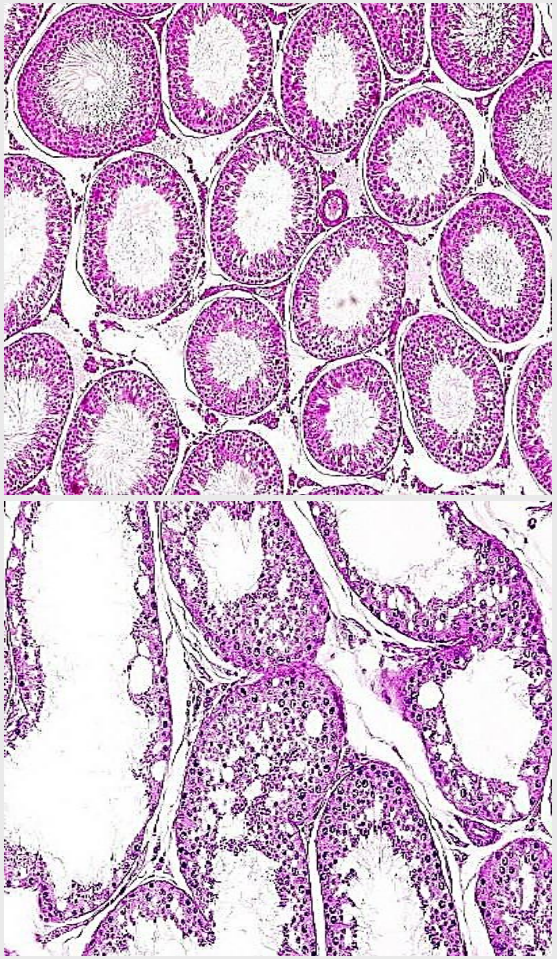
Effect on semen

- Delay
  Shorter, the more mature the target cells are
- Recovery
  Depends on dose
  Severe damage to stem cells and to SC leads to permanent infertility
- HD: high dose
  LD: low dose



Fluid imbalance

- Early signs
  - Testicular *weight*
  - *Diameter* of tubular lumen of testis, efferent ductuli and epididymal tubule
  - *Interstitial edema* in case of increased fluid production
- Later signs in case of increased fluid production
  - Pressure atrophy of germinal epithelium



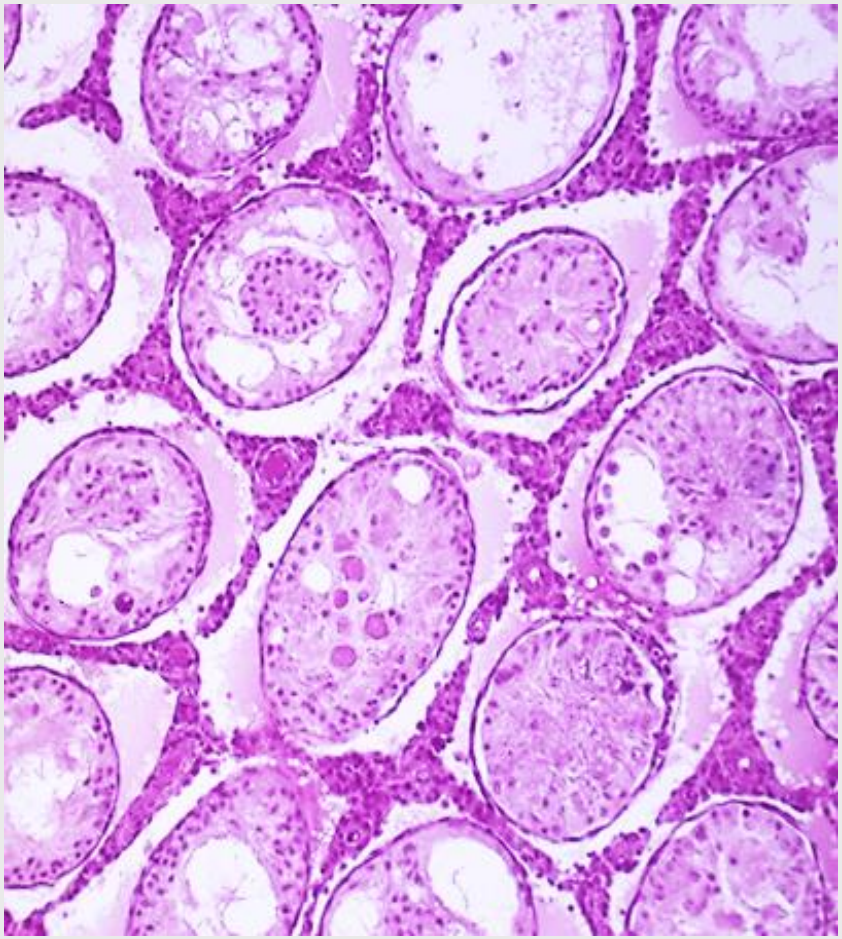
Leydig cells toxicity

- Well visible are *trophic changes*: atrophy, hypertrophy/hyperplasia and neoplasia
- Other morphological signs for primary LC toxicity are not readily evident on standard sections
  Exception:
  - *Foamy* cytoplasm following e.g. with hormonally active compounds
  - *Necrosis/apoptosis* e.g. with anticancer drugs, ethane-dimethane sulfonate
- LC changes are *frequently secondary* to changes in the seminiferous epithelium *(see next slide)*

Leydig cells – Secondary changes

Severe damage to spermatogenesis is generally associated with LC hyperplasia

- "Relative" because of decreased tubular volume
- Absolute, because of endocrine/paracrine changes associated with disturbed/absence spermatogenesis



Tubular or testicular necrosis

- Generally a consequence of *ischemia*
- Examples
  - Vascular endothelial necrosis with cadmium
    May cause ischemic necrosis of the *testis*
  - Vasoconstriction with serotonin or histamine
    May cause *focal* tubular necrosis
- Associated with inflammation and potentially with autoimmune reaction

Epididymis damage

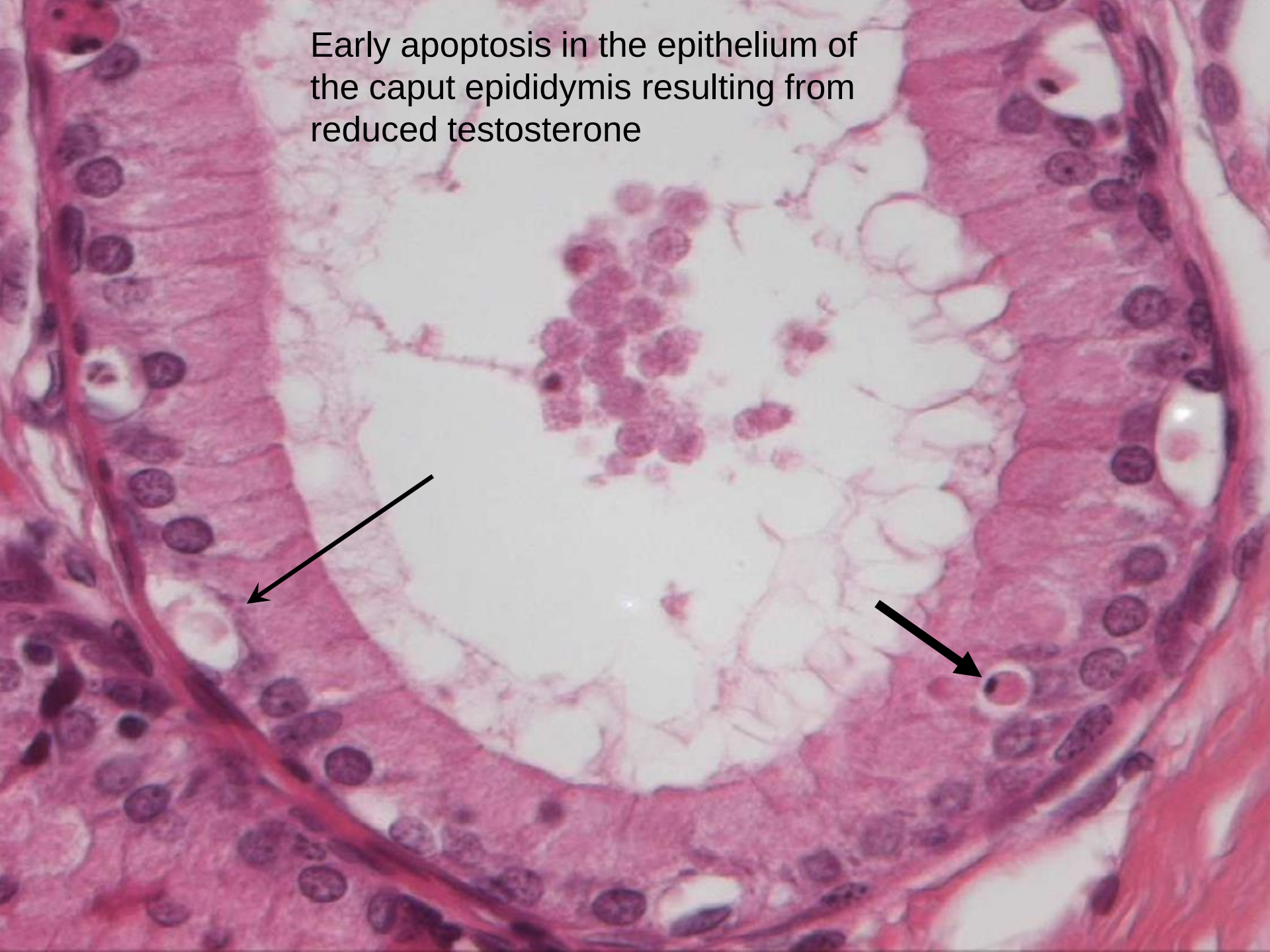
- *Vacuolation* of epididymal epithelium
  - Lack on androgen
  - Chemical injury, e.g. by oxidosqualene cyclase
- *Granulomatous* inflammation, e.g. following
  - Endothelial necrosis in caput by *cadmium*
  - Inhibition of fluid resorption by *α-chlorohydrin*

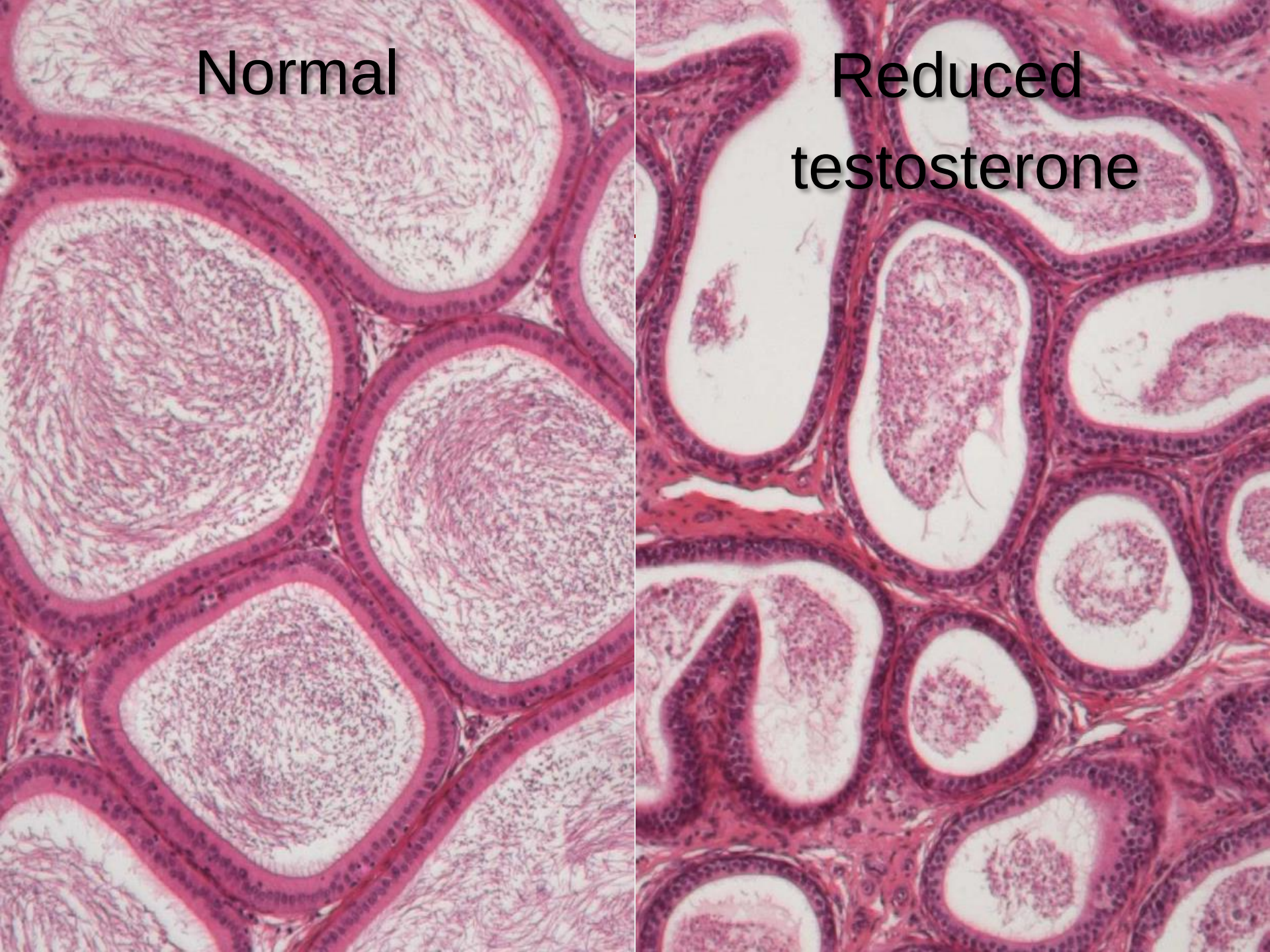
  → Breakdown of the *blood-epididymis barrier*

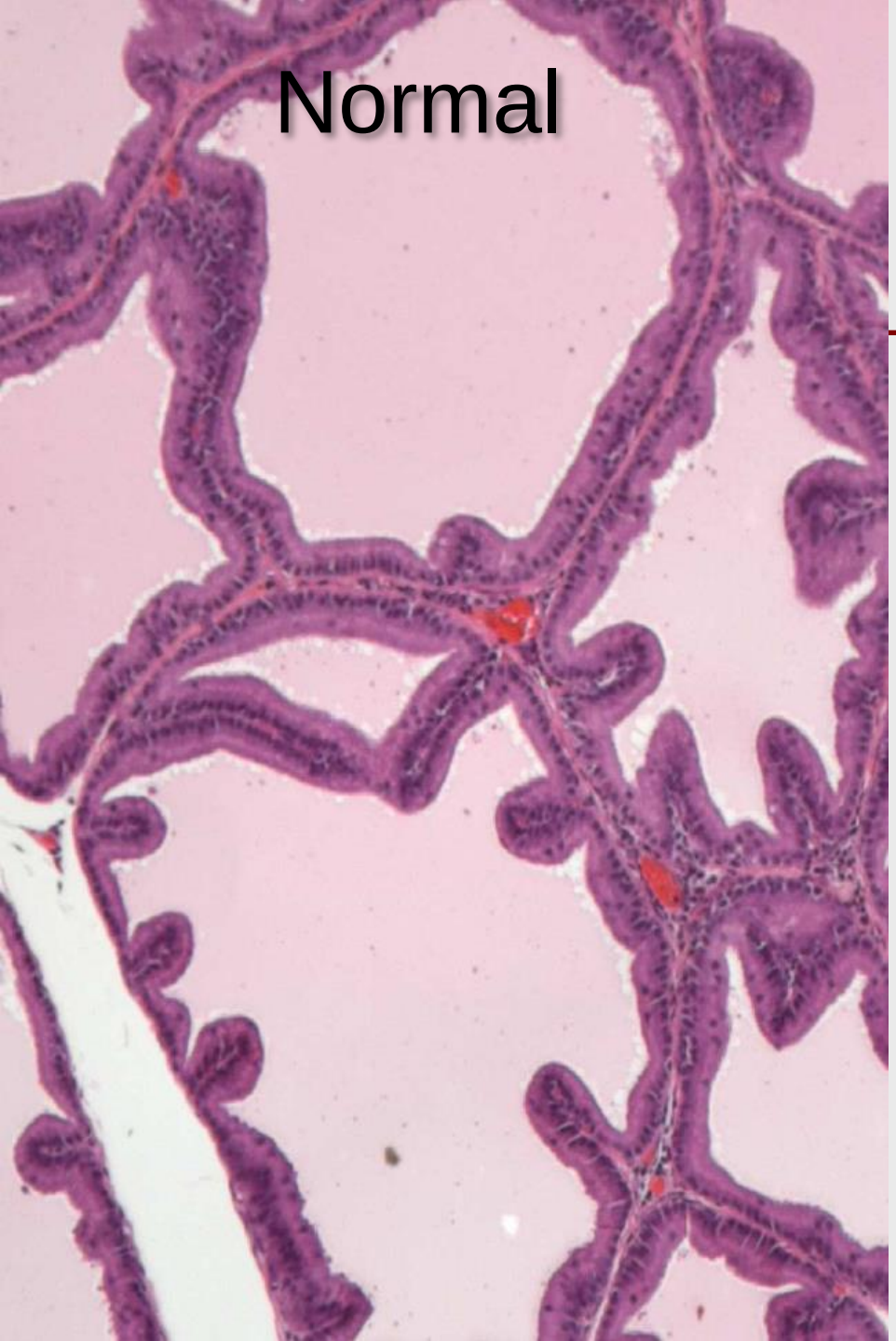
  → Immunologically competent cells attack sperm (normally not in contact with immunocompetent cells, therefore perceived as foreign)

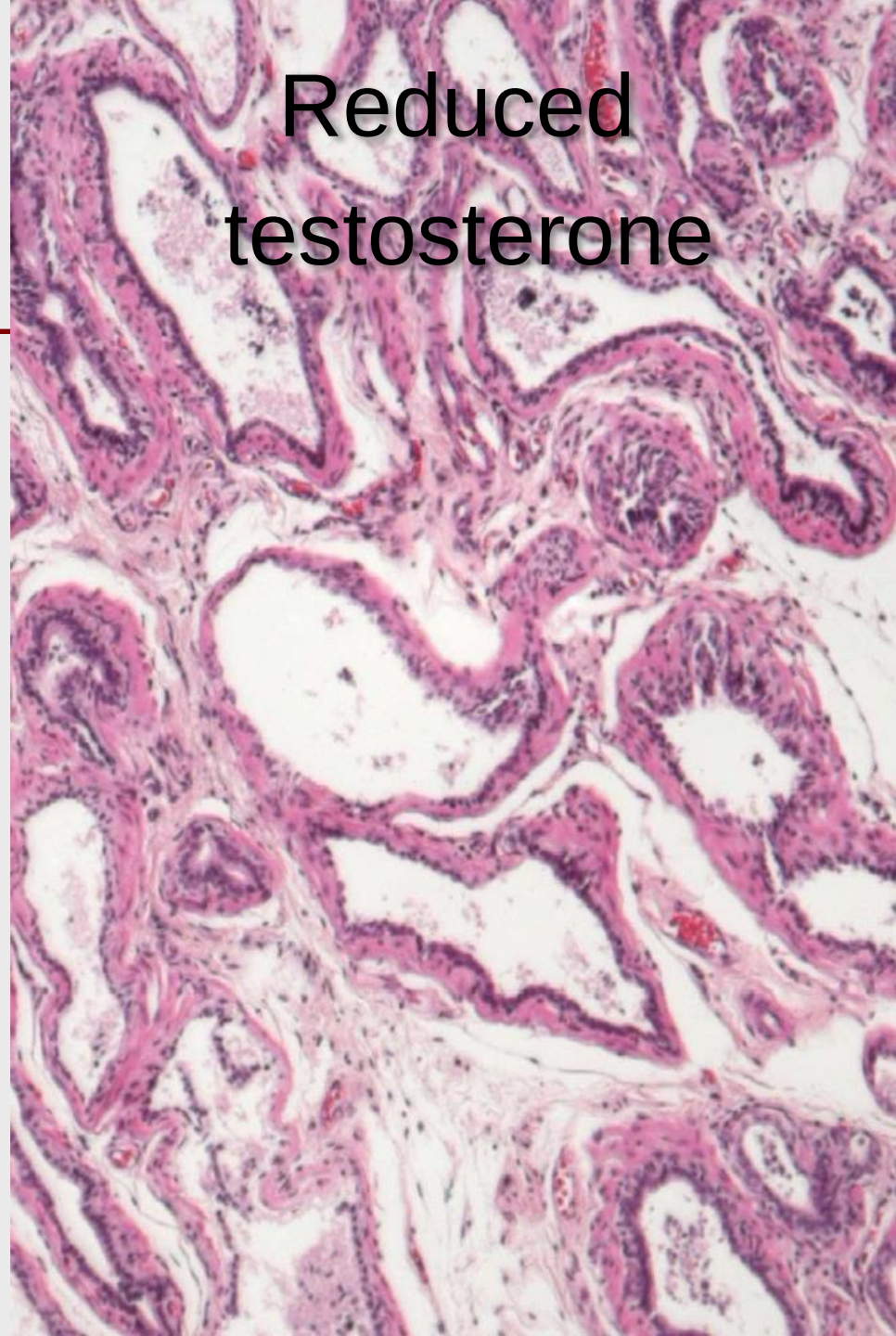
  → Sperm granuloma

  Also seen in ductuli efferentes, induced or spontaneous from blindly ending ductuli


Early apoptosis in the epithelium of the caput epididymis resulting from reduced testosterone


Normal
Reduced testosterone


Normal


Reduced
testosterone

Conclusions Topic D – General toxicity

- Toxins acting *directly* (not via endocrine regulation) primarily affect the *testis*, especially spermato-genesis
- *Early findings* are often specific for the inflicted damage and may provide insight into the MoA
- Of particular concern, because *potentially irreversible*, are
  - Stem cell toxicity (indirect assessment)
  - SC toxicity
- *Epididymal content* is an excellent and “historic” indicator of damage of spermatogenesis

Lecture 2: Practice

- C Recommended approaches for evaluation of MR organs (general methods)
- D Morphologic evaluation of MR organs
  - General toxicity
  - Endocrine disruption
    - Guidelines
    - Antiandrogens
    - Inhibition of testosterone biosynthesis
    - Gynecomastia in man
    - Effect of estrogenic compound on prostate
    - Species differences
    - Conclusions

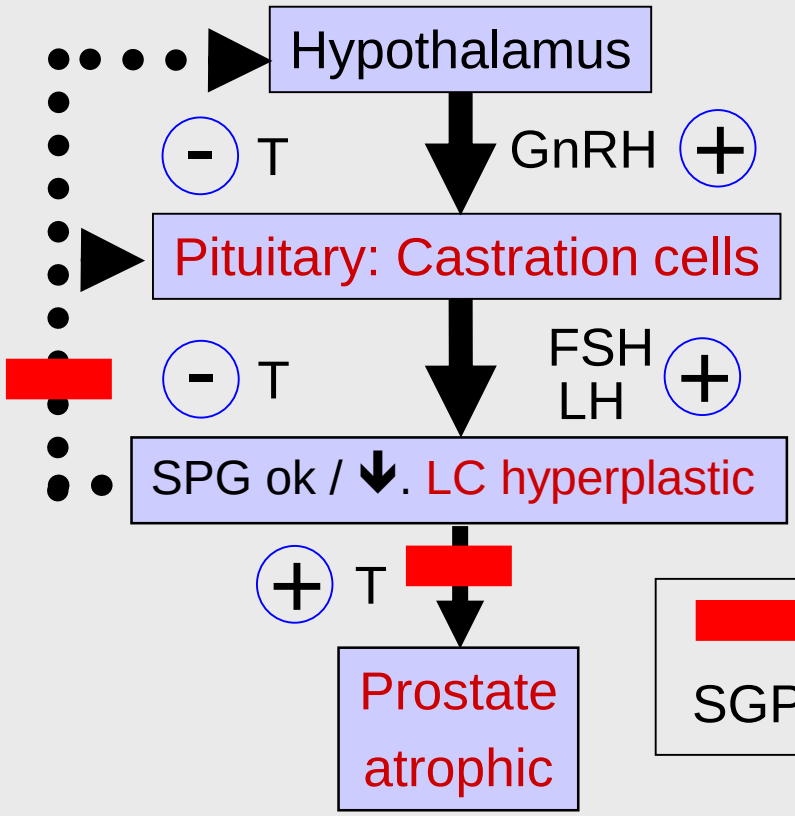
Guidelines – Chemicals

- OECD test guideline 407 for chemicals,1995 Repeated Dose 28-day Oral Toxicity Study in Rodents
  - Preliminary draft updated with Parameters for Endocrine Effects (Revised 18 December 2007)
- Endocrine disruption: a guidance document for histologic evaluation of endocrine and reproductive tests. OECD, May 2008 Website: European Society of Toxicologic Pathology (ESTP) – Guidelines – Testing Strategies. Or directly under

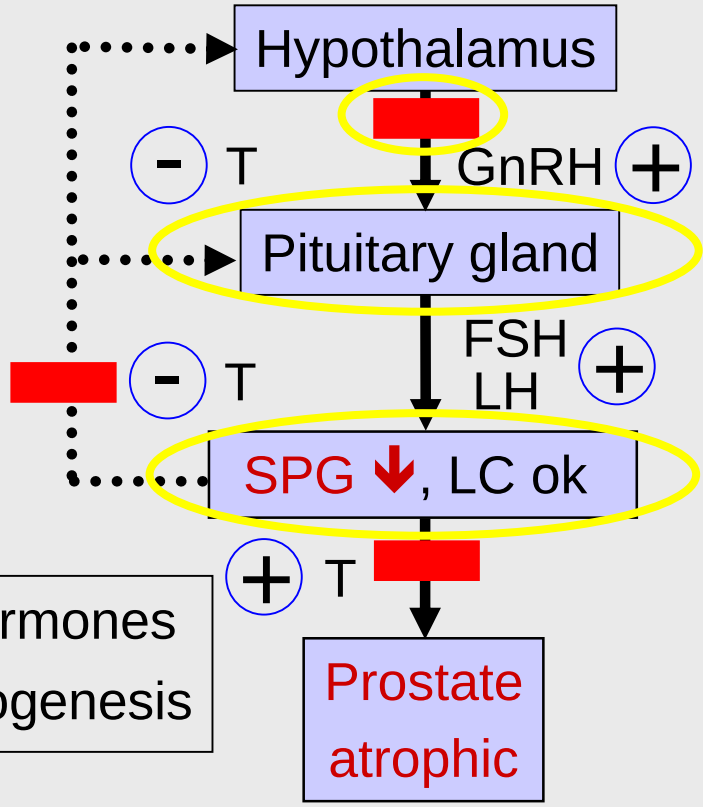
  http://www.eurotoxpath.org/guidelines/index.php?id=teststrat

Antiandrogen action - Simplified

Without antigonadotropic activity
e.g. flutamide



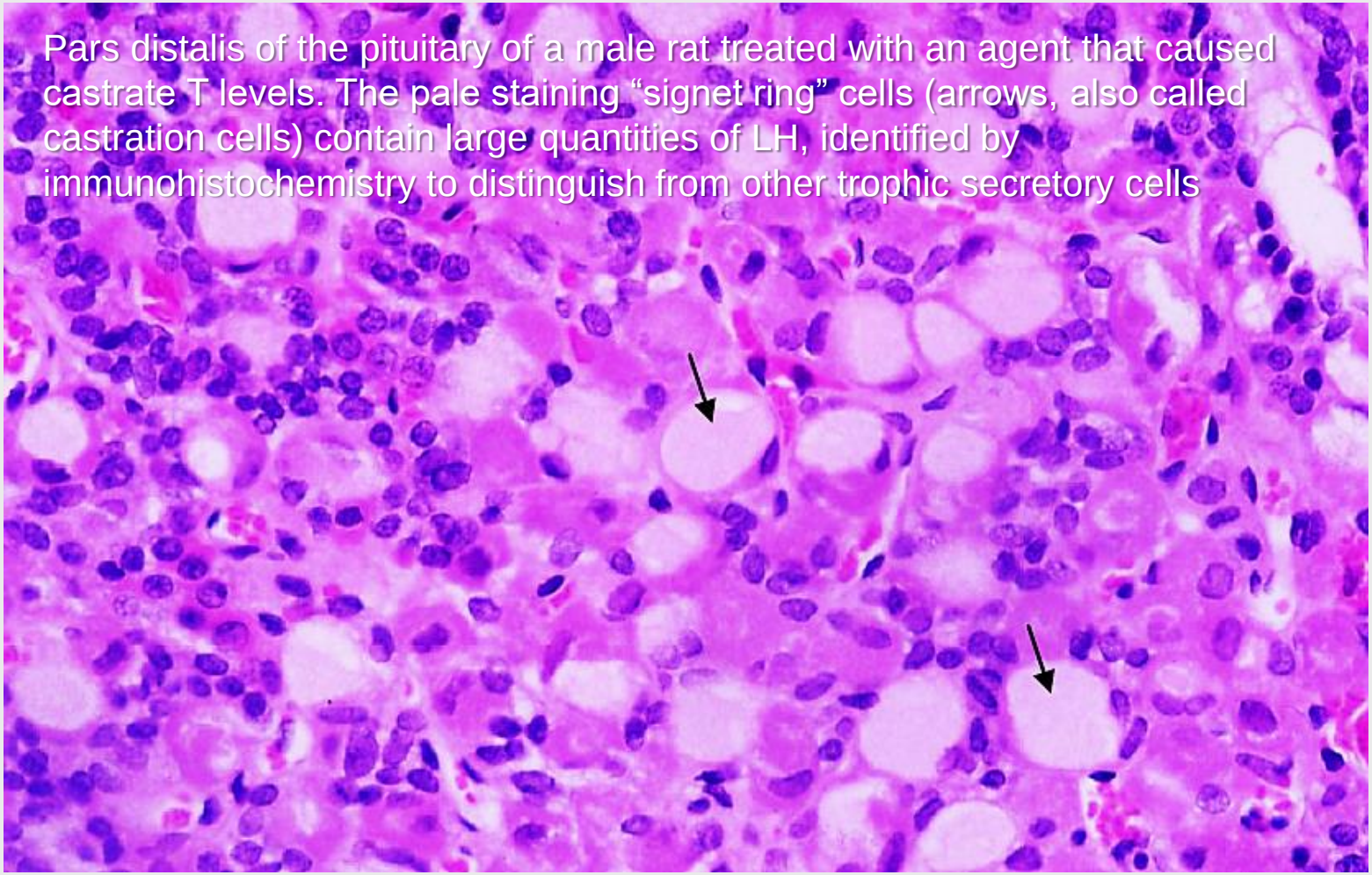
With antigonadotropic activity
e.g. cyproterone acetate



Block hormones

SGP: spermatogenesis

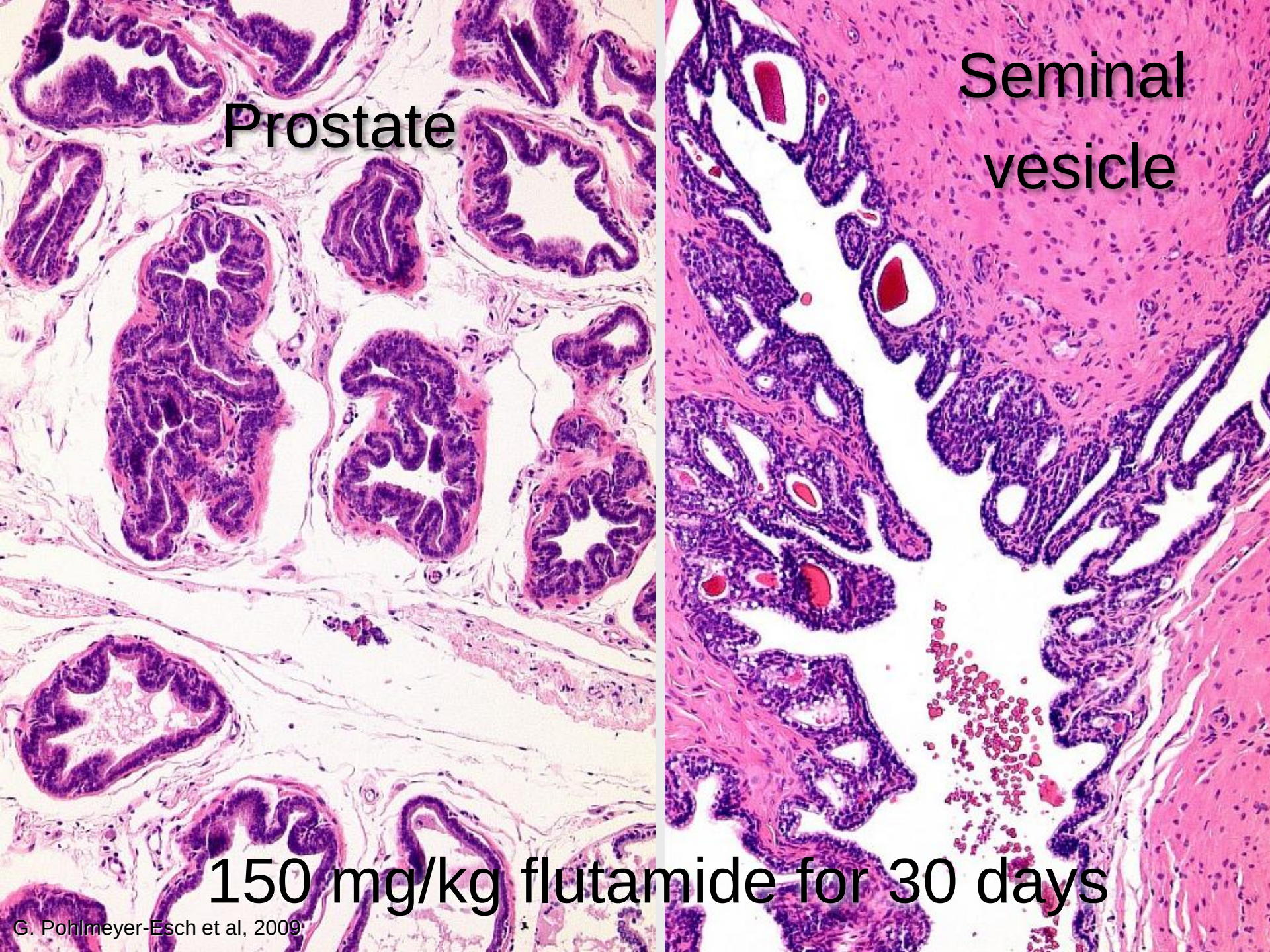
Pituitary castration cells



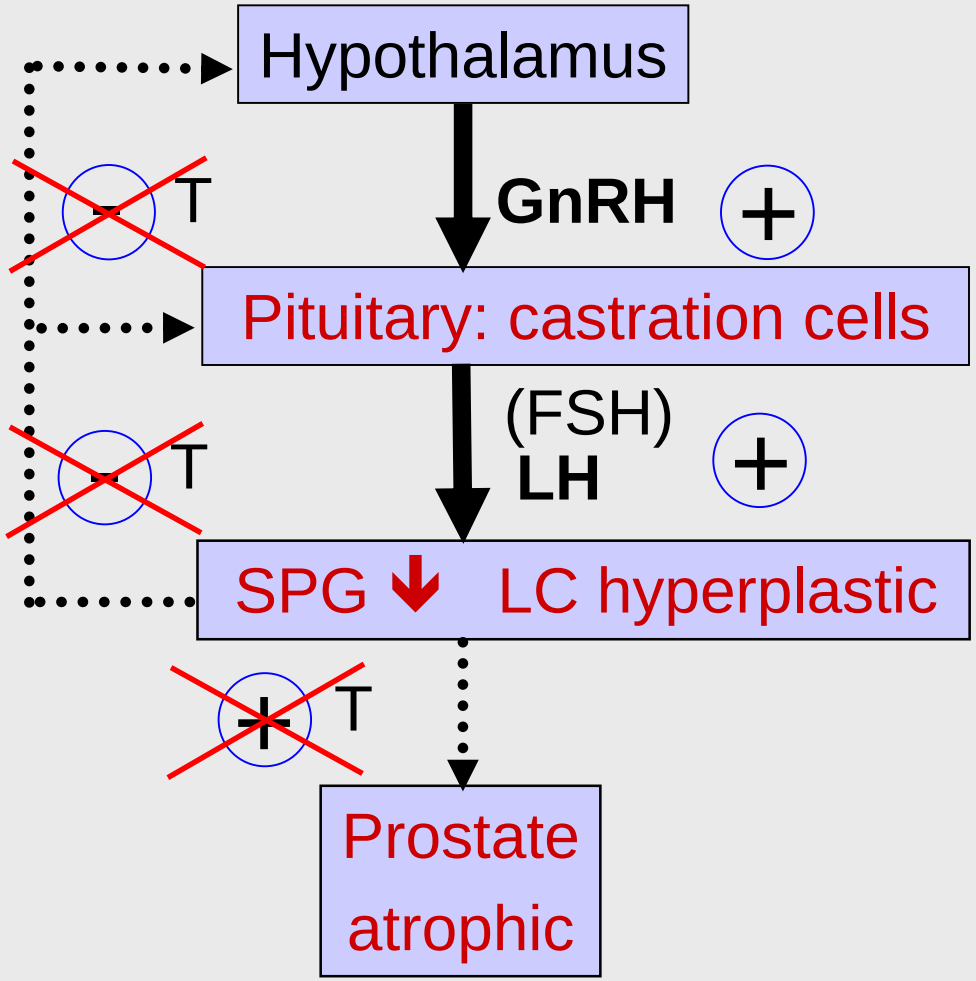
Rat, 150 mg/kg flutamide (pure antiandrogen) for 30 days

Interstitial spaces are broadened and contain more cells

G. Pohlmeyer-Esch et al, 2009


Prostate
Seminal vesicle
150 mg/kg flutamide for 30 days
G. Pohlmeyer-Esch et al, 2009

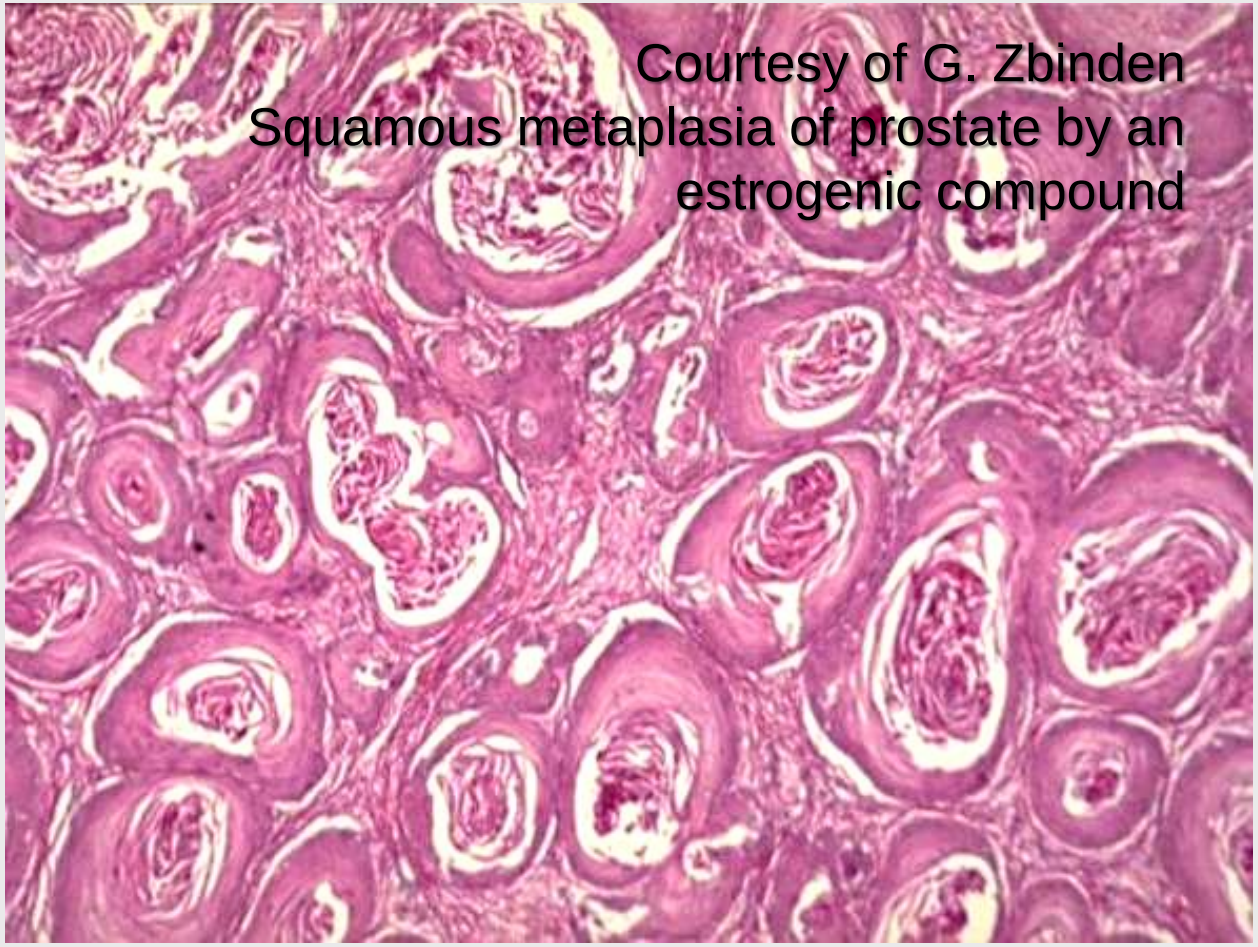
Inhibition of T biosynthesis



Gynecomastia in man

- *Benign* enlargement of the male mammary glands
- By increased/unopposed *estrogen* action on breast
- ~ 4–10% of gynecomastia in men due to *drugs*
- *Mechanisms*
  - Inhibition of androgen synthesis and/or metabolism (ketoconazole)
  - Antagonism at androgen receptor (flutamide, finasteride)
  - Direct action on estrogen receptors by estrogenic drugs (clomiphene)
  - Displacement of estrogen from binding globulin (free estrogen ↑, e.g. spironolactone)
  - Via damage the testis (anticancer drugs)

Prostate – Estrogenic compound



Species differences: rats vs. humans

- Rats lack sex hormone *binding globulin*
- Rats are more *sensitive*
- Rat Leydig cells have a high density of *LH receptors*
- Influence of *PRL* on LH receptor function in rats
- Presence of *GnRH receptors* on rat Leydig cells
- *Waning endocrine milieu* in aging women, but not in aging female rats

Conclusions Topic D - Endocrine disruption

- Endocrine disrupters not only affect the testis but generally also the *accessory MR sex organs*
- Endocrine side effects do per se not preclude further use of the chemical/drug but need a *risk evaluation:* endocrine effects are *often species-specific*
- Similar compounds can affect the MR system in different ways

Final Conclusions – 1

- Preclinical potentially adverse MR effects are *not uncommon, but of concern* → Sound and comprehensive scientific assessment is a must
- Important experimental factors
  - *Standard studies* (multiple endpoints) often sufficient
  - Good tissue *fixation*
  - *Expert histopathological examination* including knowledge of staging
  - *Confounding* factors including immature test animals
- Important risk parameters
  - *Safety ratio*
  - *Reversibility*
  - *Monitorability* in man

Final Conclusions – 2

- Identification of primary target might help to establish the MoA of MR toxin
  May need *early* time points and *time-course* studies
- Often more than one MR target:
  - Use a system's approach (may need e.g. hormonal measurements)
  - Understand patterns of adverse responses
- Affected cell type less important than *reversibility*
  Most important: survival of spg (may be difficult to find in histological sections)
- *Hormonally* mediated effects are generally *reversible*, affect early *accessory sex organs* and are often *species-specific*
- Ultimate proof often only in *man* with early markers