CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
REPRODUCTIVE SYSTEM

Third Conference

ORGANIZED BY SOCIETY FOR TOXICOLOGIC PATHOLOGY IN INDIA (STPI)

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Toxicologic Pathology of the Male Reproductive System - 2

3rd STPI Seminar, Bengalore, October 29-31, 2010

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Handout version of presentation

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Lecture 2: Practice / Application

C Recommended approaches for evaluation of MR organs (general methods) - 25 min.

- *Including* study design and sampling techniques
- *Excluding* staging and sperm evaluation

D Morphologic evaluation of the testis of laboratory animal species - 10 min.

Excluding

- Background and age related changes
- (Non-)neoplastic changes in male reproductive system

E Endocrine disruption: Guidelines for histopathologic evaluation - 10 min.

- *Excluding* effects of phytoestrogens of reproductive physiology and pathology

Evaluation of MR organs

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

Guidelines (selection)

- General safety guidelines
- Guidelines for assessing male and female reproductive 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 "reprotox" studies not addressed in this presentation
- In vivo models for male reproductive toxicology

  R. W. Tyl http://www.currentprotocols.com/protocol/tx1601

Evaluation of MR organs

- Guidelines
- **Study design**
- "Non-anatomic" parameters, esp. hormones
- Organ weights
- Tissue preparation
- Histopathological evaluation
- Dealing with unexpected findings
- Conclusions

Male-specific endpoints of reproductive toxicity

Organ weights	Testes, epididymides, seminal vesicles, prostate, pituitary
Macroscopic examination and histopathology	Testes, epididymides, seminal vesicles, prostate, pituitary
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*, ano-genital distance, external genitalia*, general 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

http://www.currentprotocols.com/protocol/tx1601

<table>
<tr><th colspan="4">Live animals</th><th rowspan="2">Necropsy</th></tr>
<tr><th>Electro-ejaculation</th><th>Blood sampling</th><th colspan="2">Unilateral orchidectomy</th></tr>
<tr><th></th><th></th><th>Culture</th><th>Homogenization resistant spt/sp</th><th>Histo-pathology</th><th></th></tr>
<tr><td rowspan="3">Cauda sperm
- Number
- Motility
- Morphology</td><td>FSH, LH, DHT</td><td rowspan="3">T, DHT
Inhibin
In culture and in testis
Morphology
Other parameters</td><td rowspan="3">Daily sperm production</td><td rowspan="3">Staging</td><td>Organ weights</td></tr>
<tr><td>If normal:
Repeat after GnRH stimulation</td><td>Testis:
- Histopathology incl. staging
- Daily sperm production
- Culture</td></tr>
<tr><td></td><td>Epididymis:
- Histopathology
- Cauda sperm

CNS incl. pituitary
Adrenals
Liver</td></tr>
</table>

Routine parameters are marked in brown

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
http://www.anthonyturton.com/admin/my_documents/my_files/3A3_EPA-HQ-OPPT-2003-0027-0009.pdf

Time

- Importance of early time points: cell-specific toxicity
- 4 week toxicity studies often sufficient, but 13 week studies are more reliable
- For tailor-made studies: duration of spermatogenic process (mouse ~ 5 weeks, rat ~ 8.5 weeks) is guiding duration, especially for recovery period
- Time-course study with serial autopsies (hours to weeks apart) may be necessary

Animals are immature at test start

Species	*Recommended starting age*	*Age of sexual maturity*
Rat	Soon after weaning = 6-7 weeks (after acclimation)	8 – 10 weeks
Mouse	Soon after weaning = 6-7 wks (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, giant cells, spermatogenesis focally incomplete
  - Epididymis: sloughed germ cells, giant cells, reduced sperm content
  - ➔ Same picture in case of toxicity!

Evaluation of MR organs

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

Hormone measurements

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

Sperm evaluation

- Epididymal sperm parameters
  See separate presentation
  - From electroejaculation in living animals or from the cauda epididymidis at necropsy
  - Number: Production, variability
  - Quality: Morphology
  - Function: Motility
- Testicular "sperm" count: homogenization resistant spermatid count (mainly step 17-19)
- Flow cytometry for evaluation of whole spermatogenic process

Evaluation of MR organs

- Guidelines
- Study design
- “Non-anatomic” parameters, esp. 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) / relative weight values
- Organ weights are sensitive indicators of hormonal balance

  In particular, accessory sex organ weights reflect well circulating testosterone levels, if receptor function is normal

Hershberger assay

- To evaluate potential (anti-)androgenic effects
- Test system: immature and castrated male rats
- Continuous 10-day exposure
- Assessment in particular of the weight of androgen-dependent tissues
  - Ventral prostate
  - Seminal vesicles (with fluid) plus coagulating glands
  - Levator ani plus bulbocavernosus muscles
  - Bulbourethral glands
  - Glans penis
- Optional: Serum testosterone + luteinizing hormone
- http://www.currentprotocols.com/protocol/tx1609

Evaluation of MR organs

- Guidelines
- Study design
- “Non-anatomic” parameters, esp. 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/

- 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

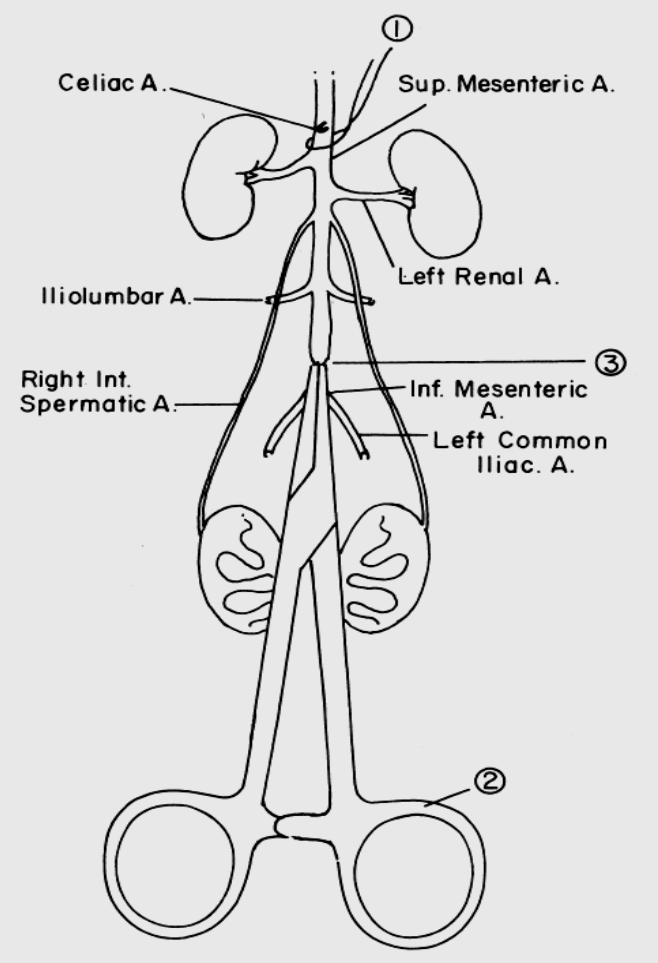
Details to be shown during presentation

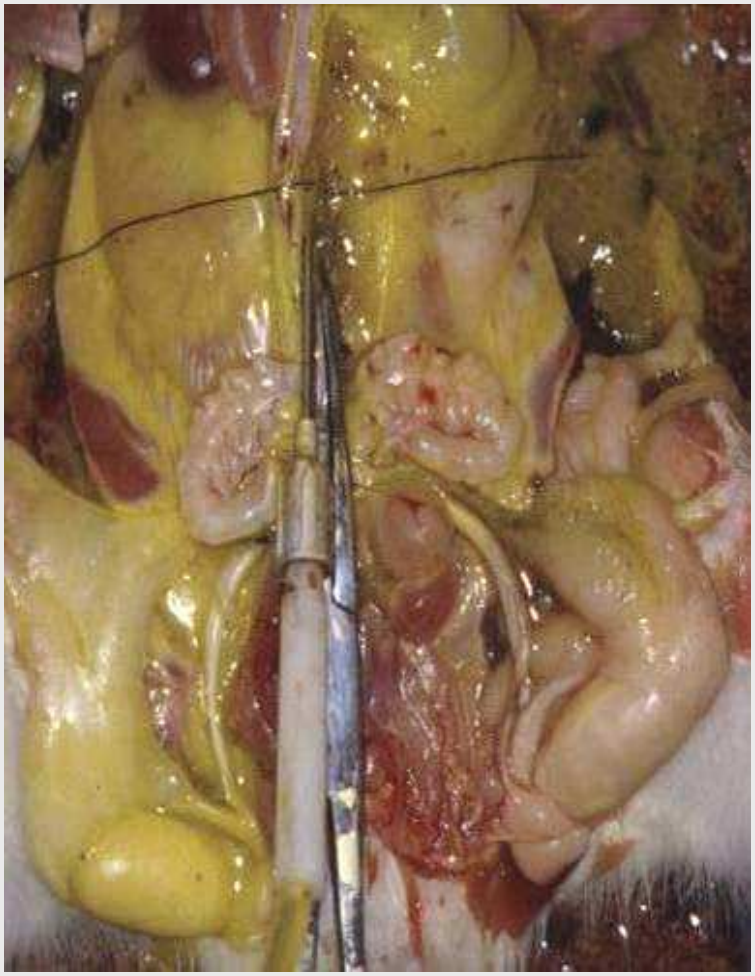
Basic histological methods for testis

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 *** or methylene blue GMA: glycol methacrylate ** or mixture of formalin and glutaraldehyde (Karnovski's)						

Histological photomicrographs illustrating the results of various fixation and embedding schemes to be shown

Part-body perfusion





Evaluation of MR organs

- Guidelines
- Study design
- “Non-anatomic” parameters, esp. 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 germ cells 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
- Vacuolation in the seminiferous epithelium, often within 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 according to spermatogenic cycle, mainly according to
  - Developmental steps of spermiogenesis and
  - Occurrence of meiosis
- Particularly important for studies up to 28 days
- Stain for acrosome: PAS (counterstain with hematoxylin)
  In dogs and non-human primates acrosomes clearly visible only around spermiation
- Also H&E allows approximate staging

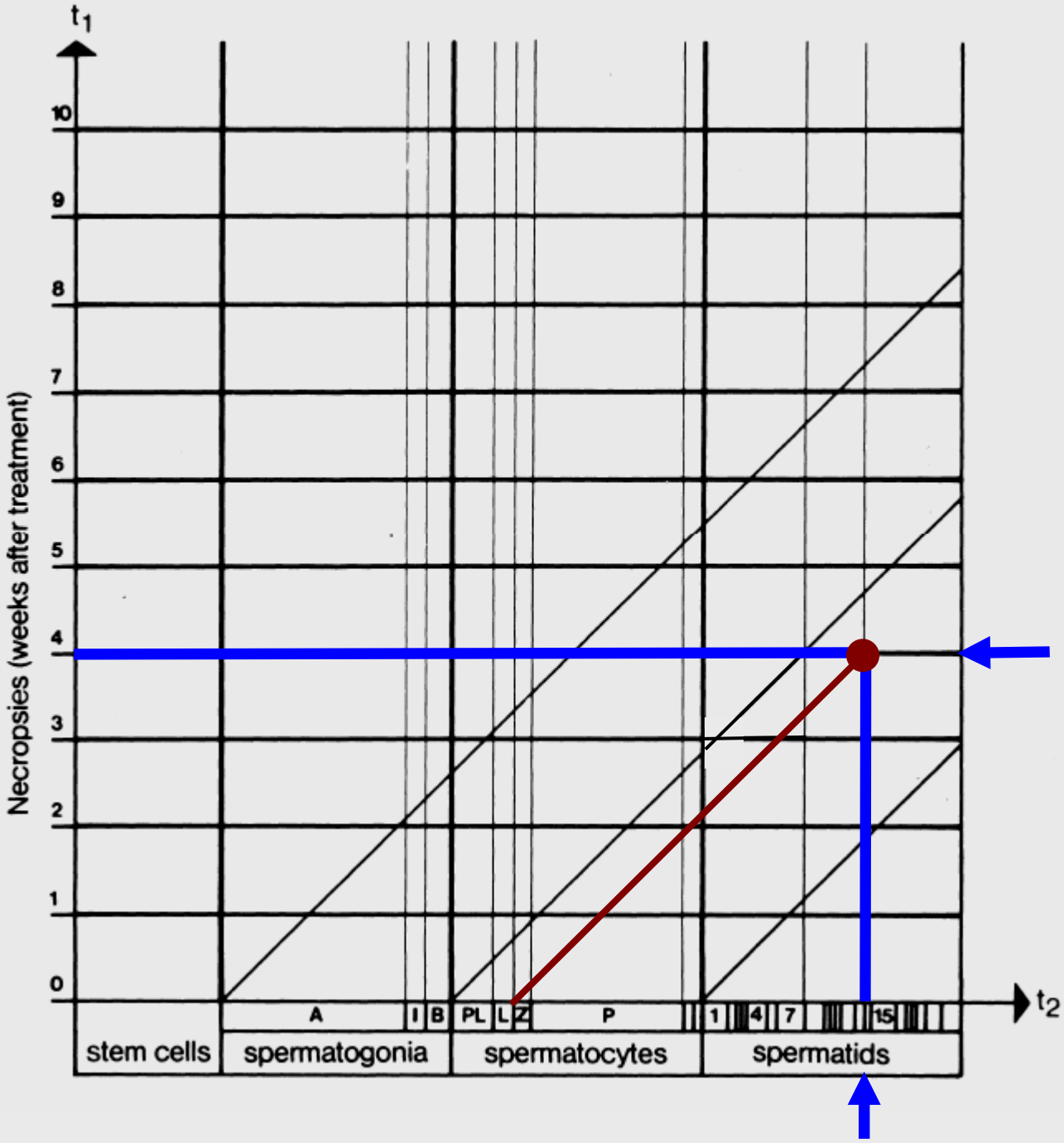
Qualitative staging – Objectives

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

Following single dose or short-term treatment:

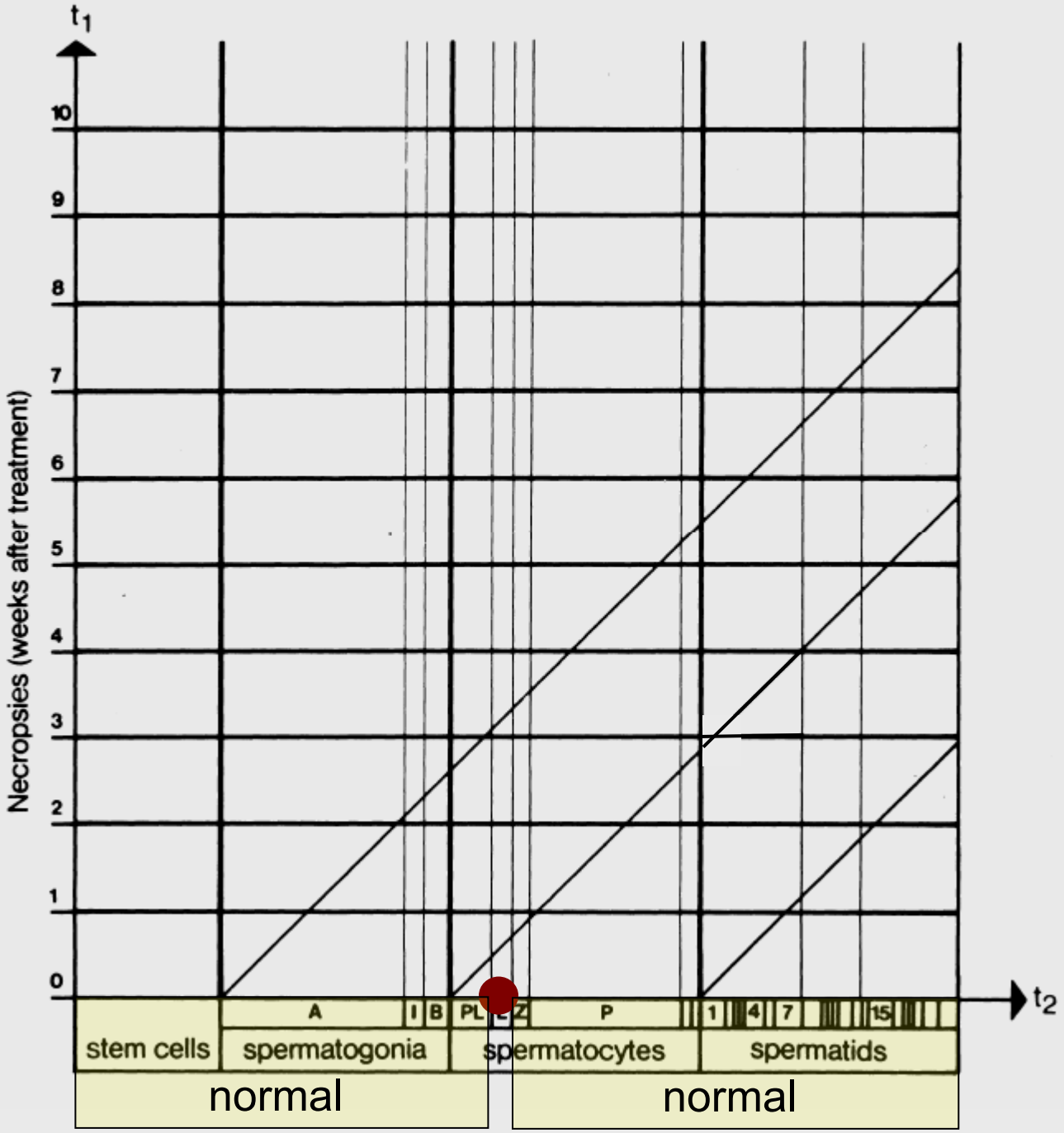
Damaged or missing germ cell type e.g. at 4 week necropsy allows to identify the primary damaged cell by extrapolating backwards

t1 and t2 axis are at same scale



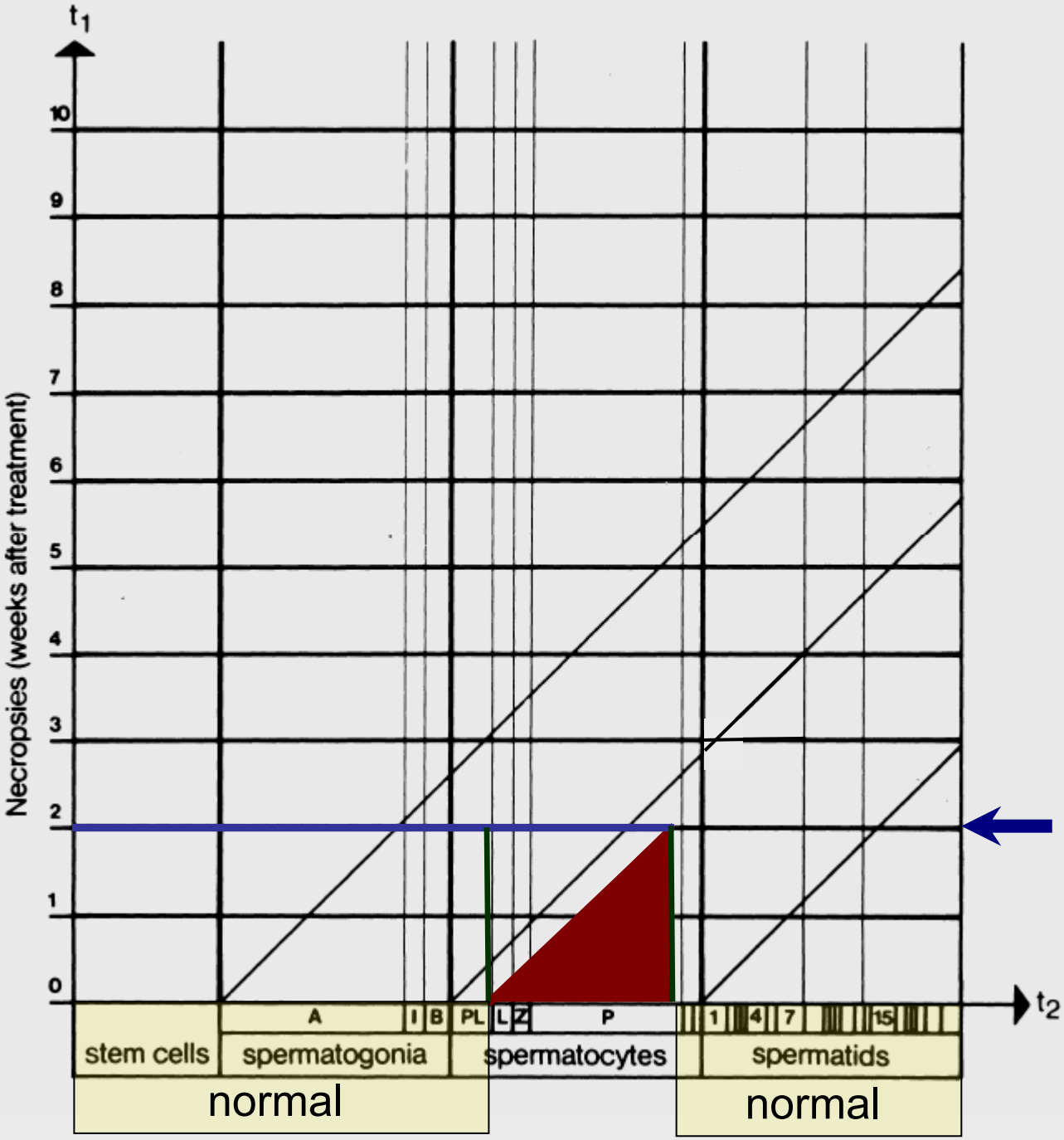
On day 0, treatment with compound X starts.

Assumption: compound X damages selectively leptotene spermatocytes



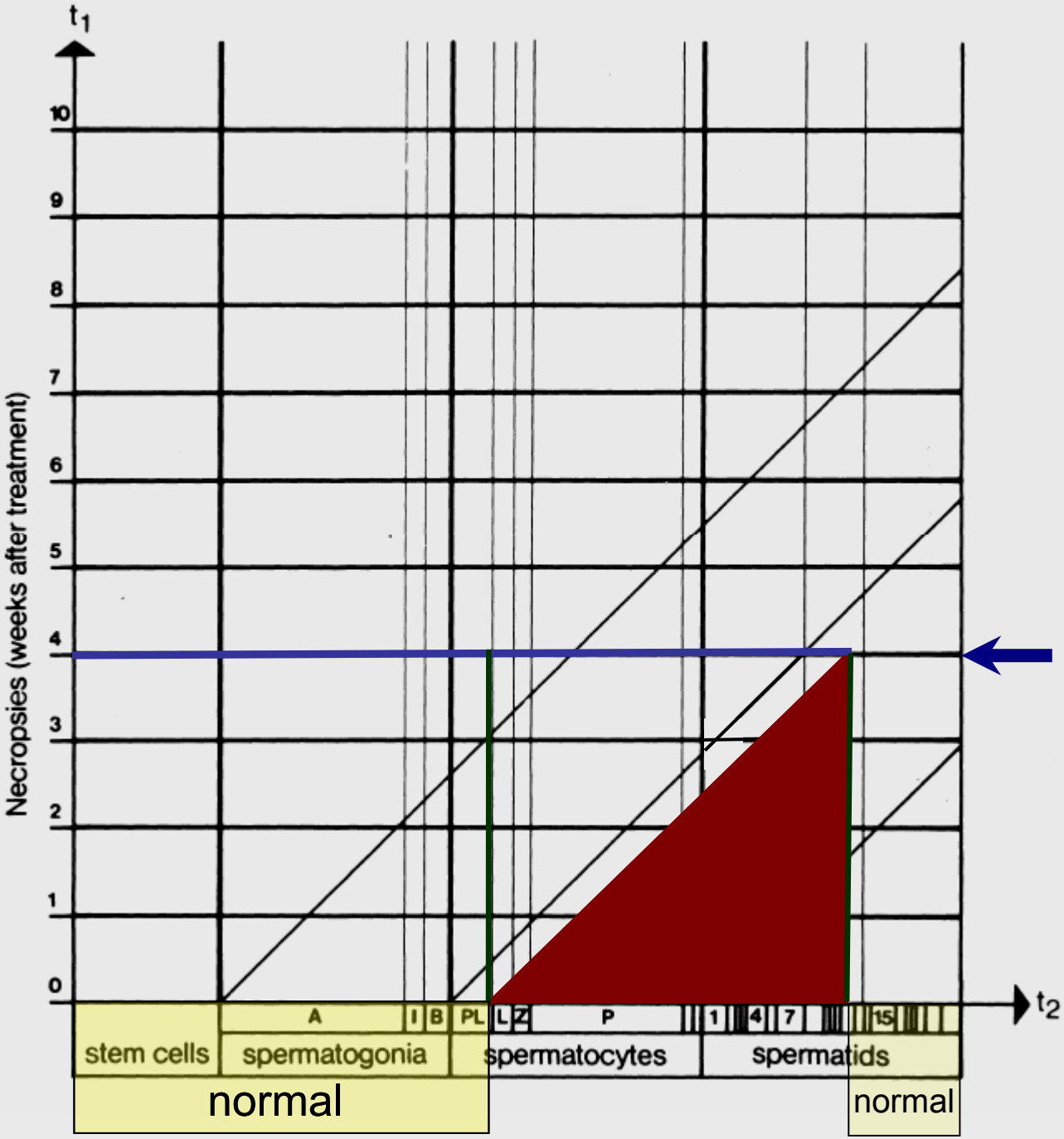
2 weeks of continuous treatment with compound X result in loss also of zygotene and most pachytene spermatocytes:

Depletion by maturation ➔ gap



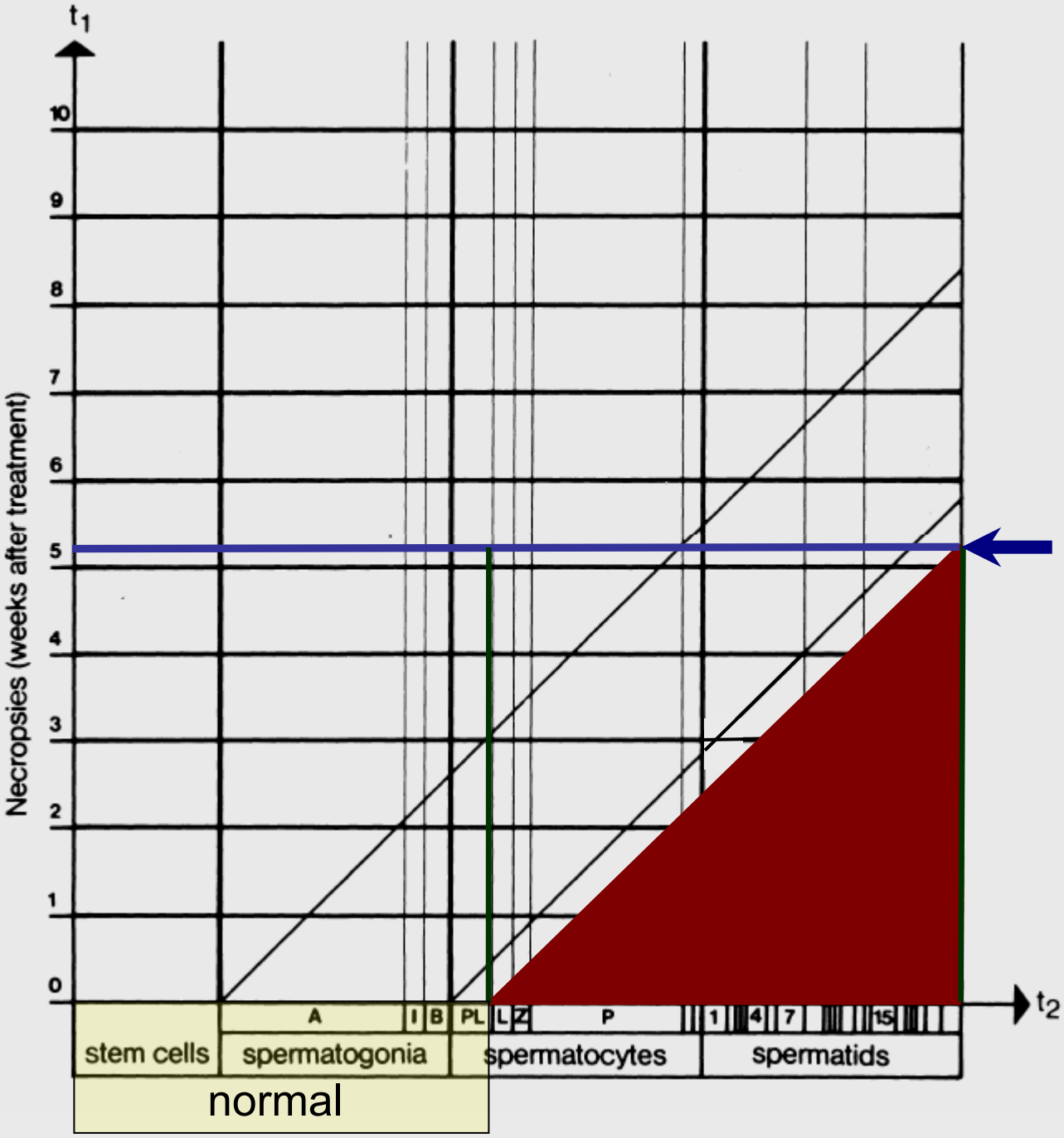
4 weeks of continuous treatment with compound X result in loss also of zygotene and most pachytene spermatocytes:

Depletion by maturation ➔ gap



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

Spermatogenic "arrest"

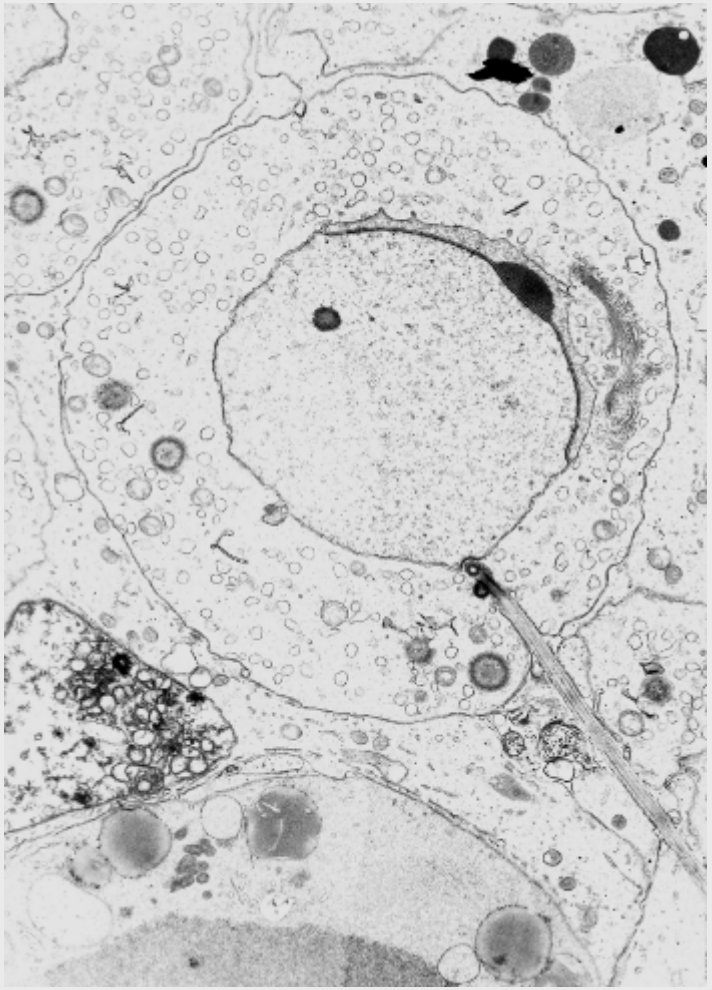


Quantitative histopathological endpoints

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

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 for those with experience 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

Evaluation of MR organs

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

1. Review of hazard identification

- Is there indeed an adverse effect?
  Unilateral vs. bilateral, spontaneous alterations, handling artifacts, variations, age-related changes, etc.
  (Details regarding spontaneous alterations covered in separate presentation)
- What else is known about the compound in question?
- Were there other relevant findings?
  System approach particularly important for MR adverse effects
- Is the study technically valid?
  E.g. influence of sexual immaturity
- Is the model valid?
- Were there other modifying factors?
  (Effects of food restriction and photoperiod covered in separate presentation)

2. Characterization of finding

- Review of older studies esp. for earlier signs
- Additional investigations on available material, e.g. hormone measurements in serum
- Additional tailor-made studies ("trouble-shooting")
  - More detailed assessment of exposure-response relationship, NOEAL, ADME, etc.
  - Sequence of pathogenetic events e.g. with serial necropsies to investigate early and "specific" lesions
  - Other species to evaluate species specificity

3. Risk evaluation

- "Qualitative" aspect:

  Mode of Action: relevance to man; human sensitivity
- "Quantitative" aspects:
  - Safety ratio: exposure at NOAEL of the most sensitive species relevant to humans for effect in question
  - Overall weight of evidence, including e.g. also
    - Risk-benefit aspects incl. in particular
      - Severity and reversibility of effect in question
      - Number of species affected
    - Alternatives on the market
    - Exposed population, in particular age
    - Exposure: how often, how much, how long
    - Can man be monitored for adverse effect?

3. Risk evaluation – Reversibility

- Relatively resistant to toxic injury are
  - Resting spg
  - Sertoli cells. However, if permanently damaged: no spermatogenesis despite spg
- Consequences of androgen (and other hormonal) deficiency are generally reversible, if hormonal climate is re-established
- Granulomatous inflammation of epididymis and sperm granuloma are considered irreversible (may progress)
  Persistent inflammation may be associated with genotoxicity

4. Management

- Surveillance of exposed human beings: ultimate proof often only possible in man, if human testing in man is acceptable

Laboratory analyses	MR performance
Sperm analysis	Function
Hormones, etc.	Pregnancy
	Children

- Limitations of the use of the compound
- Etc.

Review article on troubleshooting

Successful drug development despite adverse preclinical findings

- Part 1: Processes to address issues and most important findings
- Part 2: Examples

Journal of Toxicologic Pathology
(Japanese Society of Toxicologic Pathology)
In print in vol. 23, No. 4 (December, 2010)
http://www.jstage.jst.go.jp/browse/tox

Conclusions Topic C: Methods – 1

- For a general assessment of the MR system standard subacute toxicity studies are sufficient (plus conventional reprotox studies for function)
  - 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) and hormone measurements
  A standard 4-week recovery period is generally not sufficient
- In general terms, there is no best species

Conclusions Topic C: Methods – 2

- 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 assessment of unexpected adverse findings in the MR system following general procedures, but as always take a case-by-case approach

Lecture 2: Practice / Application

C Recommended approaches for evaluation of MR organs (general methods) - 25 min.

- *Including* study design and sampling techniques
- *Excluding* staging and sperm evaluation

D Morphologic evaluation of the testis of laboratory animal species - 10 min.

Excluding

- Background and age related changes
- (Non-)neoplastic changes in male reproductive system

E Endocrine disruption: Guidelines for histopathologic evaluation - 10 min.

- *Excluding* effects of phytoestrogens of reproductive physiology and pathology

Morphologic evaluation of the testis

- When is lesion observed
- What cells are first affected
- Morphological pattern
- ➔ Mode of action (MoA)
- Progression and maximal response
- Reversibility (partial/complete) and by when

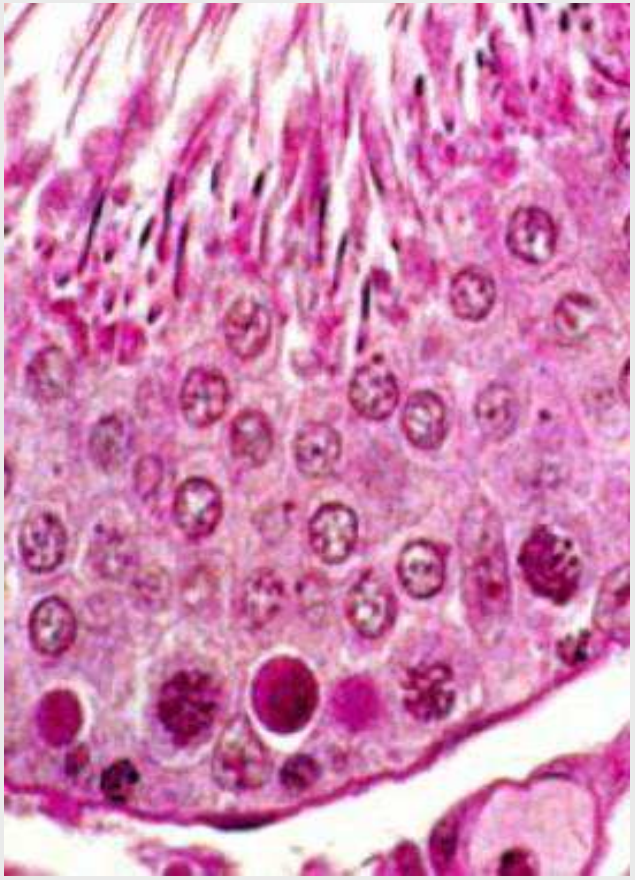
Histological photomicrographs on various induced testicular lesions to be shown

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
  - Spg A stages XI-I
  - Midpachytene spc stage VII
  - Spc in meiosis stage XIV
  - Step 7 and 19 spt in stage VII

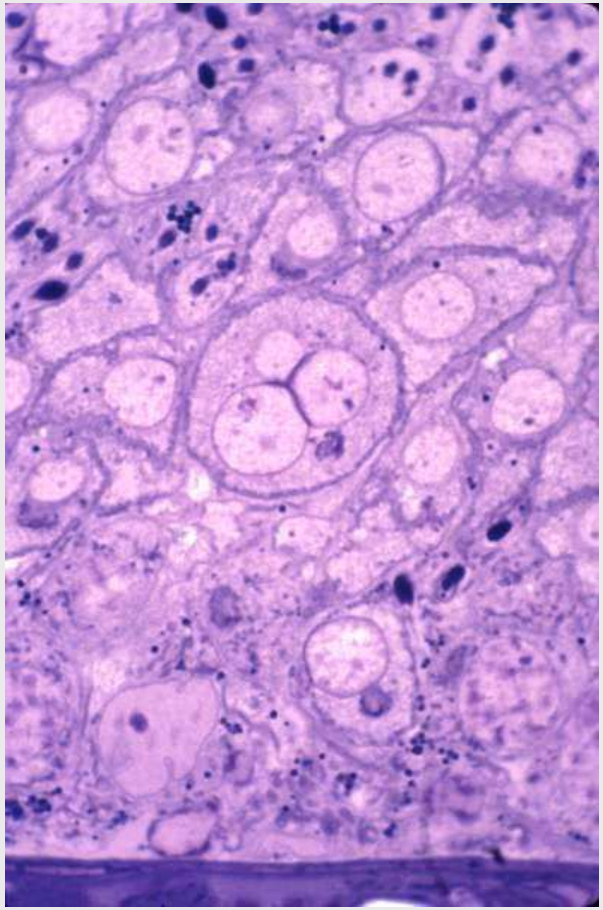


Germ cell (GC) toxicity – Early signs – 2

Spermatids (spt)

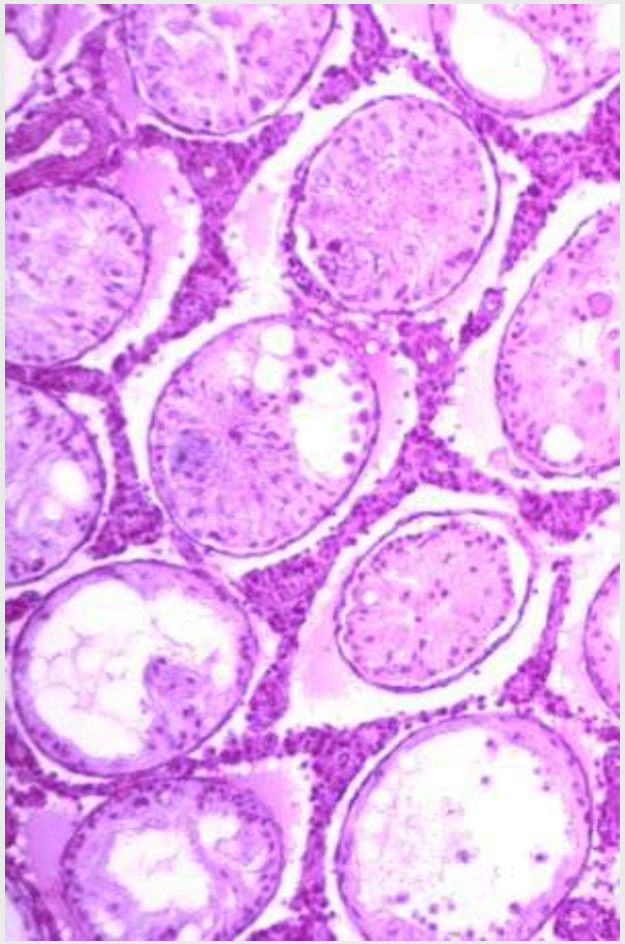
- Early spt form multinucleated giant cells*: fusion of syncytial cell groups, often with fused nuclear acrosome
- Late spt are not released but move to basal portion of tubule: spermatid retention in stages VIII to XII, an early sign of testicular toxicity

* Occasionally also arising from spc



Germ cell (GC) toxicity – mid-long term

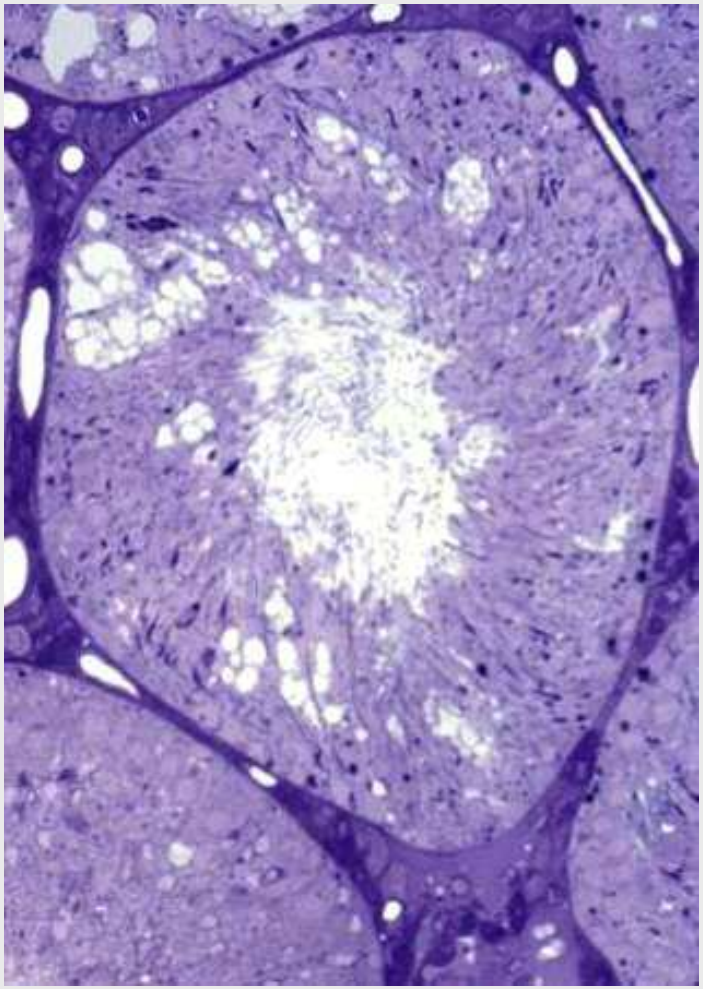
- After "a couple" of days
  - Depletion of specific GC generations: small gaps
- After "a couple" of weeks
  - Maturation depletion of target and more mature GC: larger gaps
  - Spermatogenesis may appear "arrested" at earlier cell types
- Long-term effects
  - If also spermatogonia affected: SC-only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



Histological photomicrographs of testicular lesions due to anticancer treatment of men to be shown

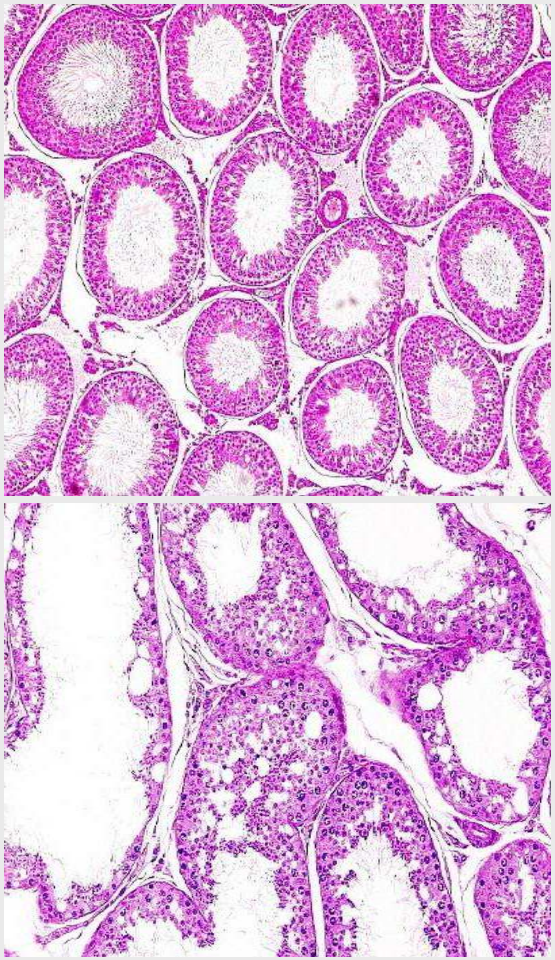
Sertoli cells (SC) – a frequent target

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



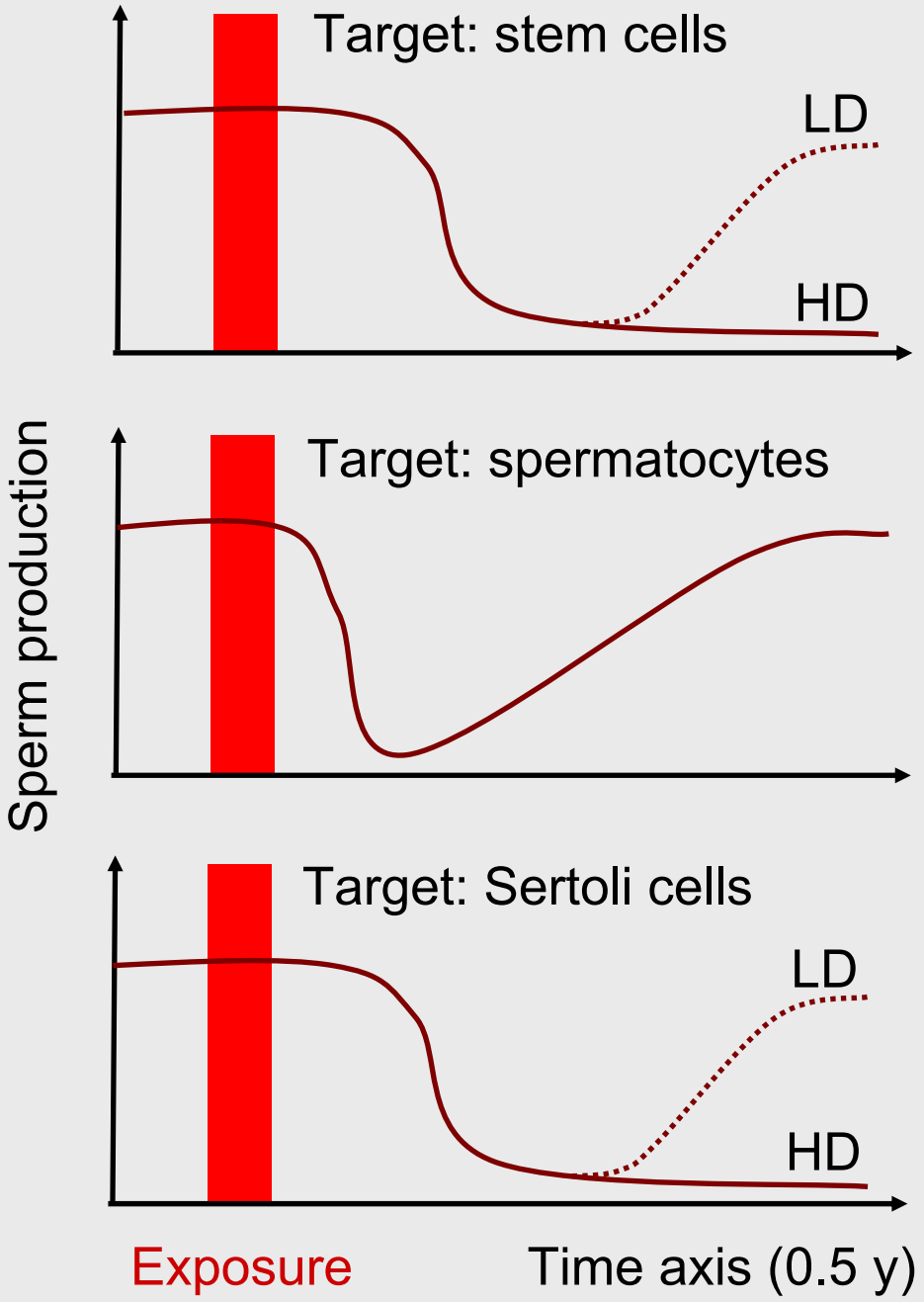
Fluid imbalance

- 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



Effect on semen

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



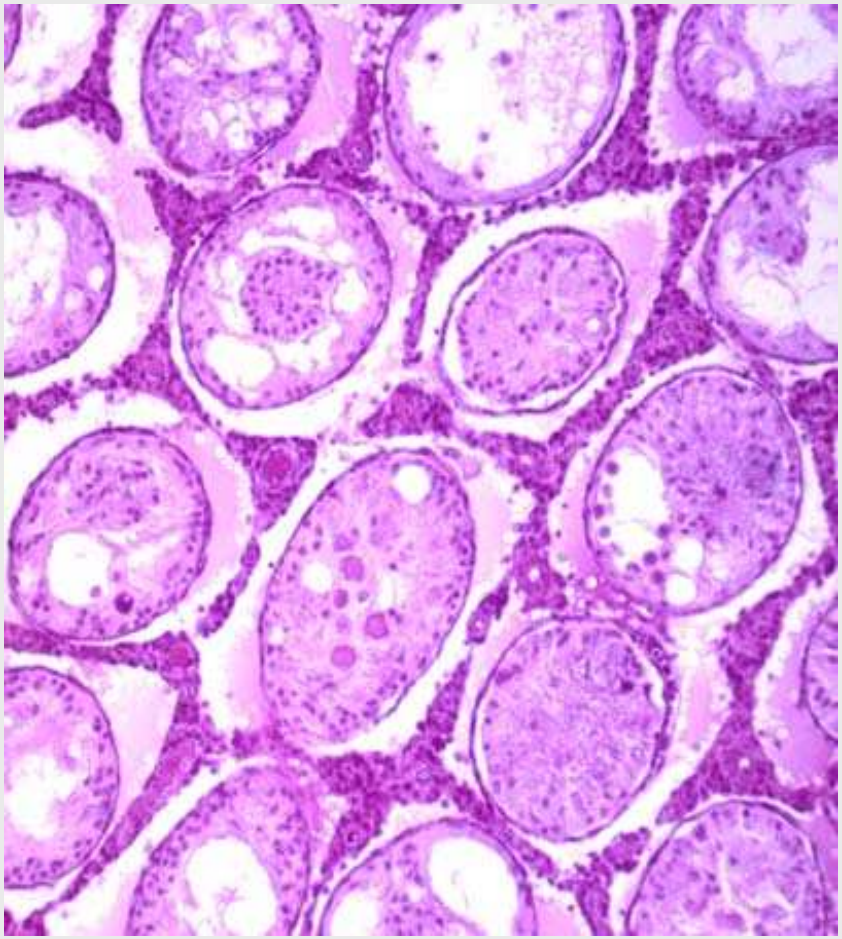
Leydig cells toxicity

- Well visible are trophic changes: atrophy, hypertrophy/hyperplasia and neoplasia
- Other morphological signs for primary LC toxicity are not readily evident on standard histological sections with exception of
  - 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

In the long-term 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

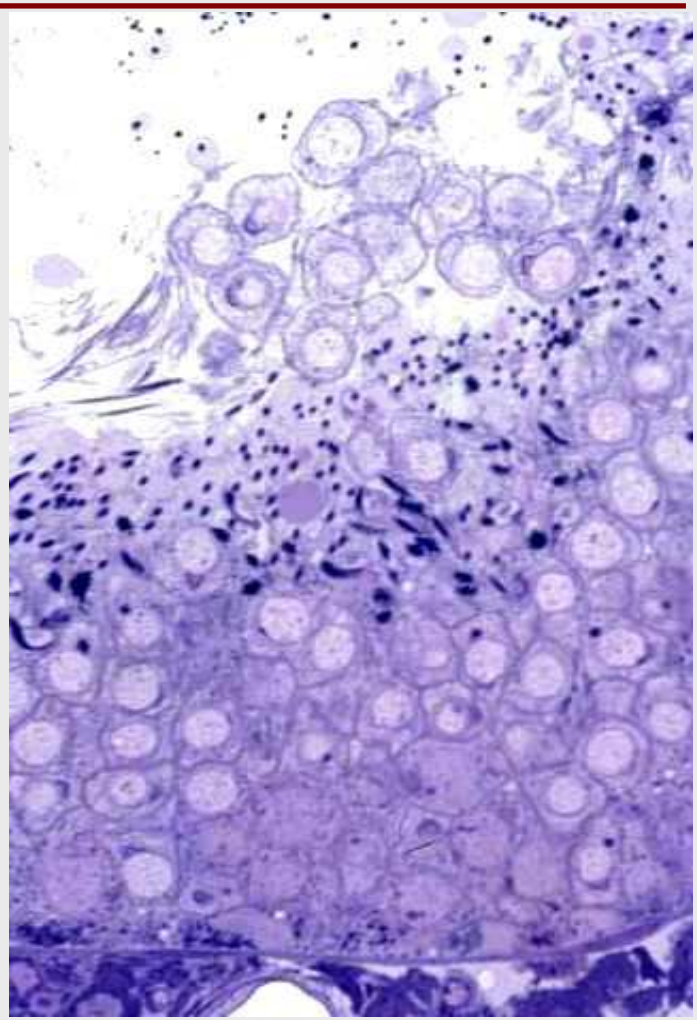
- A consequence of ischemia
- E.g. following
  - Vascular endothelial necrosis with cadmium
    May cause ischemic necrosis of the testis
  - Vasoconstriction with serotonin or histamine
    May cause focal tubular necrosis
- Associated with inflammation and potentially with autoimmune reaction

Epididymis damage

- Vacuolation of epididymal epithelium
  - Lack on androgen
  - Chemical injury, e.g. by oxidosqualene cyclase
- Granulomatous inflammation, e.g. following epithelial necrosis in the caput by cadmium (an endothelial toxin) or α-chlorohydrin (inhibition of fluid resorption)
  - ➔ Breakdown of the blood-epididymis barrier
  - ➔ Immunologically competent cells attack sperm (antigenetically foreign)
  - ➔ Sperm granuloma
    Also seen in ductuli efferentes. Occur there also spontaneously from blindly ending ductuli

Epididymis – a sensitive indicator

- Subtle testicular damage often resulting in germ cell sloughing is generally most apparent from cellular debris in the epididymis.
- Sperm reach
  - Caput within a few days
  - Cauda within 1-2 weeks
  - ➔ Cell debris might be present also after initial testicular insult is resolved



Conclusions Topic D: Morphology

- 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)
  - Sertoli cell toxicity
- Epididymal content is an excellent and “historic” indicator of damage of spermatogenesis

Lecture 2: Practice / Application

C Recommended approaches for evaluation of MR organs (general methods) - 25 min.
- *Including* study design and sampling techniques
- *Excluding* staging and sperm evaluation

D Morphologic evaluation of the testis of laboratory animal species - 10 min.
Excluding
- Background and age related changes
- (Non-)neoplastic changes in male reproductive system

E Endocrine disruption: Guidelines for histopathologic evaluation - 10 min.
- *Excluding* effects of phytoestrogens of reproductive physiology and pathology

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

OECD framework for Endocrine Disruptors

1. Prioritize testing (if at all) on existing information
2. *In vitro* testing for mechanism: e.g. QSAR, receptor binding, transcriptional analysis, steroidogenesis, etc.
3. *In vivo* for single endocrine mechanism: e.g. uterotrophic (E), Hershberger (A) assay, other endocrine organs, etc.
4. *In vivo* for multiple endocrine mechanisms: e.g. enhanced OECD 407 (4 w), pubertal assays, etc.
5. *In vivo* for other adverse effects: e.g. reprotoxicity studies, etc.

European Medicines Agency - Pharma

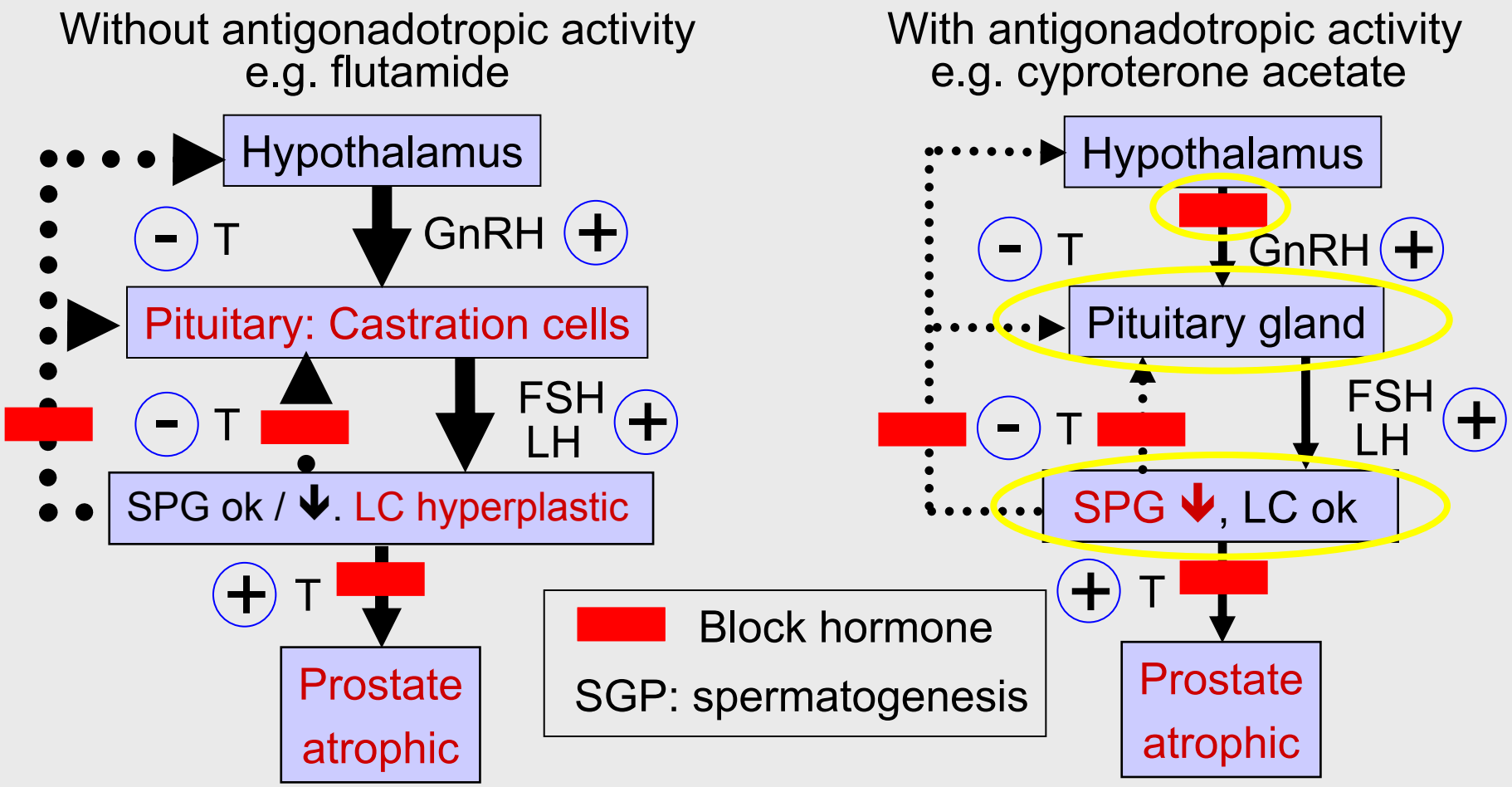
Environmental risk assessment (ERA) for human pharmaceuticals

Exemptions for certain types of chemicals are overruled when the substance has endocrine active properties

Trigger value for mandatory testing not relevant for endocrine active substances

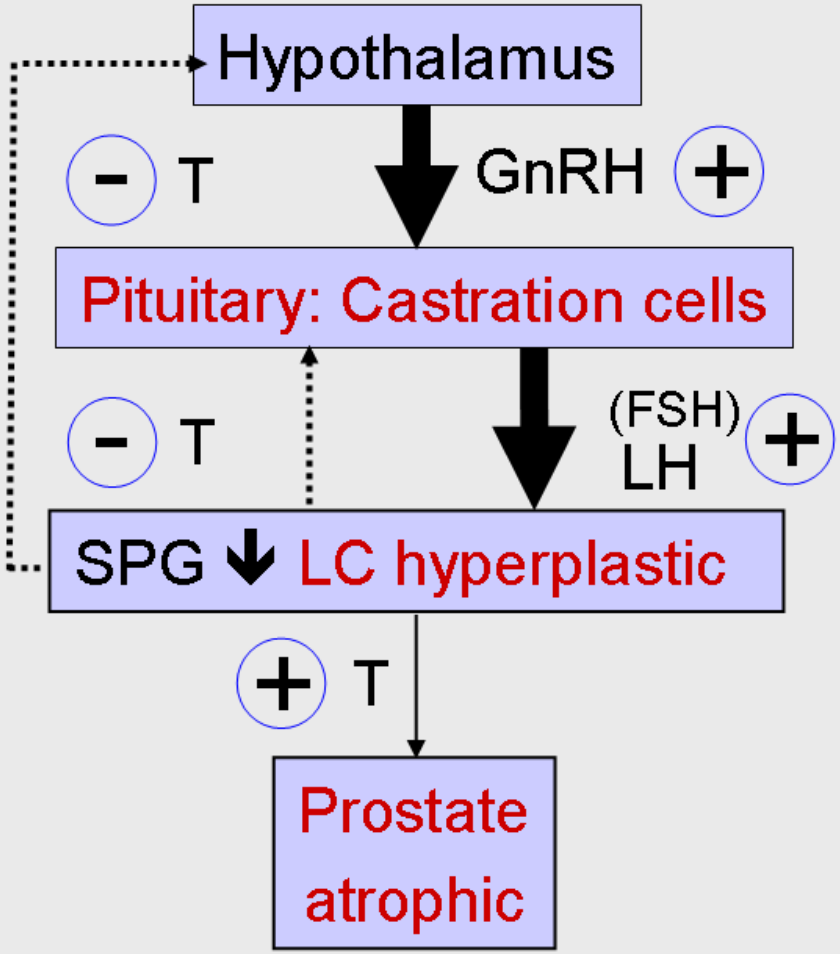
Impact on the environment should not constitute a criterion for refusal of a marketing authorization.

Antiandrogen action - Simplified



Histological photomicrographs on flutamide effect on MR system to be shown

Inhibition of T biosynthesis



Reduction of testosterone – Testis – 1

- Most sensitive testicular indicator seen within days:
  - Degenerating spc and round spt in stages VII and VIII
  - Retention of elongated spermatids in stages VIII to XII, when they get phagocytized
  - Reduced luminal diameter
- Later changes: degenerating spc and late spt in IX-XIV, a consequence of damage suffered earlier in VII-VIII

Reduction of testosterone – Testis – 2

- End stage after approx 2 weeks:
  - Maturation depletion
    - Reduction of mid/late spc and round spt
    - Loss of elongating and mature spt
  - Shrinkage of tubule: less tubular fluid produced
  - Depending of pathogenesis: LC
    - Atrophic (e.g. primarily low LH) or
    - Hypertrophic (block of steroidogenesis)

Reduction of testosterone – Other organs

- Progressive atrophy of epididymis, starting in caput
- Accessory sex organs: inactive epithelium with decreased secretion and lumina
  At EM level:
  - Reduction of RER, Golgi and secretory granules
  - Increased size of lysosomes and number of autophagic vacuoles
- Castration cells in pituitary
  Hypertrophic cells producing gonadotropins including LH and prolactin cells
- Mammary glands may become feminized

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, finastride)
  - Direct action on estrogen receptors by estrogenic drugs (clomiphine)
  - Displacement of estrogen from binding globulin (free estrogen ↑, e.g. spironolactone)
  - Via damage the testis (anticancer drugs)

Histological photomicrograph on estrogen effect on prostate to be shown

Species differences

- Rats lack sex hormone binding globulin
- Rats react more sensitively
- Rat Leydig cells have a high density of LH receptors
- Influence of prolactin on LH receptor function in rats
- Presence of GnRH receptors on rat Leydig cells
- Waning endocrine milieu in the aging rat

Conclusions Topic E: Endocrine disrupters

- Endocrine disrupters not only affect the testis but generally also the accessory MR sex organs
- Environmental endocrine disrupters are particularly feared
- For drugs endocrine side effects are a matter of risk evaluation
- Endocrine effects on MR system 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 and 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 incl. knowledge of staging
  - Confounding factors incl. immature test animals
- Important risk parameters
  - Safety ratio
  - Reversibility
  - Monitorability in man

Final Conclusions – 2

- Identification of primary target might help to establish 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 spermatogonia (may be difficult to find in histological sections)
- Hormonally mediated effects are generally reversible, affect early accessory sex organs and are often species-specific