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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Toxicologic Pathology of the Male Reproductive System – 1

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

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Handout version of presentation

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Toxicologic Pathology of the MR System

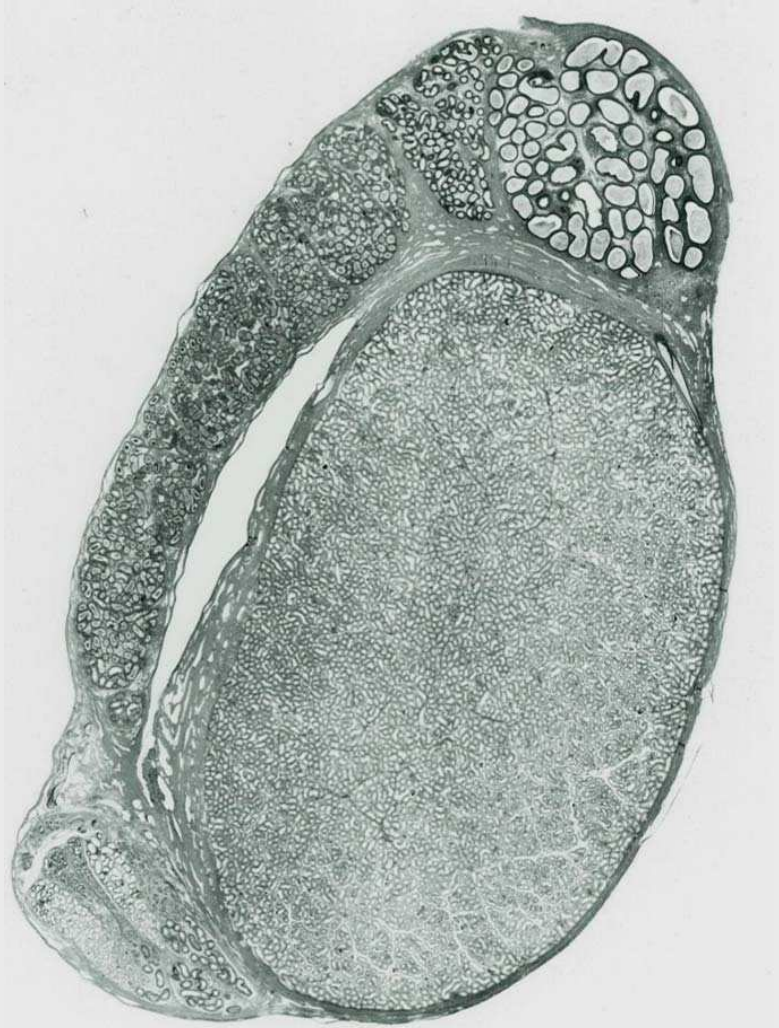
- 2 lectures
  - The basis – 45 min
    - **The male reproductive (MR) system – 25 min.**
      - Overview over MR toxicity – 20 min.
  - Practice: Methods and Examples – 45 min.
- Covered: mainly rats as well as some particularities of other species
- Not covered: Developmental reproductive toxicity

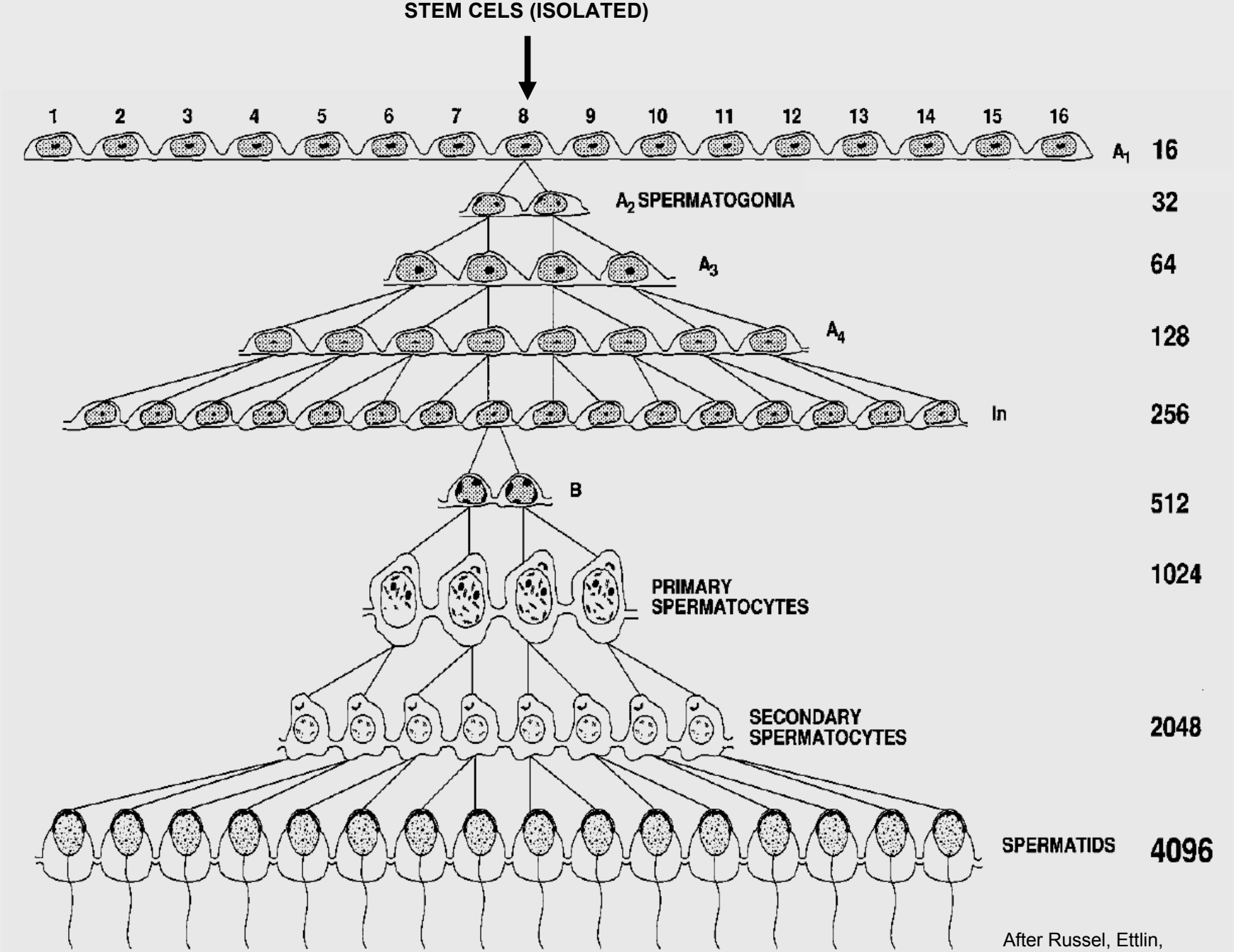
The MR System

Organ		*Function*
Main organ	Testis	Sperm and fluid production
Transport (main parts)	Rete	Transport, fluid
	Epididymis	Transport, maturation, fluid
	Vas deferens	Ejaculation
Accessory sex organs ➔ Secretions	Seminal vesicle	Nutrients
	Coagulation gland	Copulatory plug
	Prostate	Proteolytic enzymes
	Bulbourethral gland	Copulatory plugs
	Preputial gland	Pheromones
Shared with urinary system	Urethra	Transport
	Penis	Copulation

The MR System

- Testis
  - Epididymis
  - Accessory sex organs
  - Other structures
  - Endocrine, paracrine and autocrine regulation
  - Embryology - Puberty
  - Comparison laboratory animals vs. humans
  - Conclusions





After Russel, Ettlin, SinhaHikim and Clegg, 1990

Spermatogenesis – Spermatogonia

- Stems cells
  - Pale dusty nuclear structure
  - Isolated
  - Slowly replicating by mitosis, therefore less vulnerable than spermatogonia (spg) A, I, B
- Spg A
  - Syncytium (cytoplasm bridges)
  - Committed to differentiation
  - Relatively rapid proliferation by mitosis ➔ vulnerable
  - Similar morphology and difficult to see in routine sections
- Intermediate (I) and B spg
  - Increasing formation of heterochromatin clumps

Outside blood-testis barrier (BTB)

Stem cell ↓ A1 ↓ A2 ↓ A3 ↓ A4 ↓ I ↓ B

Spermatogenesis – Spc and spt

As of leptotene spc all cells inside BTB

Primary spermatocytes (spc)
Develop into the largest cells in germinal epithelium

↓

Meiosis I (reduction division), 1.5 cycles long

↓

Secondary spc
Haploid chromosome set, but double chromosome number
Smaller than primary spc and short-lived

↓

Meiosis 2 (division without S-phase): very short in stage XIV

↓

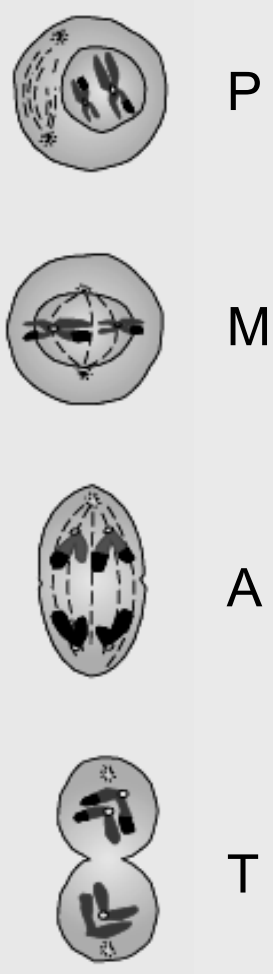
Spermatids (spt)
Haploid, single chromosomes
Spermiogenic differentiation over 1.5 cycles
Steps 1-19 based on morphologic criteria

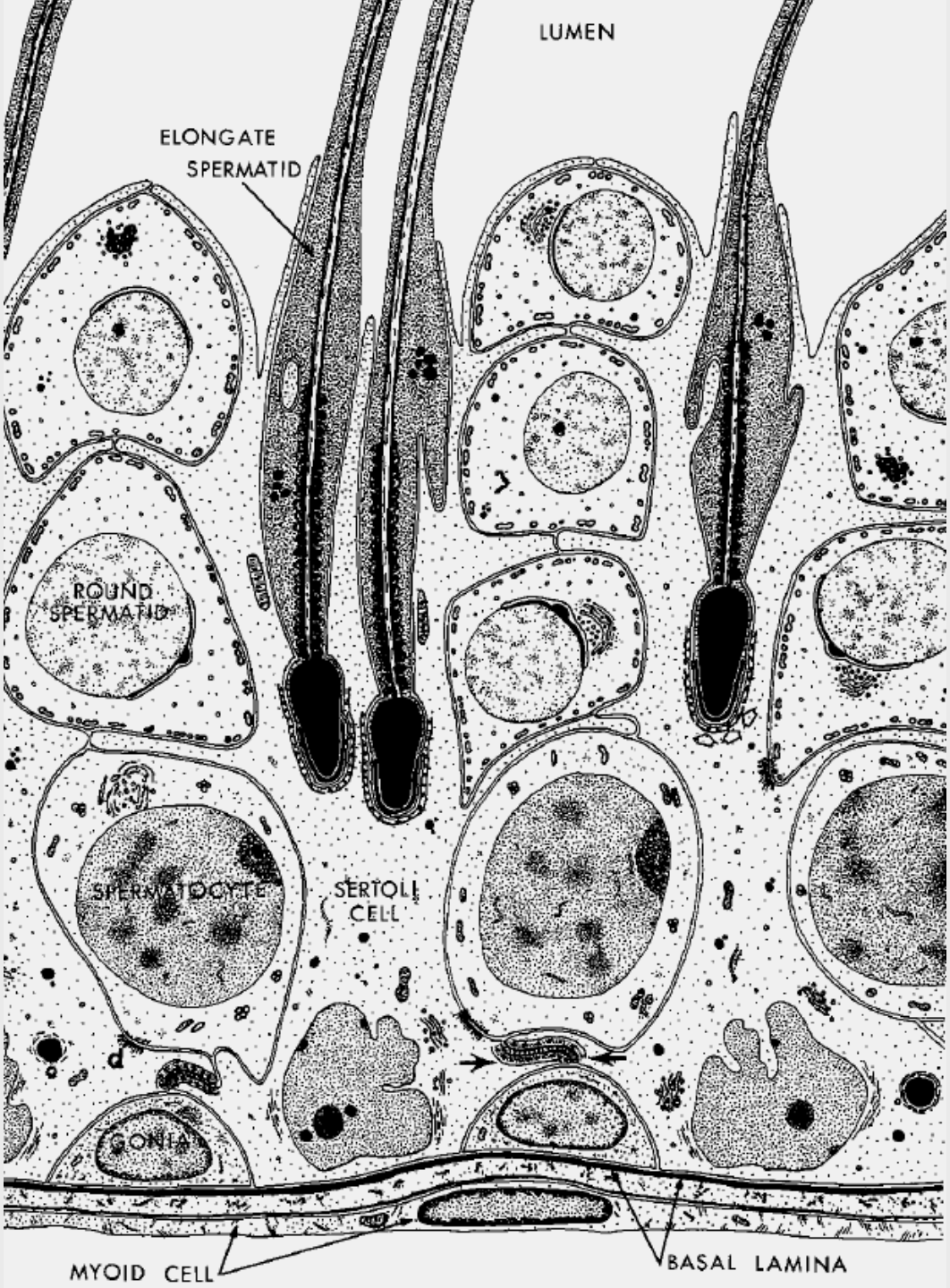
Meiosis 1

Identical phases also in meiosis 2 and mitosis

- Prophase: long – nuclear size increases
  - Preleptotene: start to move through blood-testis barrier (intermediate compartment)
  - Leptotene: chromatin threads forming
  - Zygotene: chromosomes (chr) pairing
  - Pachytene: > 1 week (depending on species), crossing over, rapid growth
  - Diplotene: short, chr separate
    largest cells in seminiferous epithelium
- Metaphase: chr in metaphase plate
- Anaphase: chr separate
- Telophase: cell division completed
  Formation of new cell membrane

Short – Stage XIV



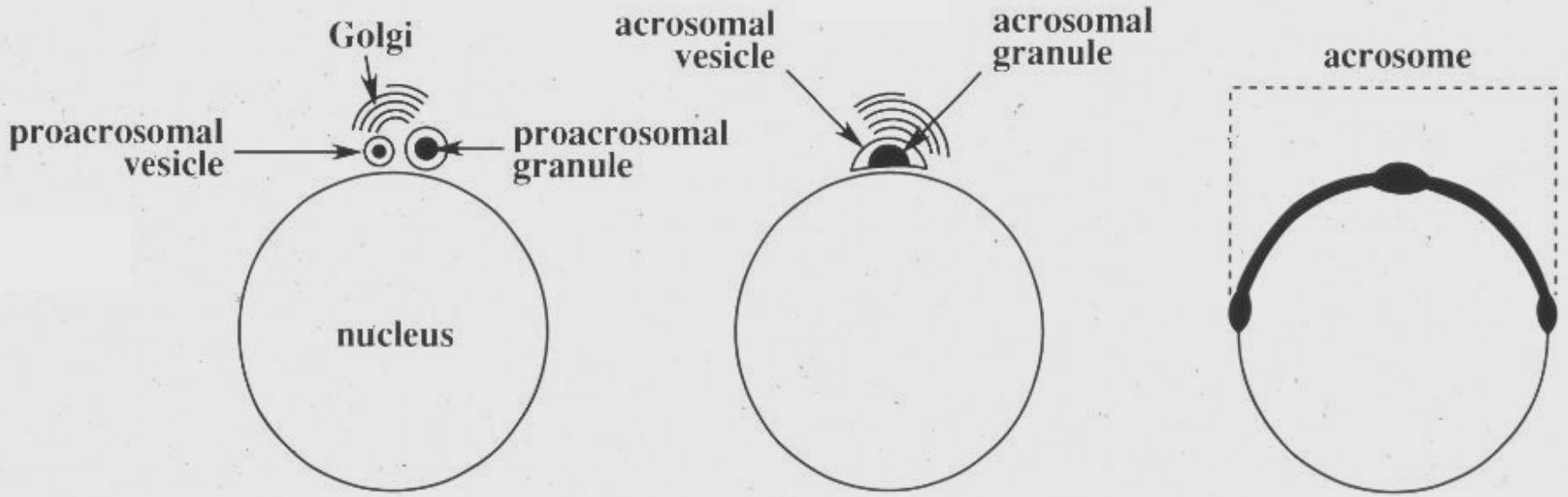


Russel, Ettlin, SinhaHikim and Clegg, 1990

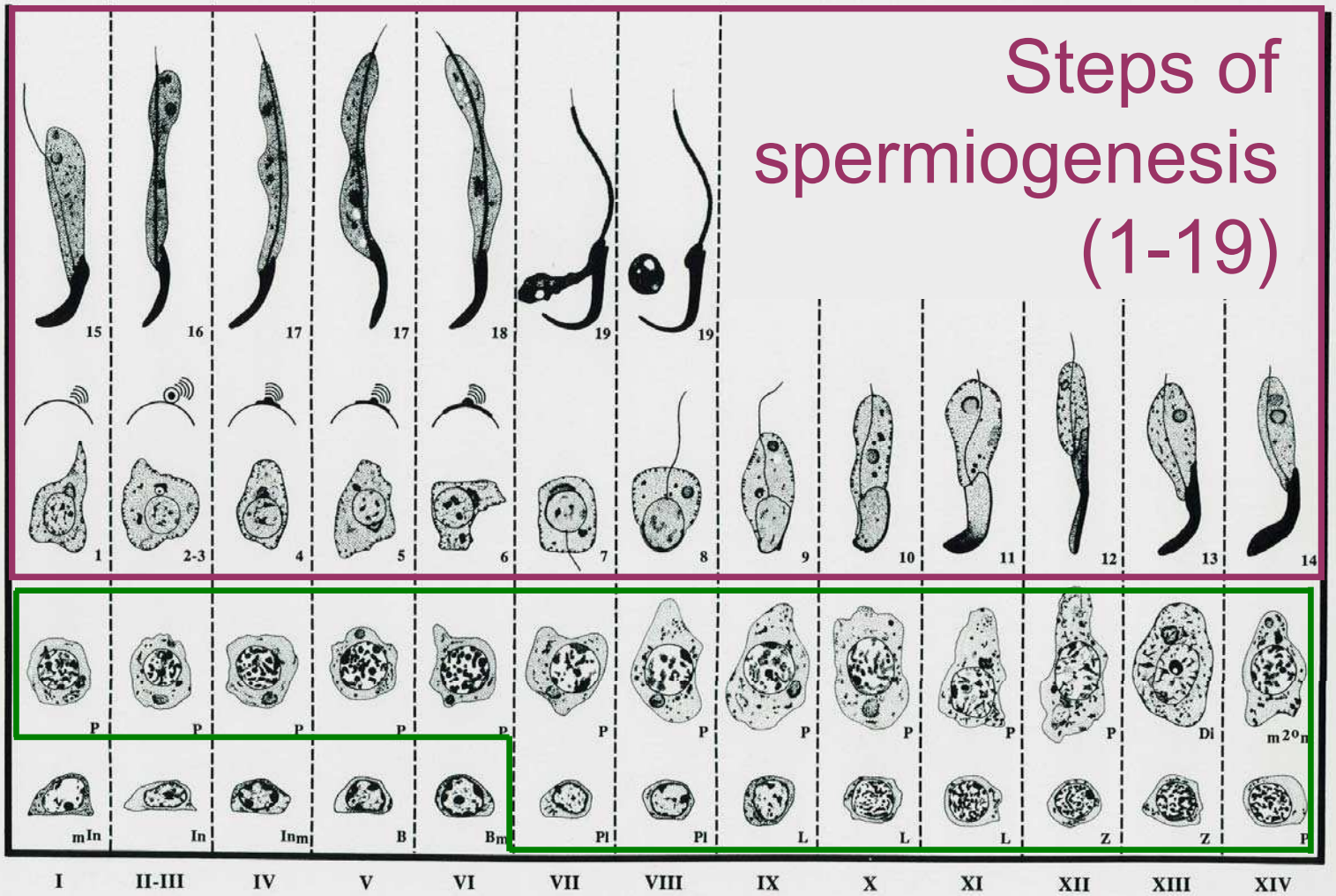
The acrosome – Key for staging

Spermatid steps (and meiosis) define stages of spermatogenesis

Staging explained in separate presentation



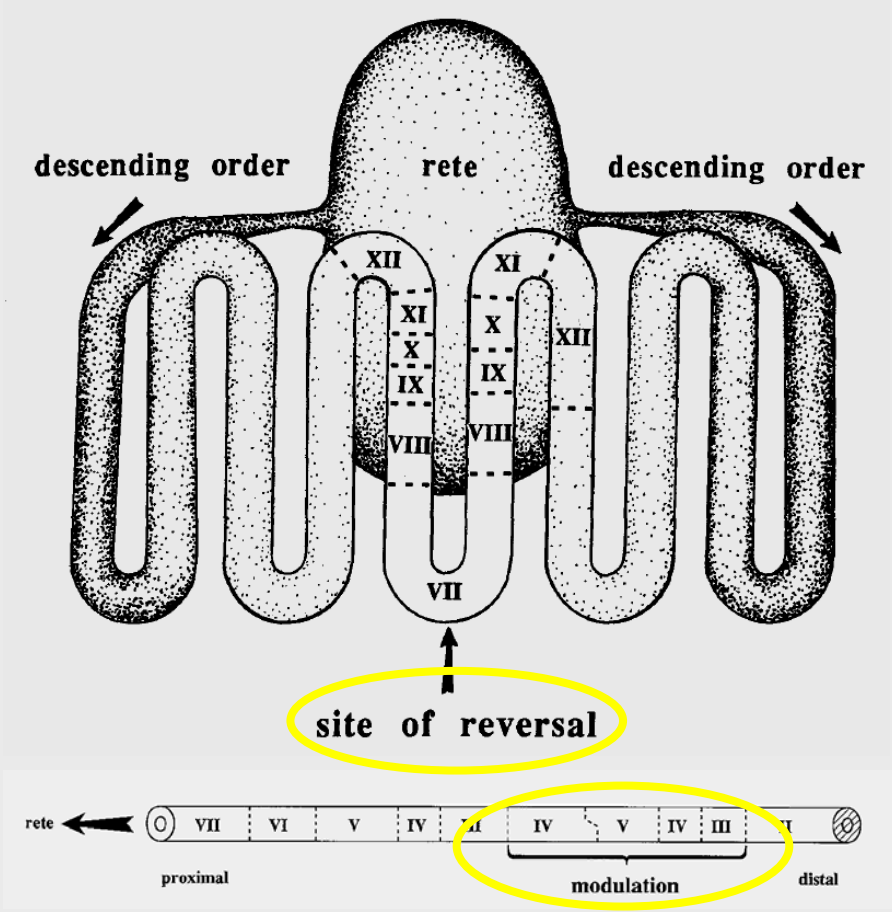
Russel, Ettlin, SinhaHikim and Clegg, 1990



RAT: stages of spermatogenesis I-XIV

Russel, Ettlin, SinhaHikim and Clegg, 1990

Tubular organization



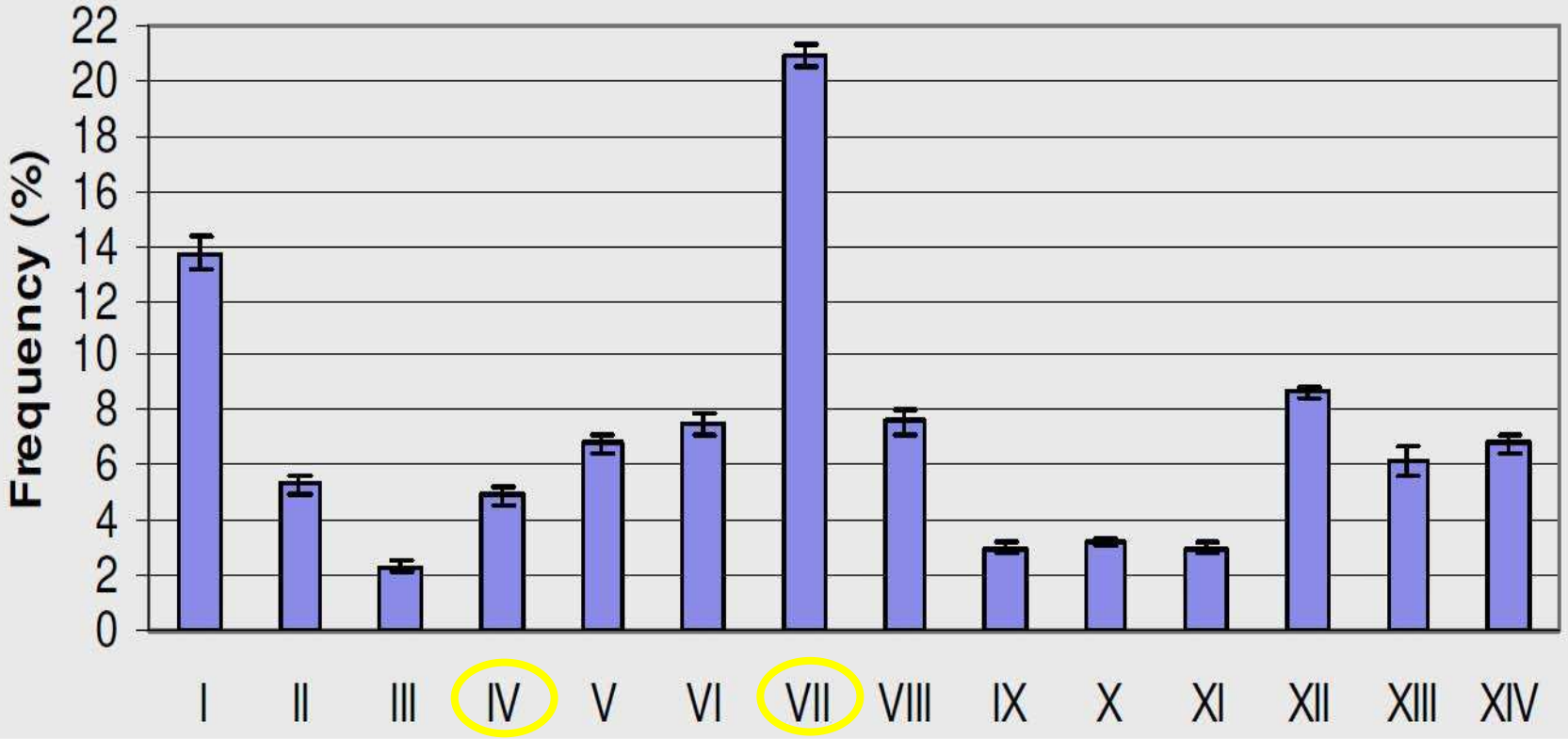
Approx. 20-30 U-shaped seminiferous tubules ending on both sides in the rete testis

Rat: ~ 12 m/g testis
Spermatogenic longitudinal wave

Man: ~ 15-25 m/g testis
Helical arrangement of spermatogenesis

Russel, Ettlin, SinhaHikim and Clegg, 1990

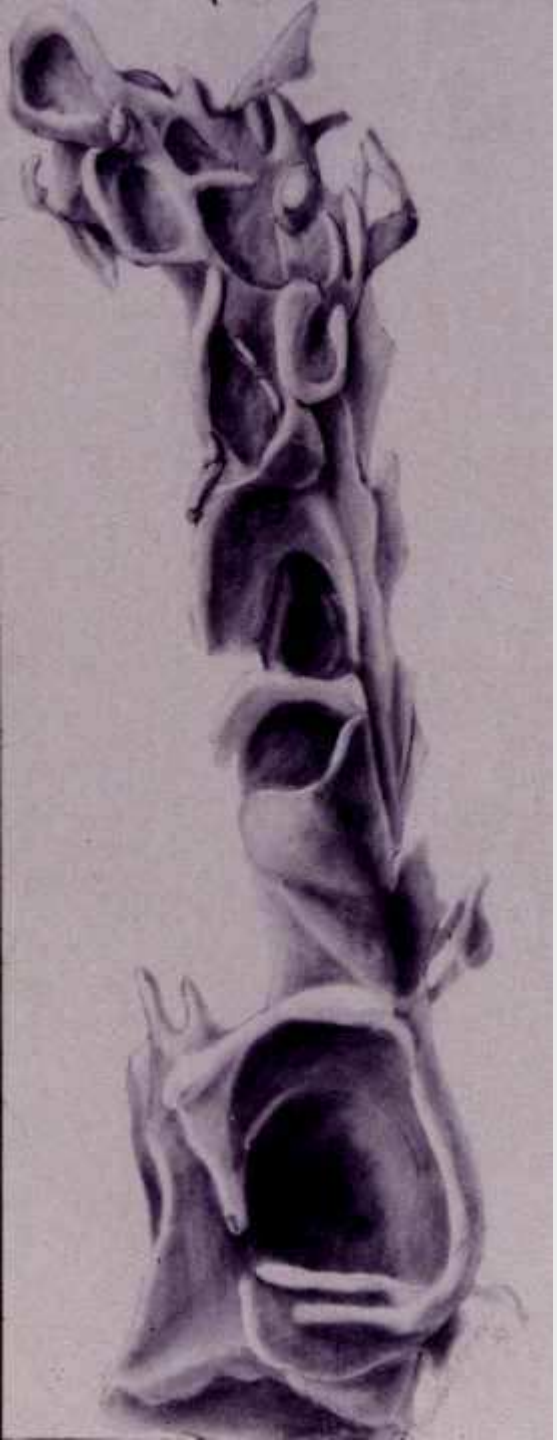
Frequency of stages in rat testis



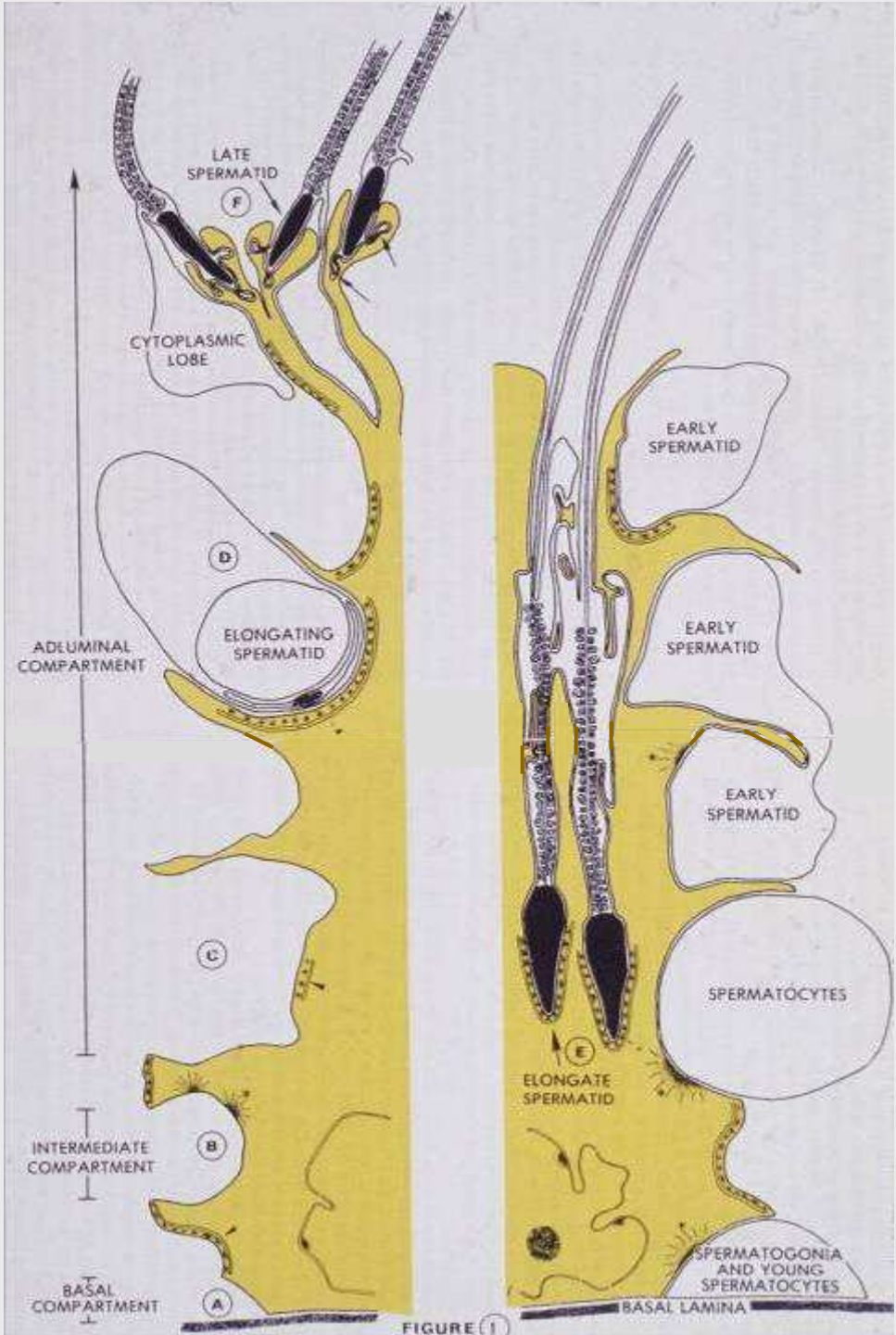
The duration of a stage determines the frequency of its occurrence
Example: stage VII lasts approx. 58 hours, stage IV approx. 12 hours in rats

Hess, 1990

Histological photomicrographs to be shown

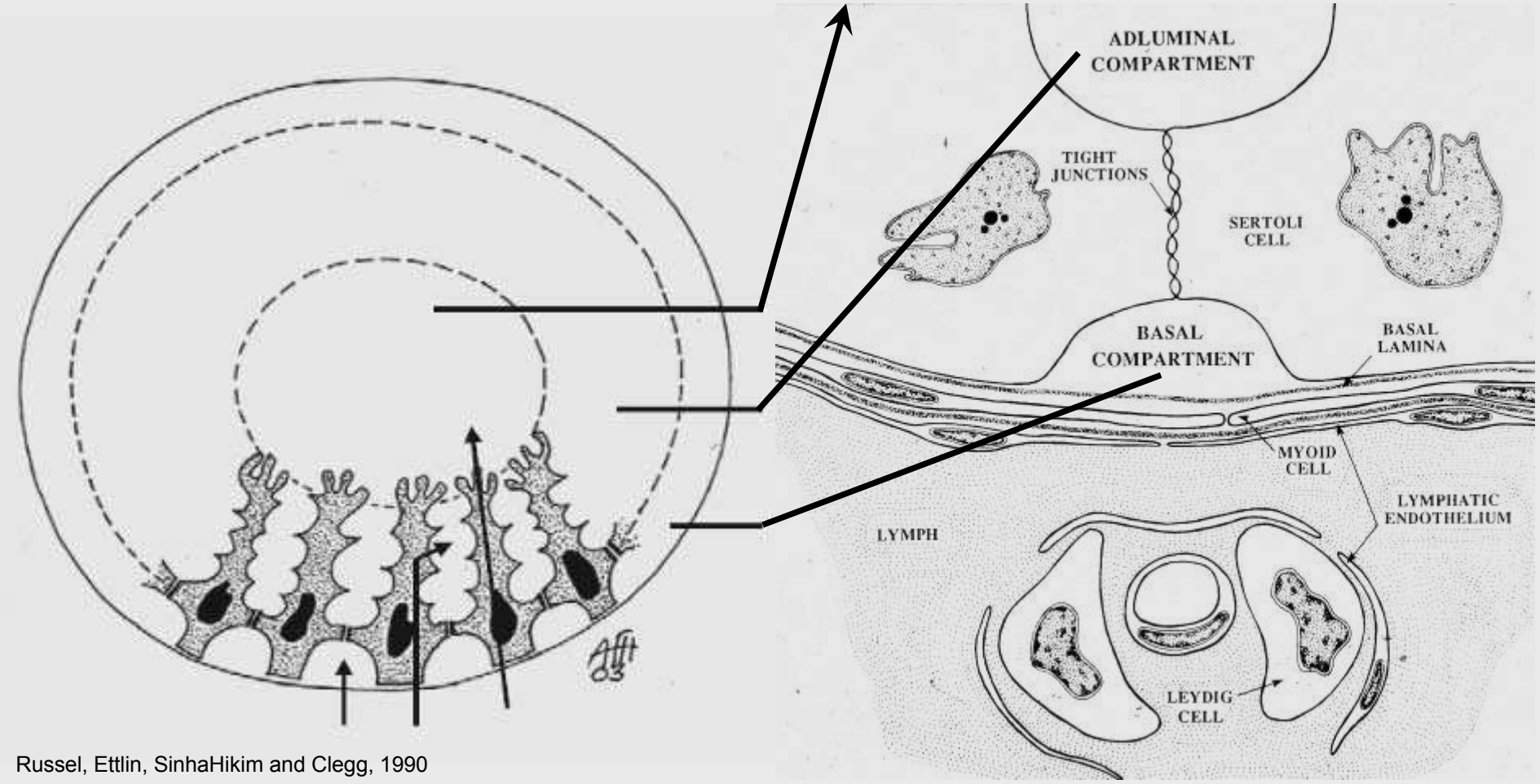


Sertoli cell



Russel, Ettlin, SinhaHikim and Clegg, 1990

Sertoli cells – 3 testis compartments



Russel, Ettlin, SinhaHikim and Clegg, 1990

Sertoli cells (SC)

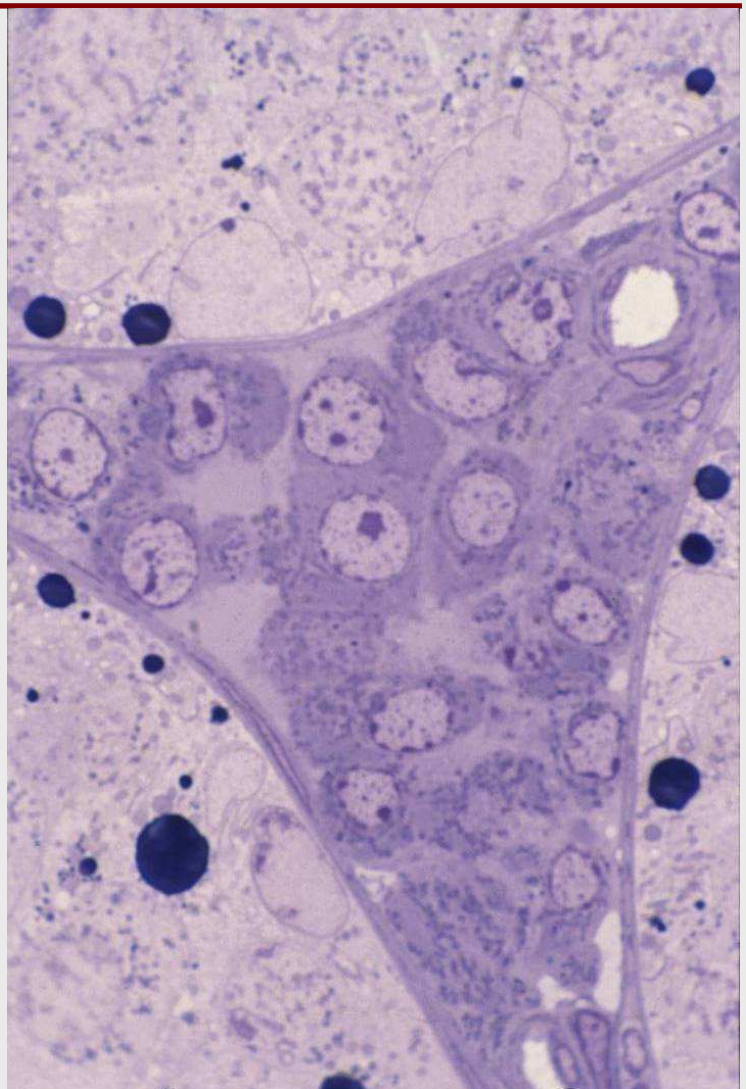
- Divide in rats until 18-21 days of age
- Blood-testis (tubule) barrier
- Structural support to germinal epithelium
- Nutrition of germ cells: no blood vessels in seminiferous epithelium
- Phagocytosis, e.g. of apoptotic bodies and of degenerating germ cells
- Secretion
  - Androgen binding protein, inhibin, activin and other factors
  - Seminiferous fluid

Peritubular cells

- Myoid cells with actin filaments
  ➔ Propulsion of sperm along tubules
- Under control of testosterone (T)
- Modulate SC function, e.g. regarding production of androgen-binding protein

Interstitial space

- Leydig cells (LC) arise from fibroblast-like, often peritubular cells, throughout life
- Localization and number species-specific
- Macrophages (up to 25%), specific function unknown
- Other cells and structures incl. blood and lymphatic vessels



Testis … Rete testis … Epididymis

- Both ends of a seminiferous tubule
  - ➔ Tubuli recti (SC only, valve function ?)
  - ➔ **Rete testis**
  - ➔ Efferent ductuli: 6 (rodents, merge into single entry duct) – 20 (larger mammals incl. humans) *Often discarded with fat pad!*
  - ➔ Epididymis
- Rete testis
  - Single or several interconnected channel(s)
  - Cuboidal or columnar epithelium
  - On cranial outer aspect of rat and mouse testis Central in dogs and rabbits
- Rete testis function: Reservoir, fluid resorption

Microscopic and histological photomicrographs to be shown

For an excellent review article in particularly on the connecting ductal system between testis and epididymis

see

George L. Foley
Overview of male reproductive pathology
Toxicol Pathol 29/1: 49-63, 2001

or

Papers by RA Hess

The MR system

- Testis
- Epididymis
- Accessory sex organs
- Other structures
- Endocrine, paracrine and autocrine regulation
- Embryology - Puberty
- Comparison laboratory animals vs. humans
- Conclusions

Epididymis – 1

- Abrupt transition between efferent ductuli (from rete testis) and epididymis with basal, apical and principal epithelial cells
- One single coiled duct
- Parts
  - Caput and corpus
    - Produce and secrete macromolecules, e.g. of steroids, carnitine, inositol
    - Reabsorb tubular fluid (up to 90%)
  - Caudal part is dilated with stored sperm
    Epithelium also contains clear cells (endocytic activity incl. resorption of remaining sperm cytoplasm)

Epididymis – 2

- Epididymal blood-lumen barrier (not as effective as BTB)
- Sperm reach
  - Caput approx. 2-5 days after release
  - Cauda approx. 1-2 weeks after release

  ➔ “Memory” of damage to spermatogenesis
- During epididymal passage sperm mature (morphology and metabolism)

  ➔ Capacitation: surface antigens enable fertilization

The MR system

- Testis
- Epididymis
- Accessory sex organs
  - Seminal vesicles
  - Prostate
  - Bulbourethral glands
  - Preputial glands
- Other structures
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Accessory sex organs - Occurrence

Species	Prostate	Seminal vesicles	Bulbour. gland	Preputial gland
Rat	+	+	+	+
Mouse	+	+	+	+
Rabbit	+	+	+	+
Dog	+	-	-	-
Man	+	+	+	-

Macroscopic and histological photomicrographs to be shown

Secretions

- Seminal vesicles: ~ 60% of semen; yellow-white and viscous, alkaline, nutrients for sperm survival (e.g. fructose, citric acid)
- Coagulating gland: proteins for copulatory plug
- Prostate gland: ~ 30% of semen, colorless, proteolytic enzymes
- Bulbourethral glands (Cowper): ~ 5% to semen, mucoid and clear, contributes to copulatory plug
- Preputial gland (sebaceous): pheromones and β-glucouronidase

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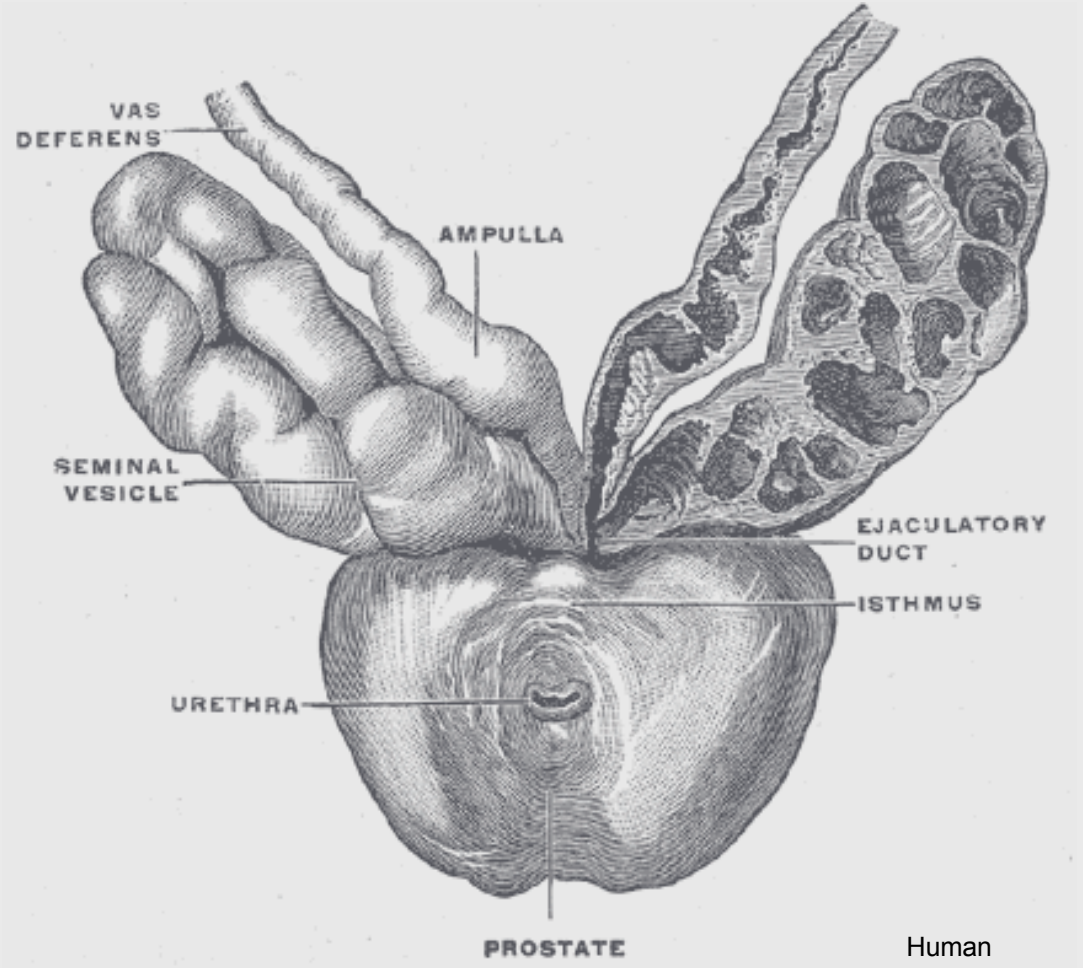
Efferent ducts

- Ducts deferens (also called vas deferens)

  Widens at the end: ampulla
- Ejaculatory duct

  Joining of ductus deferens and seminal vesicle
- Urethra



Human

Penis

- Tactile or psychological stimulation leading to
  - Erection due to vasodilation of blood vessels mainly controlled by parasympathetic nervous system (NS)
  - Emission and ejaculation due to contraction of ductus deferens and “pelvic” muscles mainly controlled by sympathetic NS
- Erectile dysfunction in man is often multifactorial

The MR system

- Testis
- Epididymis
- Accessory sex organs
- Other structures
- **Endocrine, paracrine and autocrine regulation**
- Embryology - Puberty
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“Sex” hormones regulation – 1

- Gonadotropin releasing hormone (GnRH), also called LHRH
  - From hypothalamus by pulsatile release and SC
  - Acts on pituitary (gonadotropins ↑), LC (rat only)
- Luteinizing hormone (LH), formerly also called ICSH
  - From pituitary by pulsatile release
  - Acts on LC (steroidogenesis ↑)
- Follicle stimulating hormone (FSH)
  - From pituitary by pulsatile release
  - Maintenance of spermatogenesis (esp. spg, spc) directly and indirectly (via SC)
    Acts on SC to produce regulatory proteins, etc.

“Sex” hormones regulation – 2

- Prolactin (PRL):
  - From pituitary by pulsatile release
  - Acts on prostate and seminal vesicles (regulation of secretion)
    LC (maintenance of GnRH receptors in rats)
- Inhibin:
  - From SC
  - Acts on pituitary (FSH production ↓, LH unchanged)
    Possibly also paracrine effect within testis
- Activin
  - From SC
  - Acts on pituitary (FSH production ↑)

“Sex” hormones –T and metabolites

- Testosterone (T):
  - From LC by pulsatile release
  - Acts on SC to produce androgen binding protein
    ➔ T concentration in testicular fluid can be up to 100 times higher than in plasma
    Acts also directly on spermatids, accessory sex organs, associated blood vessels and peritubular myoid cells
- Dihydrotestosterone (DHT):
  - Produced from T e.g. in epididymis and accessory sex organs
  - Acts on epididymis, prostate and seminal vesicles
- Estradiol etc. (E):
  - Produced from T by aromatases e.g. in adipose tissue, LC, SC (neonate only)
  - Acts on LC (T ↓), pituitary (LH ↓), etc.

Other hormones influencing MR system

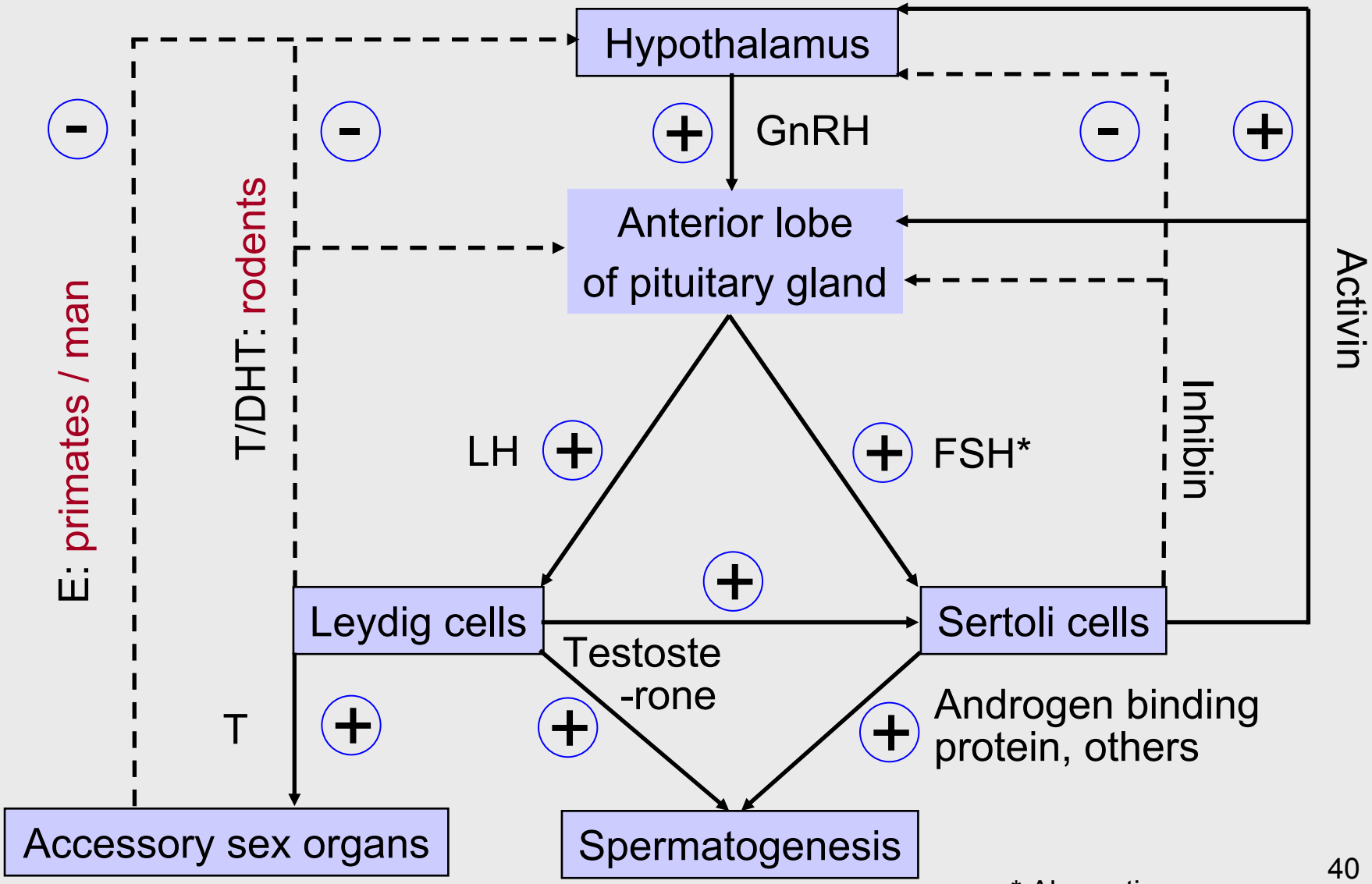
- Thyroid hormones T4, T3:
  From thyroid, act on SC (maturation of prepubertal SC)
- Androstenedione:
  From adrenal cortex, act on human prostate (growth promotion)
- Aldosterone:
  From adrenal cortex, act on epididymis (fluid regulation)
- Oxytocin:
  From LC und pituitary, act on peritubular myoid cells of testis and on epididymis (sperm transportation ↑)
- Insulin:
  From pancreas, act on SC (glucose uptake)
- Etc.

Paracrine regulation of MR

- Some of the aforementioned hormones have also paracrine effects, e.g. inhibin, activin, T, DHT, E
- Growing list of further factors with functions often not well understood, including e.g. endorphin, ACTH and MSH produced by LC
- Production of such factors seems often stage-dependent
- etc.

Hormonal regulation of the MR system

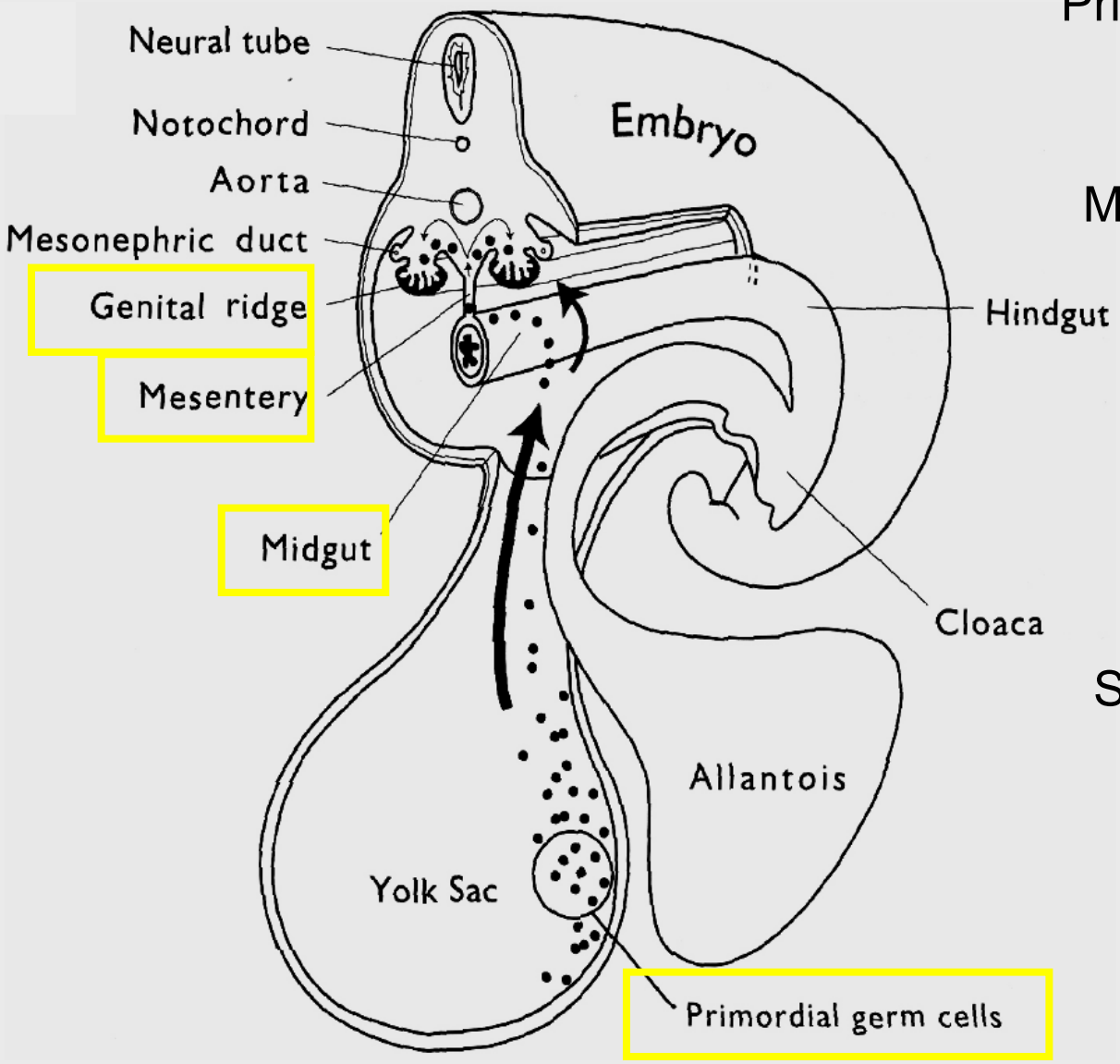
Simplified



* Also acting on spg

The MR system

- Testis
- Epididymis
- Accessory sex organs
- Other structures
- Endocrine, paracrine and autocrine regulation
- **Embryology** (see handout) - **Puberty**
- Comparison laboratory animals vs. humans
- Conclusions



Primordial GC from yolk sac endoderm

via

Midgut region / mesentery

to

Genital ridge

+

Somatic cells from mesonephros (SC)

⬇

Seminiferous (medullary) cords

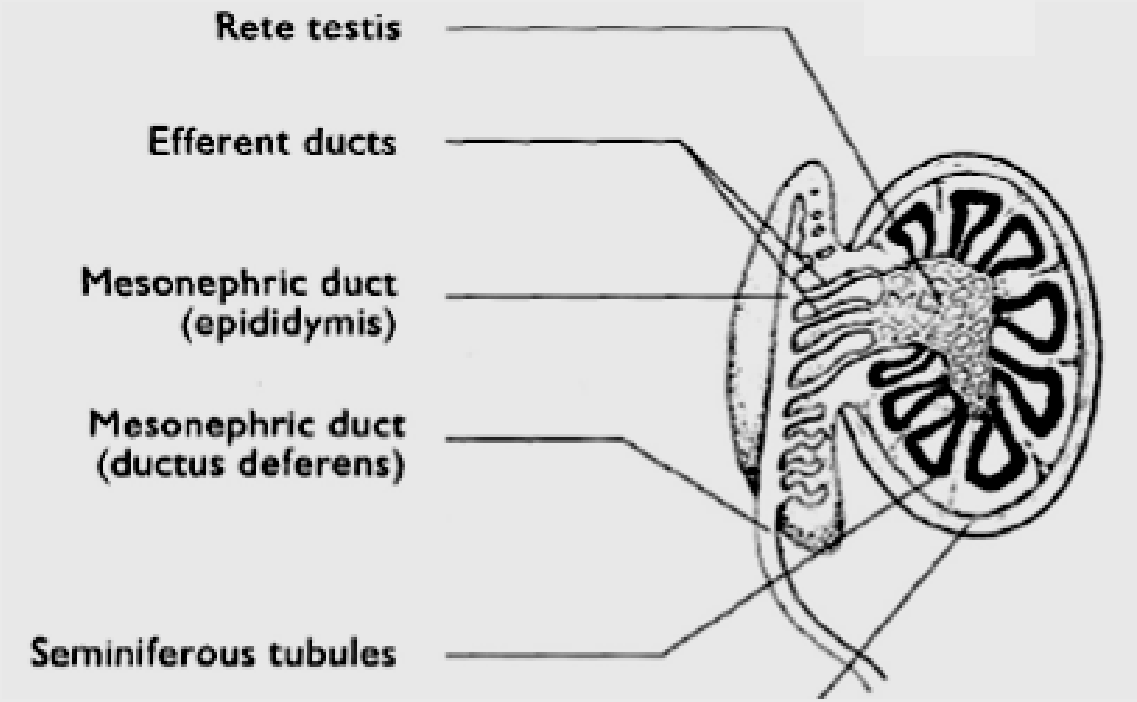
+

Interstitial cells (mesenchymal, LC)

⬇

Testis

Development of testis and efferent ducts



Wolffian (mesonephric) duct

↓

Efferent ducts

Seminal vesicles

Fetal LC produce temporarily T ➔ MR system

Are later replaced by adult LC (rat > PN day 10)

Puberty in laboratory animals

Species	Puberty
Rat	8 - 10 weeks
Mouse	7 - 8 weeks
Dog	7 - 12 months
Monkey	3.5 - 4.5 years

The MR system

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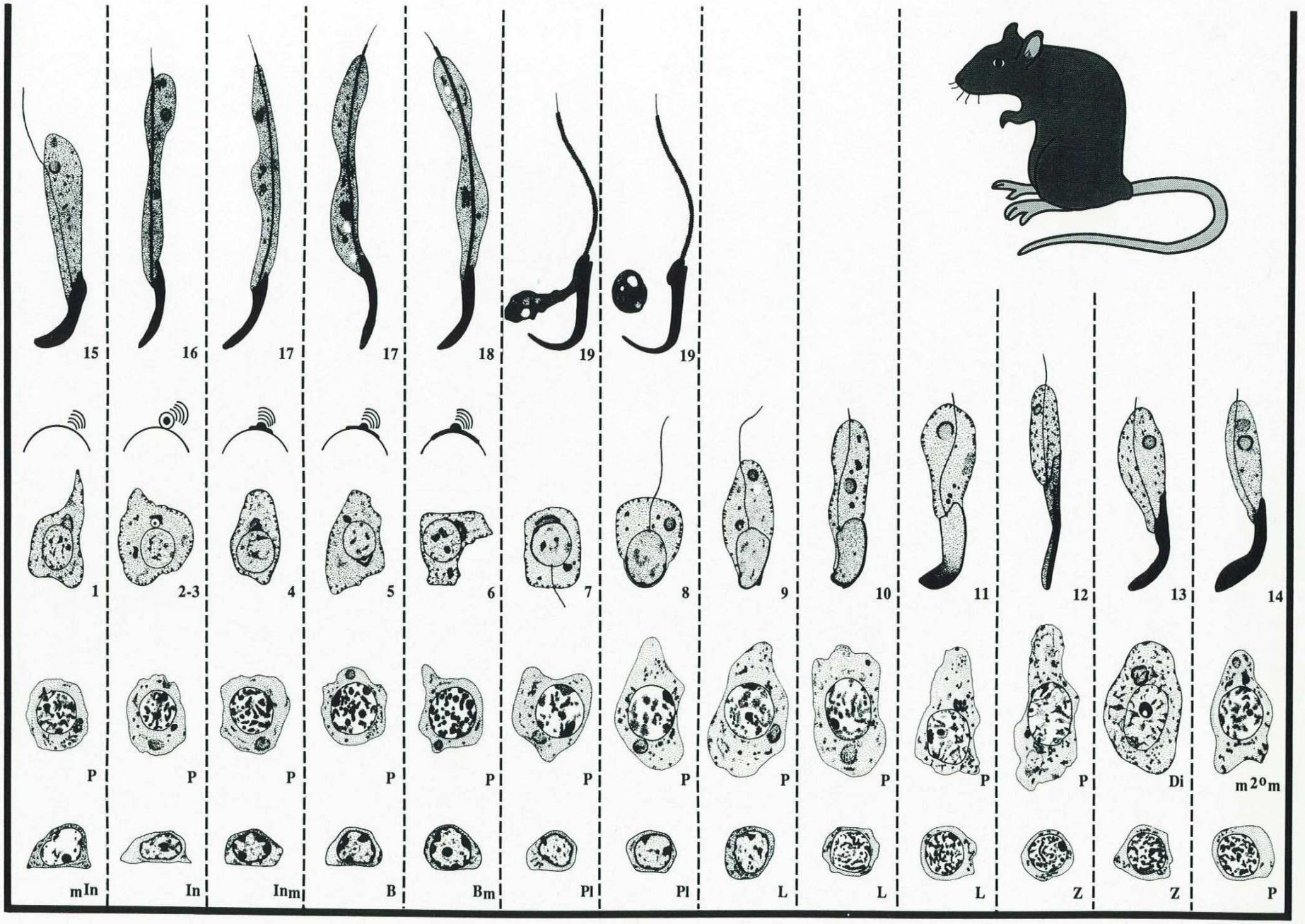
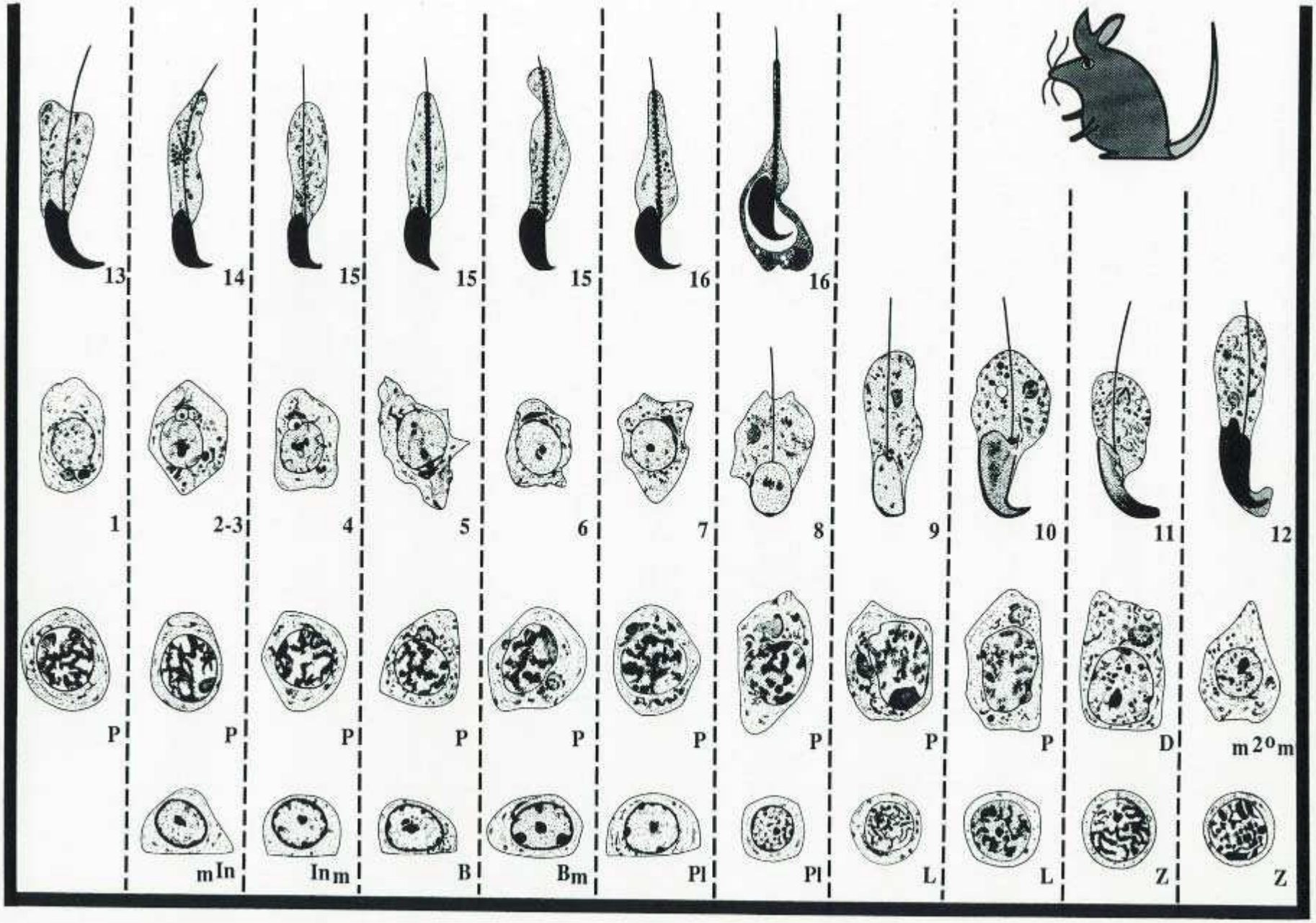
  Introduction. Further examples provided in connection with discussion of MR toxicity later in the presentation
- Conclusions



Russel, Ettlin, SinhaHikim and Clegg, 1990



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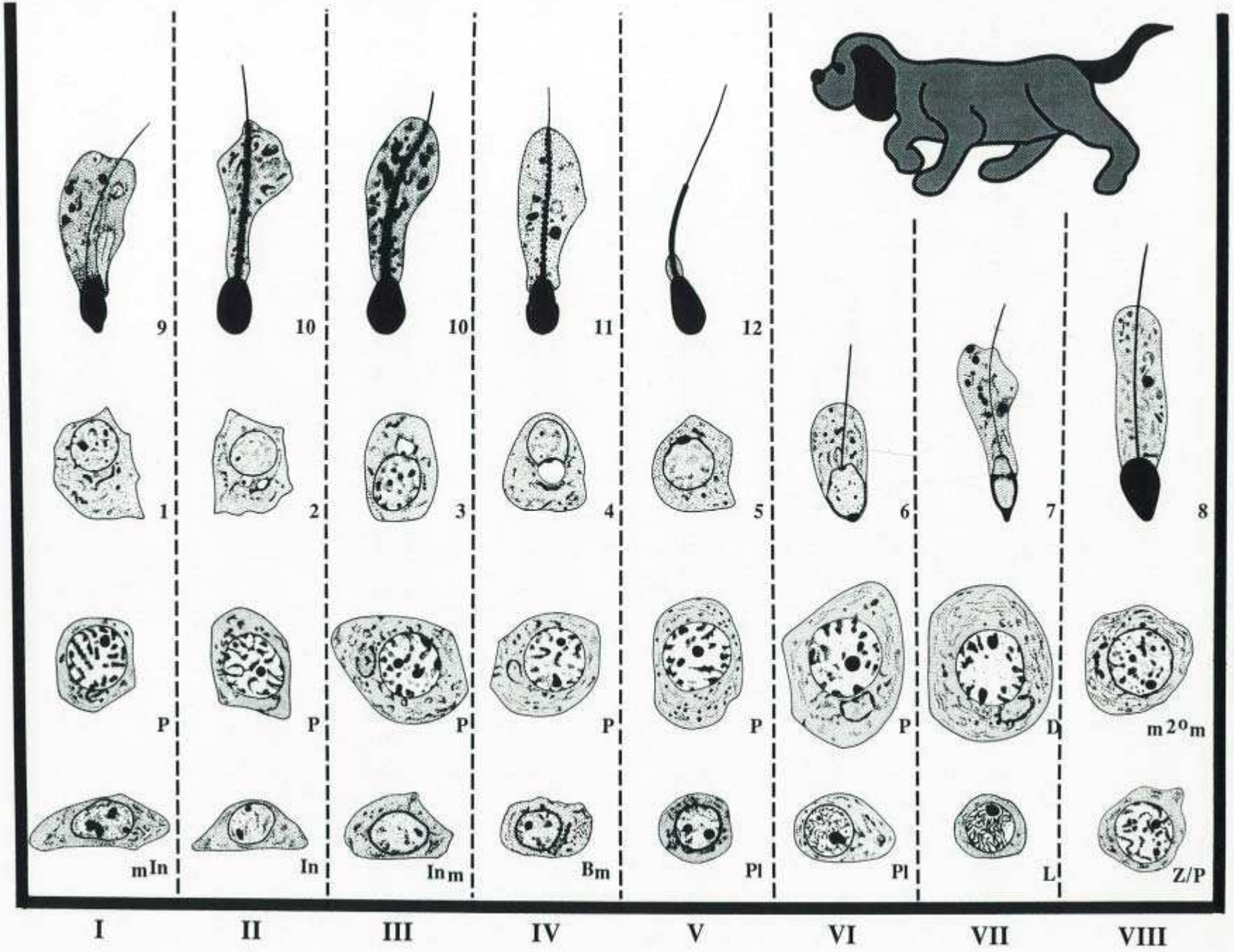


Russel, Ettlin, SinhaHikim and Clegg, 1990

Spermatogenesis in different species

Species	# stages	Duration of one cycle (days)	Duration of spermatogenesis (days)
SD Rat	14	12.9	51.6
Wistar Rat	14	13.3	53.2
Mouse	12	8.6	34.5
Dog	8	13.6	54.4
Monkey	12	9.5	38
Hamster	8-13	8.7	35
Rabbit	8	10.9	51.8
Man	6	16.0	64

Tomas James Rees, Thesis: Literature Review in Applied Toxicology. Portsmouth University, 1993
http://www.brighton73.freeserve.co.uk/tomsplace/scientific/msc-review/msr-top.htm

Sperm production

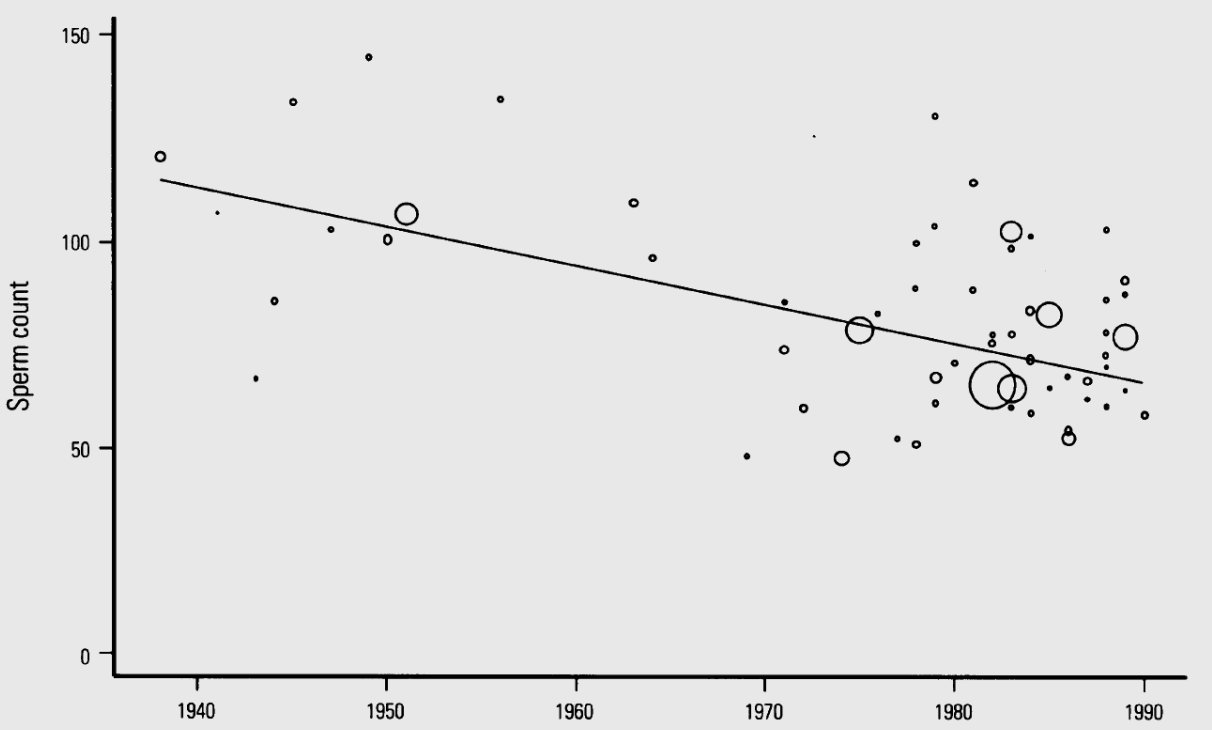
Species	Daily sperm production	
	Per g testis (x 10^6)	Per male (x 10^6)
Rat	18	48
Mouse	28	5
Dog	20	na
Monkey	23	1‘100
Human	4.4	125

Toppari et al, 1996. Env Health Perspect 104, suppl 4: 741-803

Human sperm numbers decline

Rat reproduction functions with 10% sperm

Human sperm production is borderline



Toppari et al, 1996.
Env Health Perspect 104, suppl 4: 741-803

Conclusions Topic A: Normal MR

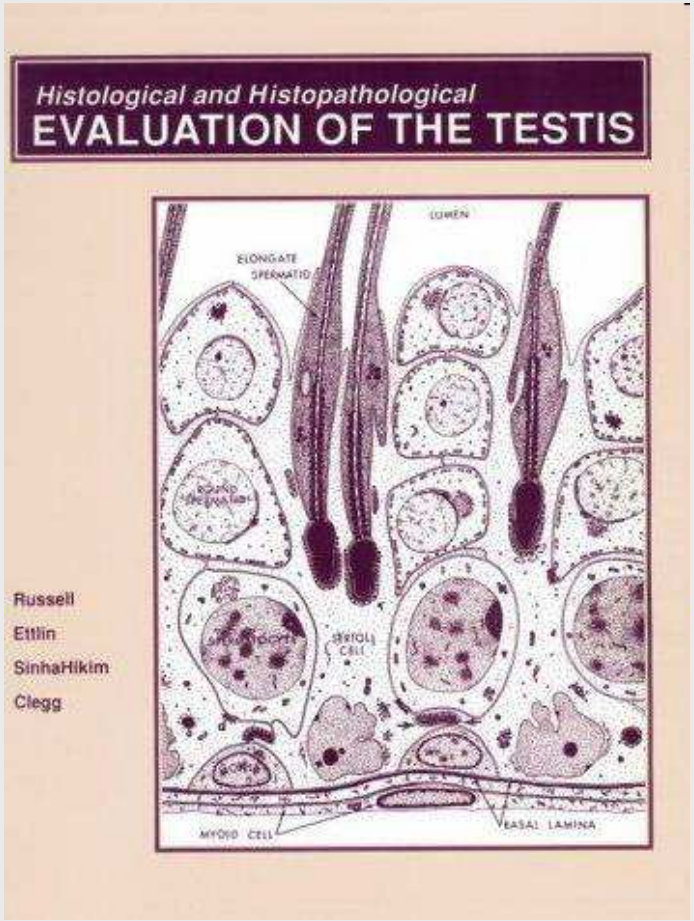
- A system consisting of various, rather complex organs
- Complex regulation
- Species differences, but overall many similarities
- MR system is key for survival of the species
  Declining human sperm number/quality

➔ Concerns

L.D. Russell, R.E. Ettlin, Amiya P. Sinha Hikim and E.D. Clegg

Histological and histopathological evaluation of the testis

Cache River Press, Clearwater, 1990



Toxicologic Pathology of the MR System

- 2 lectures
  - The basis – 45 min
    - The male reproductive (MR) system – 25 min.
    - **Overview over MR toxicity – 20 min.**
  - Practice: Methods and Examples – 45 min.
- Covered: mainly rats as well as some particularities of other species
- Not covered: Developmental reproductive toxicity

Overview over MR toxicity

- MR toxins – Introduction
  - Targets of MR toxicity - General aspects
  - Disruption of MR endocrine regulation
  - Non-endocrine MR toxicity
  - Conclusions

The morphological appearance of adverse effects produced by MR toxins will be discussed in the second lecture

MR toxins

- Chemicals: environment, workplace
- Drugs, including social drugs, alcohol and smoking *(addressed later in more details)*
- Physical factors: radiation, heat, noise, trauma, vibration, nanoparticles, etc.
- Pathophysiological conditions: infection, exhaustion, varicocele, hormonal dysregulation, spinal alterations, etc.
- Psychological factors

Occupations at risk

Employment	Sperm parameters	Pregnancy	
		Rates	Outcome
Agriculture	+	+	+
Construction			+
Military	+		
Plastic production	+		
Printing			+
Mechanics	+		+
Blacksmiths			+
Taxi drivers	+		
Tobacco industry			+
Welding	+	+	+

P. Claman. Men at Risk: Occupation and Male Infertility. Sexuality, Reproduction & Menopause, 2/1: 19-26, 2004

Human-relevant MR chemicals

- Proven: e.g.

  Aromatic hydrocarbons, carbaryl, chlordane, carbon disulfide, disulfonic acid, DBCP, EDB, *ethylene glycol ethers*, ethylene ether, PCE, dioxins such as TCDD, p-nitrophenole, Pb, *phthalate esters, 2,5 hexanedione*

- Suspected: e.g.
  - Anesthetic gases
  - Other heavy metals: Al, As, B, Cd, Chr, Co, Hg, Mg, Ni, V
  - Other pesticides: DDT
  - Other organic solvents

Key reference for workplace hazards

Workplace Hazards to Reproduction and Development: A Resource for Workers, Employers, Health Care Providers, and Health & Safety Personnel

Sharon L. Drozdowsky, Stephen G. Whittaker,

Safety and Health Assessment and Research for Prevention (SHARP). Washington State Department of Labor and Industries

P.O. Box 44330, Olympia, WA 98504-4330

http://www.lni.wa.gov/Safety/Research/files/repro_dev.pdf

Overview over MR toxicity

- MR toxins – Introduction
- Targets of MR toxicity – General aspects
- Disruption of MR endocrine regulation
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Hierarchy of toxicity targets

- Molecular targets e.g. DNA and proteins
  Effects e.g. by alkylation, intercalation, antimetabolites, alkaloids
- Specific subcellular targets e.g. receptors
  Affected e.g. by agonists and antagonists of receptors
- Specific cell targets, e.g. Sertoli cells
  Affected e.g. by chemical disruption of cell-specific functions and processes
- Organ-specific targets: e.g. endocrine organs, CNS, PNS, vascular system
  Affected by endocrine disrupters, agents acting on innervations and blood vessels

One MR toxin can have various targets

Functional MR targets

Sperm production	*Fluid production*	*Hormonal regulation*	*Sperm passage*	*Sperm quality*	*Sperm delivery*
Sertoli cells	Sertoli cells	Central NS	Ductal obstruction	Sperm motility	Libido
Germ cells	Rete testis	Peripheral: LC, SC, …	Ductal motility	Sperm mal-formation	Erection
Vessels	Accessory organs	Receptors	Fluid	Capacita-tion	Ejaculation
Leydig cells		Metabolism		Gene damage	
		Clearance		Cytotoxicity	

Toxicity in the male reproductive system is often due to multiple pathways

Effects of various MR toxins – 1

Toxin	Endocrinology	Spermato-genesis	Maturation and Storage	Accessory Sex Glands	Ejaculation
Environmental					
EDS	X				
Cadmium	X	X	X		
DBCP	X	X			
Boric acid	X	X		X	
TCDD	X	X			
Benomyl		X			
Ethylene oxide		X			
Fluoride		X			
Acrylamide		X			
MeCl		X	X		
EDB	X		X	X	
Anticholin.pesticides					X

Tomas James Rees, Thesis: Literature Review in Applied Toxicology. Portsmouth University, 1993
http://www.brighton73.freeserve.co.uk/tomsplace/scientific/msc-review/msr-top.htm

Effects of various MR toxins – 2

Toxin	Endocrinology	Spermato-genesis	Maturation and Storage	Accessory Sex Glands	Ejaculation
Therapeutic					
Gossypol		X			
DES	X	X		X	
Radiation		X			
Radio-mimetics		X			
Anti-hypertensives					X
Recreational					
Ethanol	X	X	X	X	X
Cannabis	X	X			X

Modified after: Tomas James Rees, Thesis: Literature Review in Applied Toxicology. Portsmouth University, 1993. http://www.brighton73.freeserve.co.uk/tomsplace/scientific/msc-review/msr-top.htm

Spermatogenic susceptibility

Depends on spermatogenic stage, e.g.

- Hormonal requirements
  - High FSH levels needed in stages I, II, XI and XIV
  - High T requirement of spc and round spt in stages VII and VIII
- Cell divisions with increased metabolism
  Mitosis, meiosis

Further examples to be discussed under mechanisms of toxicity

Overview over MR toxicity

- MR toxins – Introduction
- Targets of MR toxicity – General aspects
- **Disruption of MR endocrine regulation**
- Non-endocrine MR toxicity
- Conclusions

Example: Impairment of androgen action

Target	Mechanism of action	Compound
Regulation: Hypothalamus-pituitary axis	Feedback on GnRH secretion	Estrogens, progestin
Production: Androgen synthesis	Key enzymes	Steroid analogues, diphenylmethylanes (incl. DDD), pyridine derivatives, glutethimides, triazines, hydrazines, thiosemicarbazones
Metabolism: DHT synthesis	5α-reductase	Androstene-17-carboxylic acid, estrogens
Plasma binding	Ratio of free/bound androgen	Estrogens
Receptor binding	Action block of DHT	Cyproterone acetate, spironolactone, dihydroprogesterone
Receptor function	Signal transduction	Cyproterone acetate, 17 α-methyl-ß-testosterone, flutamide

CNS effects

- GnRH receptor agonists (affect also peripheral receptors, e.g. in rats), e.g.
  - Buserelin, leuprolide (GnRH agonists)
    Paradox inhibition of gonadotropin release following long-term continuous receptor stimulation
    ➔ Atrophy of regulated organs
- Modulators of prolactin levels: e.g.
  - Neuroleptic drugs
  - Ergot compounds such as muselergine, norprolac
  - Tricyclic antidepressants
  - Cimetidine
  - Gemfibrozil

Example: Dopaminergic drugs

Target organ axis	*Mesulergine treatment – Sequence of events*	
Hypothalamus		③ Reduced testosterone feedback ➔ LHRH ↑ (also through production ↑ in Sertoli cells)
Pituitary	① Prolactin secretion ↓	④ LH ↑
Testis (LC)	② ➔ **Number of LH receptors on LC** ↓ ➔ Testosterone production ↓ ➔ T serum levels ↓	⑤ LH and LHRH ↑ have a tropic (proliferative) effect on LC ➔ LC hyperplasia and neoplasia

After R.A. Ettlin et al. Dopamine agonists In: Classic Examples in Toxicologic Pathology, 3rd ed. E Karbe, W Drommer, PG Germann, G Morawietz, and R Kellner (eds). European Society of Toxicologic Pathology, Hannover. CD-ROM. 2009

Histological photomicrograph to be shown

Peripheral effects – 1

- Antagonists of peripheral androgen receptors, e.g.
  - Cimetidine
  - Linurol, procymidone
  - Vinclozolin
  - Flutamide, finastride
  - Cyproterone acetate
- LC GnRH receptor agonists in rats (acts also centrally), e.g.
  - Buserelin, leuprolide

Peripheral effects – 2

- Estrogen agonists
  - ➔ LC tumors in mice
    May decrease incidence of LC tumors in rats
    (not by perfluorooctonate)
  - Diethylstilbestrol, ethinyl estradiol, ammonium perfluorooctonate, digoxin
- Direct Leydig cell impairment, e.g.
  - Alkylating agents
  - Ethanedimethane sulfonate
    Selective destruction of LC

Disturbance of steroidogenesis

Examples

- Modulators of drug metabolizing enzymes, e.g. P450 inducers or CYP17 inhibitors, such as Conazole
- Interaction with histamine receptors involved in steroidogenesis, e.g. proton pump inhibitors lansoprazole, isradipin, cimetidine
- Raising of estradiol level and/or inhibit testosterone biosynthesis e.g. by peroxisome proliferators buserelin, leuprolide, histrelin, nafarelin
  (relevant especially for rats)

Disturbance of testosterone metabolism

Examples

- Aromatase inhibitors, e.g. letrozol, formestane
  - ➔ Block conversion of T to estradiol
  - ➔ In dogs (not in rats) LC hypertrophy / hyperplasia (aromatization of T important in dogs, non-human primates and man, but not so much in rodents)
- 5^{α}-reductase inhibitors, e.g. finasteride ➔ block conversion of T to DHT (dihydrotestosterone)

Histological photomicrograph to be shown

Example: Aromatase inhibitors

	Letrozole		Formestane	
	Rat	Dog	Rat	Dog
Testes				
• Leydig cell atrophy			+	
• Leydig cell hypertrophy / hyperplasia, diffuse		+++		+++
• Disturbance of spermatogenesis		++		
• Leydig cell hyperplasia, multifocal	+			
Prostate, seminal vesicles				
• Atrophy	+	–	+	–

After U. Junker. Aromatase inhibitors. In: Classic Examples in Toxicologic Pathology, 3rd ed. E Karbe, W Drommer, PG Germann, G Morawietz, and R Kellner (eds). European Society of Toxicologic Pathology, Hannover. CD-ROM. 2009

Leydig cell responsiveness

Parameter	Rat	Man
Plasma LH levels with age	↑	↓
Number of LH receptors/Leydig cell	20'000	1'500
GnRH receptors on Leydig cells	Present	Absent
GnRH production by Sertoli cells	Yes	No
LH ↑	LC tumors	No tumors*
Cryptorchidism	No LC tumors	LC tumors
Drug associated Leydig cell tumors	Many	None
* Klinefelter syndrome (XXY, XXXY)		

Disruption of MR endocrine regulation

General remarks

- Endocrine disruption
  - Generally affects various MR targets
  - Results in diverse alterations depending on the predominant effects
- In rodents (particularly rats) prolonged disruption of the hypothalamic-pituitary-gonadal axis generally results in Leydig cell tumors of minimal relevance to man (increased stimulation of LC)

  Exception: sustained GnRH stimulation (physiologically pulsatile) may result in paradox inhibition

Overview over MR toxicity

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Non-hormonal MR toxins - Testis

	Germ cells	Sertoli cells	Fluid balance	Leydig cells*
Examples	Anticancer drugs Chemothera-peutics „Male antifertility pill“ Dinitropyrroles Methylchloride	Vincristine „Male antifertility pill“ Phthalates Hexadione	Psychopharmaca (ACTH Cortisone) Oxytocin Endothelin	Anticancer drugs Ketoconazole Isradipine Ethanol Tri-o-cresyl-phosphate Ethane-dimethane sulfonate
MoA, e.g.	Alkylation Intercalation Antimetabolites Free radicals Apoptosis ↑ Cell division	Cytoskeleton Metabolism *See also fluid production*	Fluid production and resorption Tubular emptying Obstruction of efferent ducts	Cytotoxicity Inhibition of steroidogene-sis

* Results in secondary effects in the rest of the seminiferous epithelium and in accessory sex organs, mediated by hormonal imbalance
(Partly also acting by endocrine mechanisms)

Non-hormonal MR toxins – Other organs

	Epididymis	Blood vessels	Unspecific effects	Function
Examples	α-chlorohydrin Epichlorohydrin Benomyl Guanethidine Antifertility agents Carbendazim Methyl chloride Ethyl chloride Trichloroethylene	5-hydroxy-tryptamine Histamine Cadmium	Zn deficiency Hypovitaminoses Diseases of other organs	(CNS/PNS drugs)
MoA, e.g.	Damage to ductal epithelium Sperm granuloma Sperm maturation Sperm integrity	Hypoperfusion	Malnutrition Photoperiod Eye lesions	Perfusion Erection Sex drive
(Partly also acting by endocrine mechanisms)				

Germ cell toxins – 1

- Affecting among others spermatogonia
  - Alkylating agents: busulfan, cyclophosphamide (protective effect of cooling)
  - DNA-intercalating: doxorubicin, bleomycin
  - Antimetabolites: cytosin arabinoside, 6-mercaptopurin, methotrexate, 5-fluorouracil
  - Production of free radicals: bleomycin, 1,3-dimethyl-benzantracene
  - Induction of apoptosis: 2-methoxyethanol, methoxyacetic acid
  - DBCP ➔ epichlorohydrin ➔ α-chlorohydrin

Germ cell toxins – 2

- Affecting dividing cells (spermatogonia and spermatocytes): alkaloids, e.g. vincristine
- Affecting spermatocytes: 2-methoxyethanol , dinitropyrroles, ethylene glycol monoethyl ether, heat
- Affecting spermatids incl. genetic damage: ethylmethane sulfonate, methyl chloride
  Late spt: boric acid, dibromoacetate
- Affecting various GC:
  - Chemotherapeutics: metronidazole, nitrofuran, sulphasalazin, artesunate, benomyl, ciprofloxacin
  - Antispermatogenic drugs: antabus, diamine,
  - Industrial chemicals, e.g. dinitropyrrole, tolnidamine

Sertoli cell toxins

Sertoli cell are “easy to impair, but difficult to kill

- Cytoskeleton: e.g. vincristine, vinblastine, colchicine, cytochalasin A, 2,5-hexanedione*,
- Metabolism: e.g. antispermatogenic substances
- *See also fluid production*
- Mechanism not well understood: 1.3-dinitrobenzene*, phthalate esters incl. DEHP*, tri-o-cresylphosphate, thiamphenicol

* Decreases also fluid production

Disturbance of fluid balance

- Fluid production by SC (androgen dependent)
  - Increased ➔ Testicular swelling
    e.g. as consequence of psychopharmaca, ACTH, cortisone
  - Decreased e.g. by antiandrogens
- Disturbed resorption in rete testis or caput epididymidis (normally > 90% re-absorbed in caput epididymidis). *See also under epididymis*
- Reduced empting
  - Of seminiferous tubules. Role of tubular contraction? Influence e.g. of oxytocin and endothelin
  - Obstruction of efferent ductuli:
    e.g. by sperm granuloma induced e.g. by carbamates pesticides. *See also under epididymis*

Leydig cell toxins

- Leydig cell toxicity
  - Direct toxicity
    - Busulfan etc.
    - Ethane-dimethane sulfonate
    - Ethanol (may also interfere with steroidogenesis etc.)
  - Inhibition of steroidogenesis
    - Tri-o-cresylphosphate
    - Ketoconsole
    - Isradipine

Epididymal toxins

- Epithelium: α-chlorohydrin*, epichlorohydrin, 6-chloro-deoxyglucose*, methyl chloride, gossypol. May lead to:
- Sperm granuloma, seen .e.g. with
  - Carbendazim acting mainly on *efferent ductules (see there)*
  - Ethyl chloride by necrosis of epididymal epithelium
  - α-chlorohydrin and benomyl by inhibition of fluid resorption especially in the caput
  - Guanethidine by adrenergic ganglion blockade at the epididymis-vas deferens junction
- Sperm maturation: (e.g. estrogenic compounds)
- Sperm: trichloroethylene, alkylating substances, gossypol

* Affects also sperm respiration

MR toxins with various targets

- Levamisol
- Blood vessels
  - 5-hydroxy-tryptamine and histamine → reduced blood flow with anoxia
  - Cadmium → endothelial necrosis
  - Circulatory disturbances

Unspecific effects on MR

- Malnutrition, in particular Zn deficiency
- Hypovitaminosis; A, B_6, E, biotin, thiamin
- Diseases of other organs, in particular
  - Liver
  - Kidney
  - CNS, PNS
- Decreased photoperiod:
  e.g. hamster < 14 hours of light
  Eye lesions

Functional effects

- Action on CNS e.g. opiates, phenothiazine, hydantoin, benzodiazepine, tricyclic antidepressants, xanthine
- Action on PNS e.g. by
  - Sympathomimetic action: anorectica
  - Sympatholytic action: guanethidine, propranolol
- Decrease of organ/compartment perfusion e.g. by
  - Vasodilation: antihypertensive therapy
  - Diuretic action, e.g. thiazide (chlorthalidon)

Many articles about male reproductive toxicity in general and due to endocrine effects by

Dianne Creasy

e.g.

Pathogenesis of male reproductive toxicity
Toxicol Pathol 29/1: 64-76, 2001

or

OECD guidance document on endocrine disruption (co-author)

Conclusions Topic B: MR toxicity

- MR system can be affected in many different ways
- Often more than one MR target adversely affected
- Large array of diverse chemicals and drugs have MR adverse effects.
- Have a basic understanding of class effects
- Though, similar chemicals might have diverse MR effects depending on the predominant effect
- Endocrine adverse effects are often species-specific (see also Topic E)