Testicular stem cells

Dirk G. de Rooij

Department of Endocrinology
Faculty of Biology, Utrecht University

Berlin, June 2004

1. Knowledge on the development of the spermatogenic stem cell lineage
2. Principals of the nature of spermatogonial stem cells in primates and non-primates
3. Understand the principles of the regulation of differentiation and self-renewal
4. Possible important new development - Side population of stem cells

primordial germ cells

↓

migration to genital ridge
proliferation

↓

enclosure in seminiferous cords — **6 weeks p.c.**

↓

gonocytes

↓

proliferation

↓

cell cycle arrest — **9 weeks p.c.**

↓

adult type spermatogonia

↓

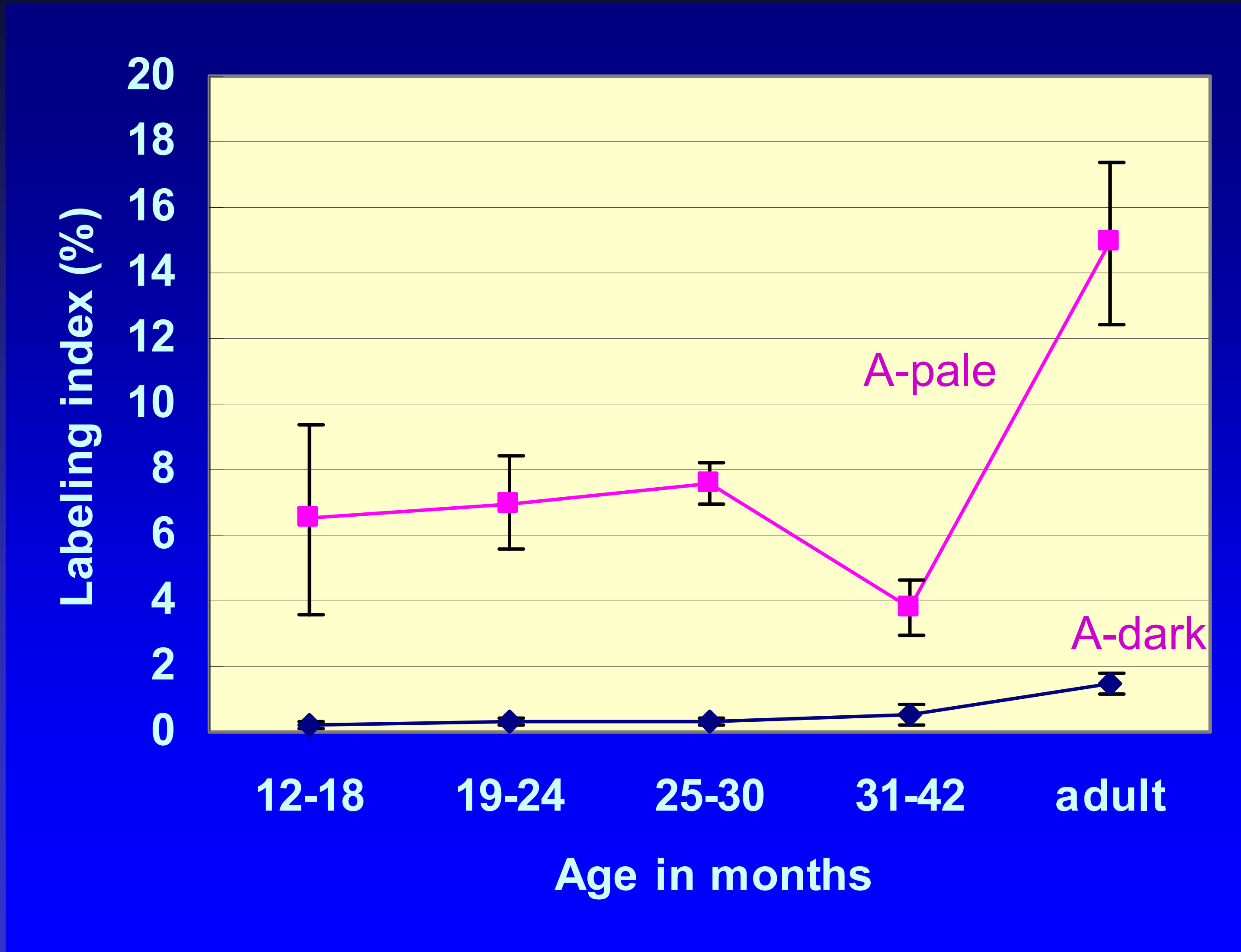
start of spermatogenesis

Numbers of spermatogonia / spermatocytes in the developing monkey testis

age (months)	A-dark	A-pale	A-dark/A-pale	B	spc
day of birth (2)	0.5	0.3	1.9	0	0
12-18 (6)	0.9	0.2	4.7	0.03	0
19-24 (14)	1.2	0.4	3.6	0.02	0.02
25-30 (8)	1.2	0.5	2.9	0.04	0.09
31-42 (4)	2.2	0.6	4.5	0.02	0.01
puberal (1)	2.2	1.6	1.4	0.4	5.1
adult (6)	2.5	2.4	1.0	8.7	31.3

Kluin et al., 1983

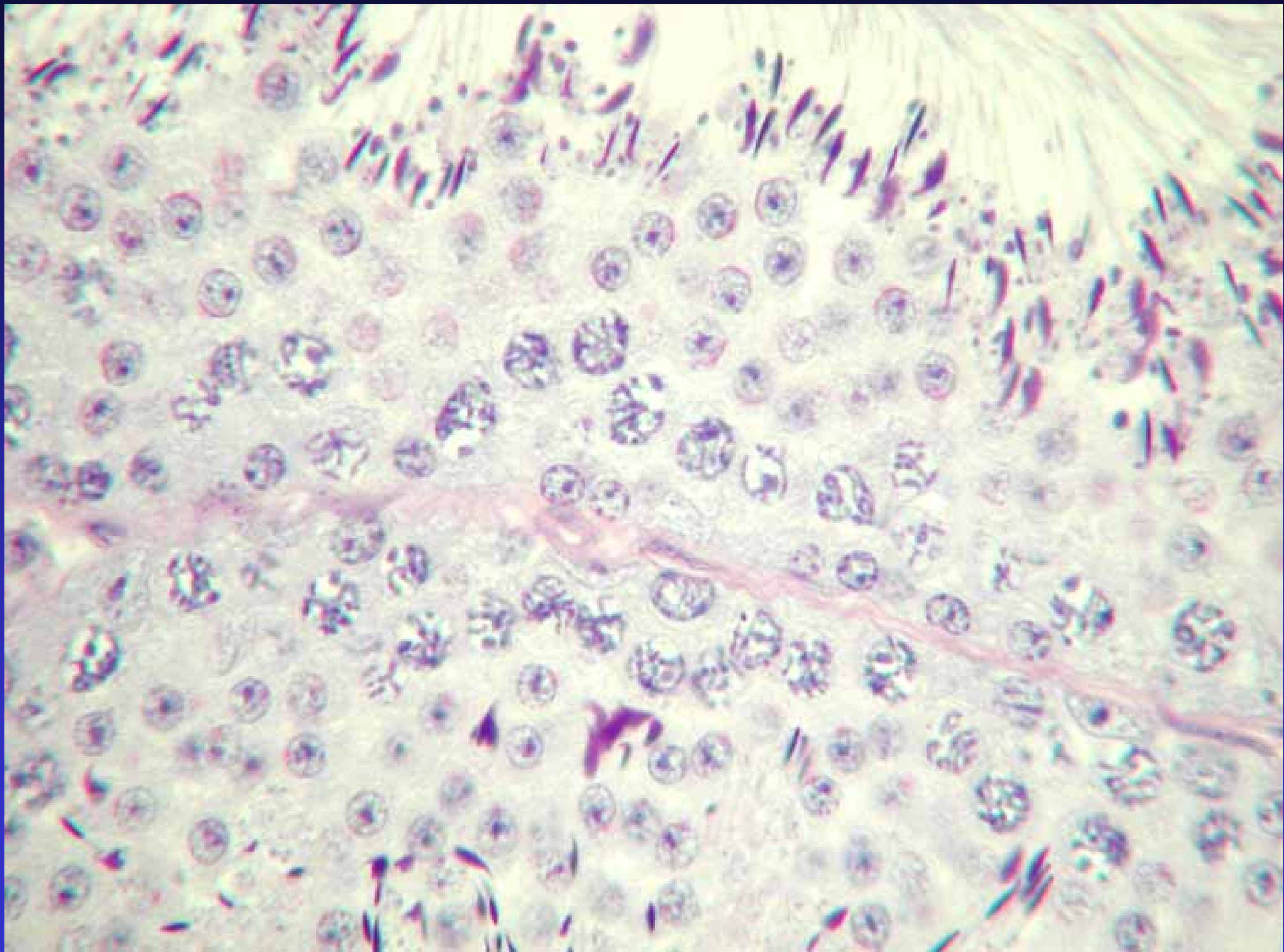
^{3}H-thymidine labeling index of monkey spermatogonia during development



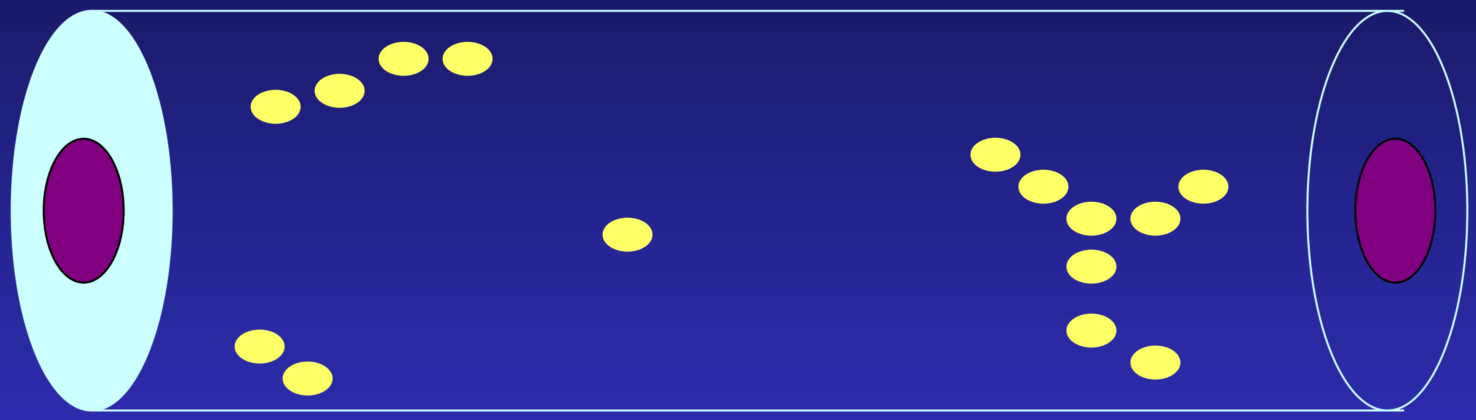
Kluin et al., 1983

1. Knowledge on the development of the spermatogenic stem cell lineage
2. **Principals of the nature of spermatogonial stem cells in primates and non-primates**
3. Understand the principles of the regulation of differentiation and self-renewal
4. Possible important new development - Side population of stem cells

Normal mouse spermatogenesis



Cell patterns in whole mounts of seminiferous tubules



Non primate mammals

$A_s \rightarrow A_{pr} \rightarrow A_{al}$

$A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_4 \rightarrow In \rightarrow B$

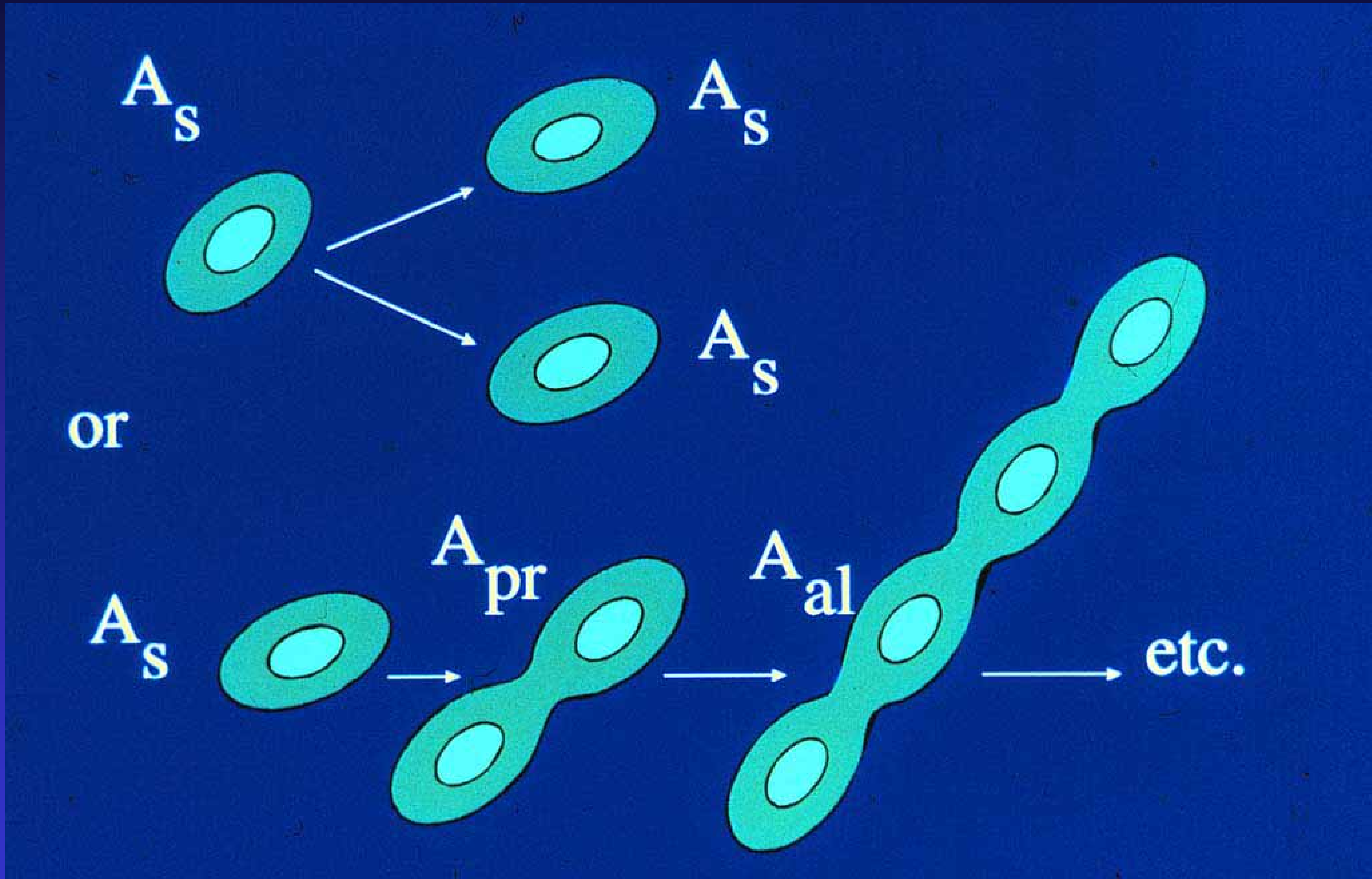
spermatocytes

$\downarrow$

spermatids

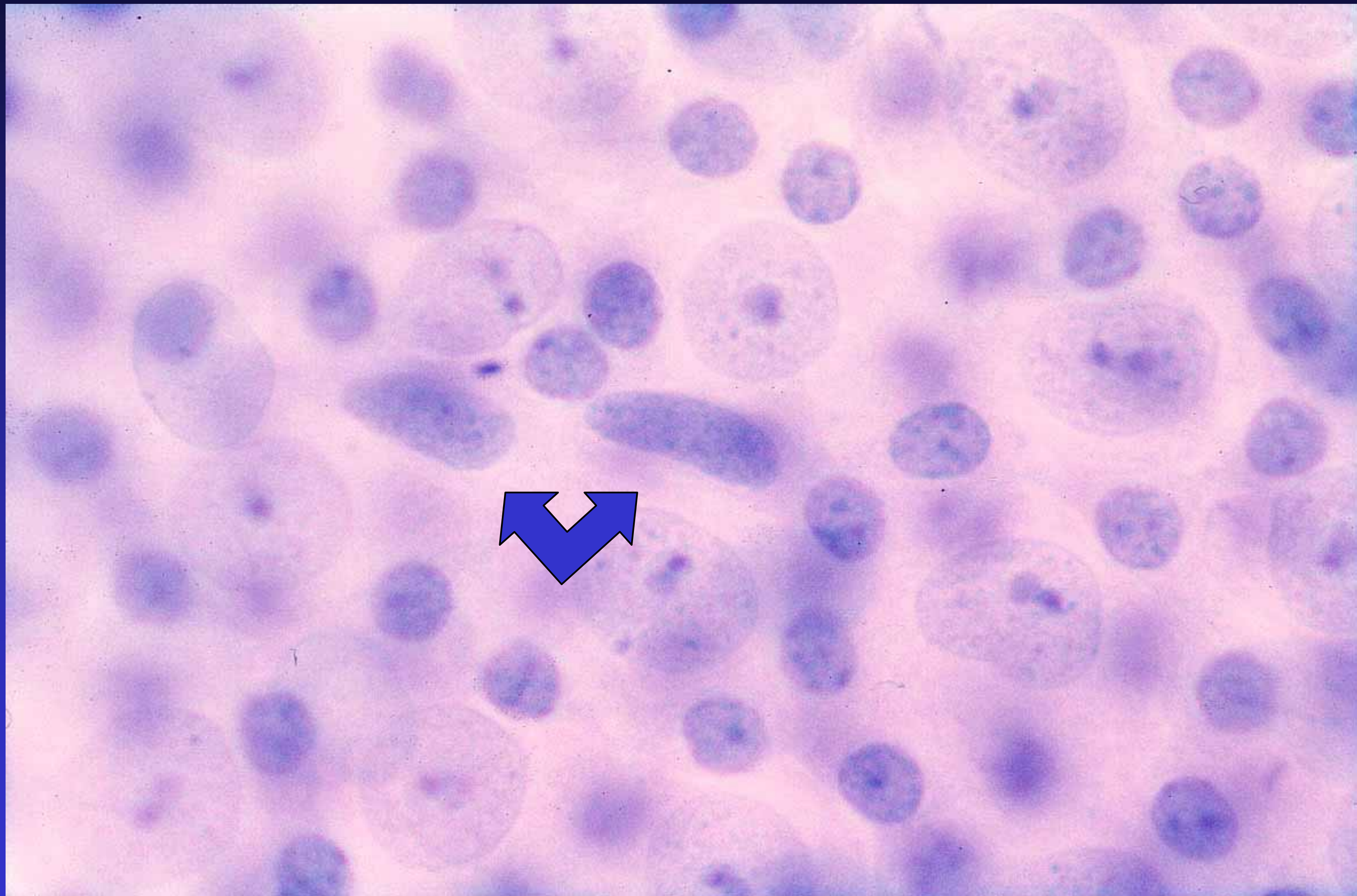
(round → elongated)

Stem cell renewal and differentation

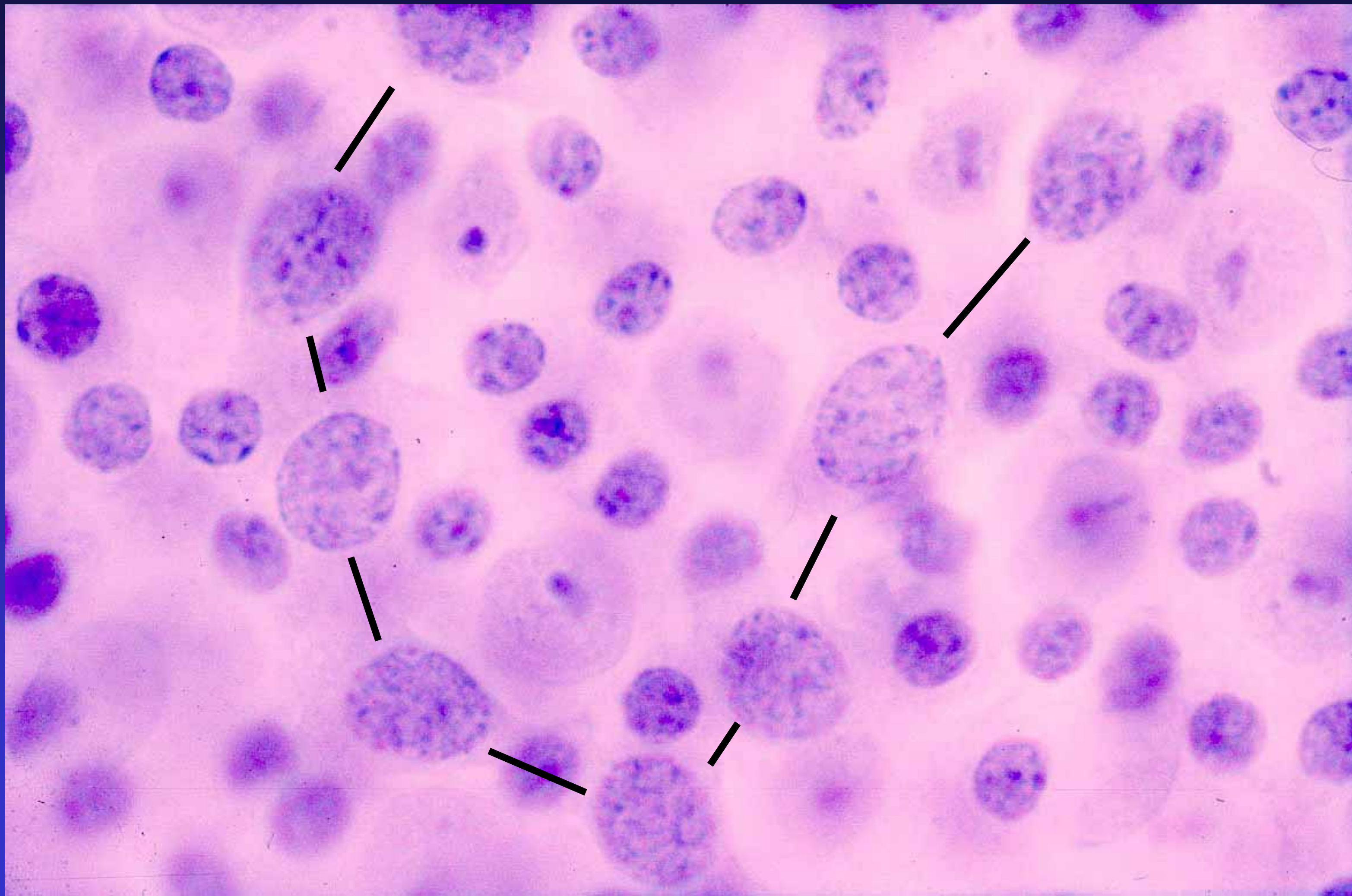


Spermatogonial stem cell

First differentation step – formation of a pair



Chain of 8 spermatogonia in prophase of mitosis



$A_s \rightarrow A_{pr} \rightarrow A_{al}$

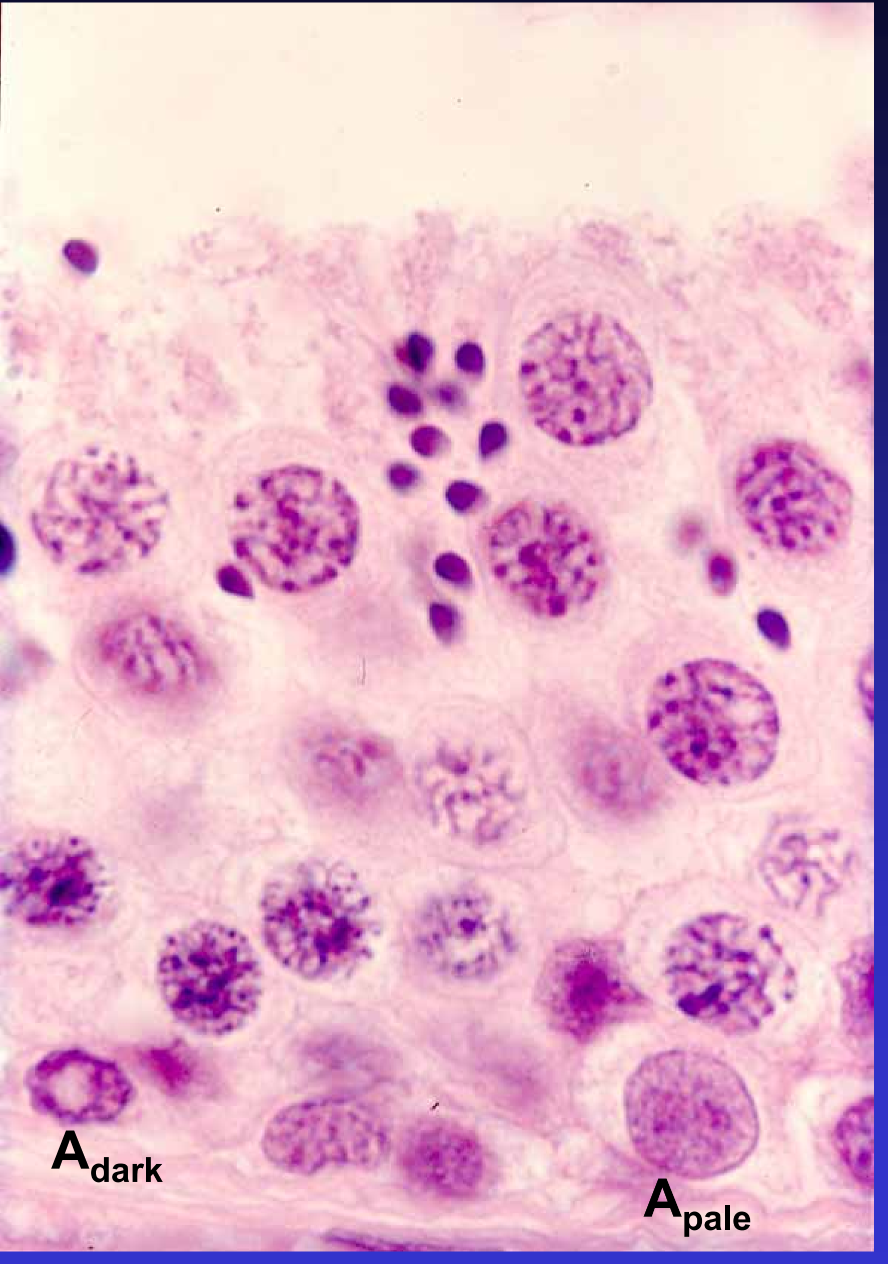
$A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_4 \rightarrow \mathrm{In} \rightarrow \mathrm{B}$

spermatocytes

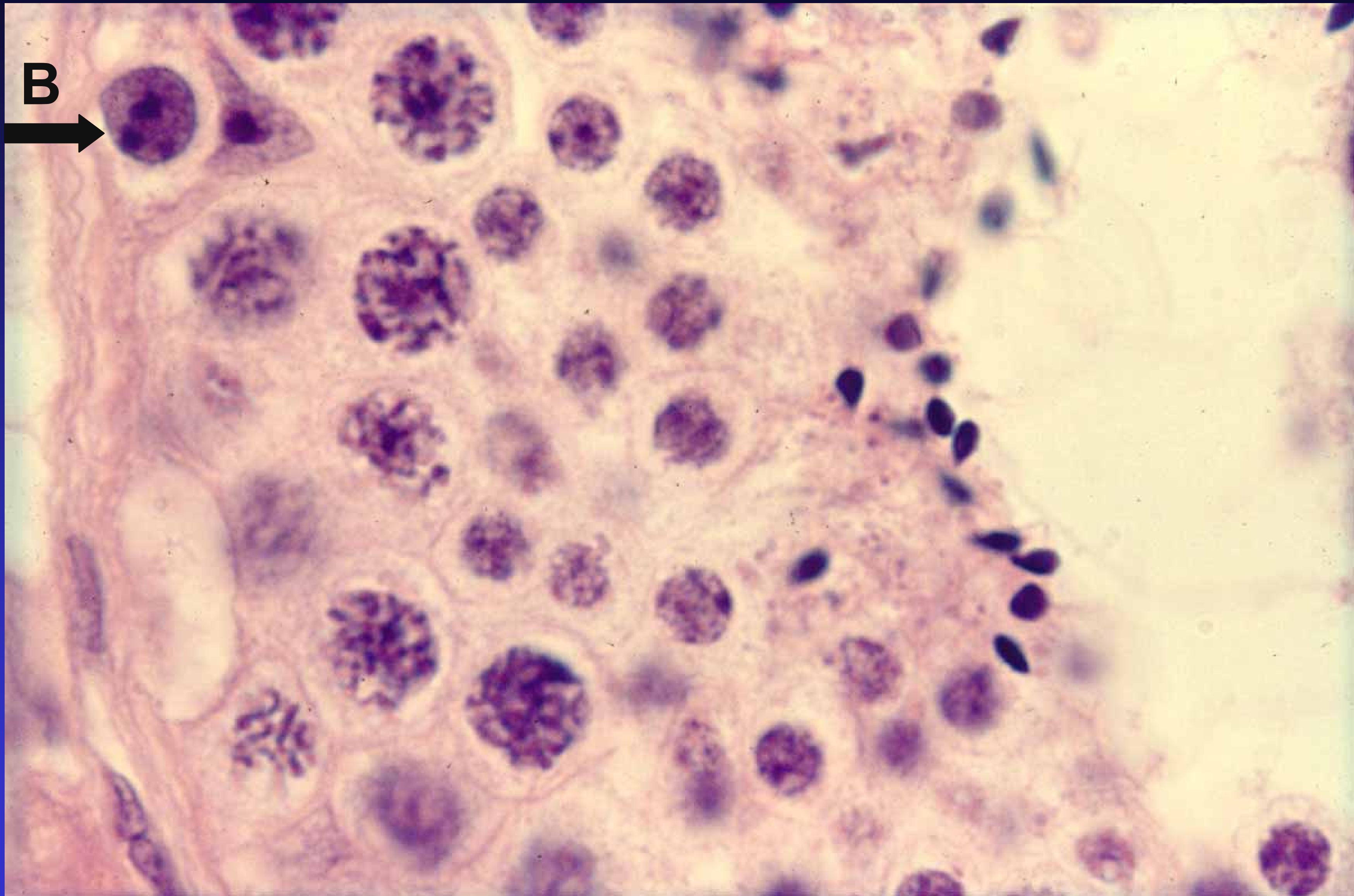
$\downarrow$

spermatids

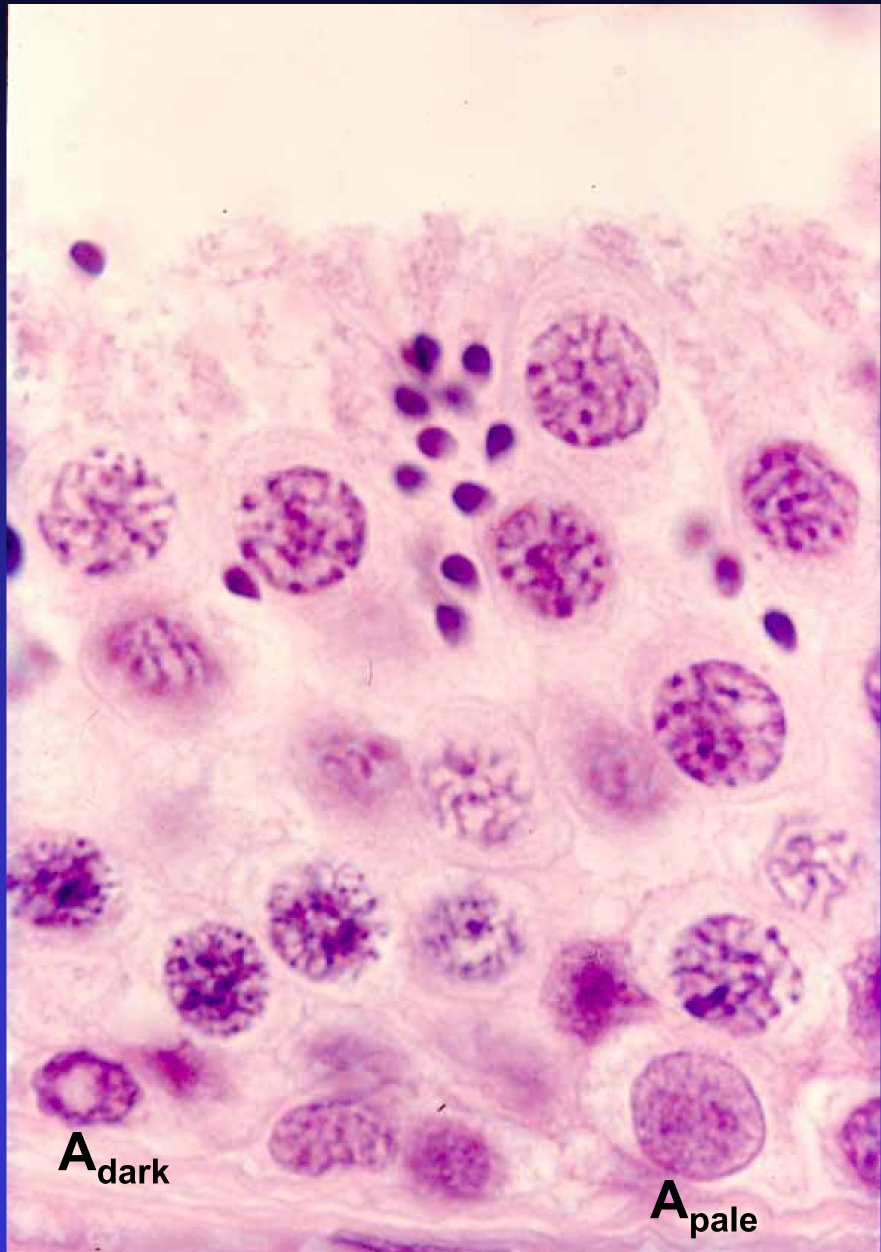
Seminiferous epithelium in the human



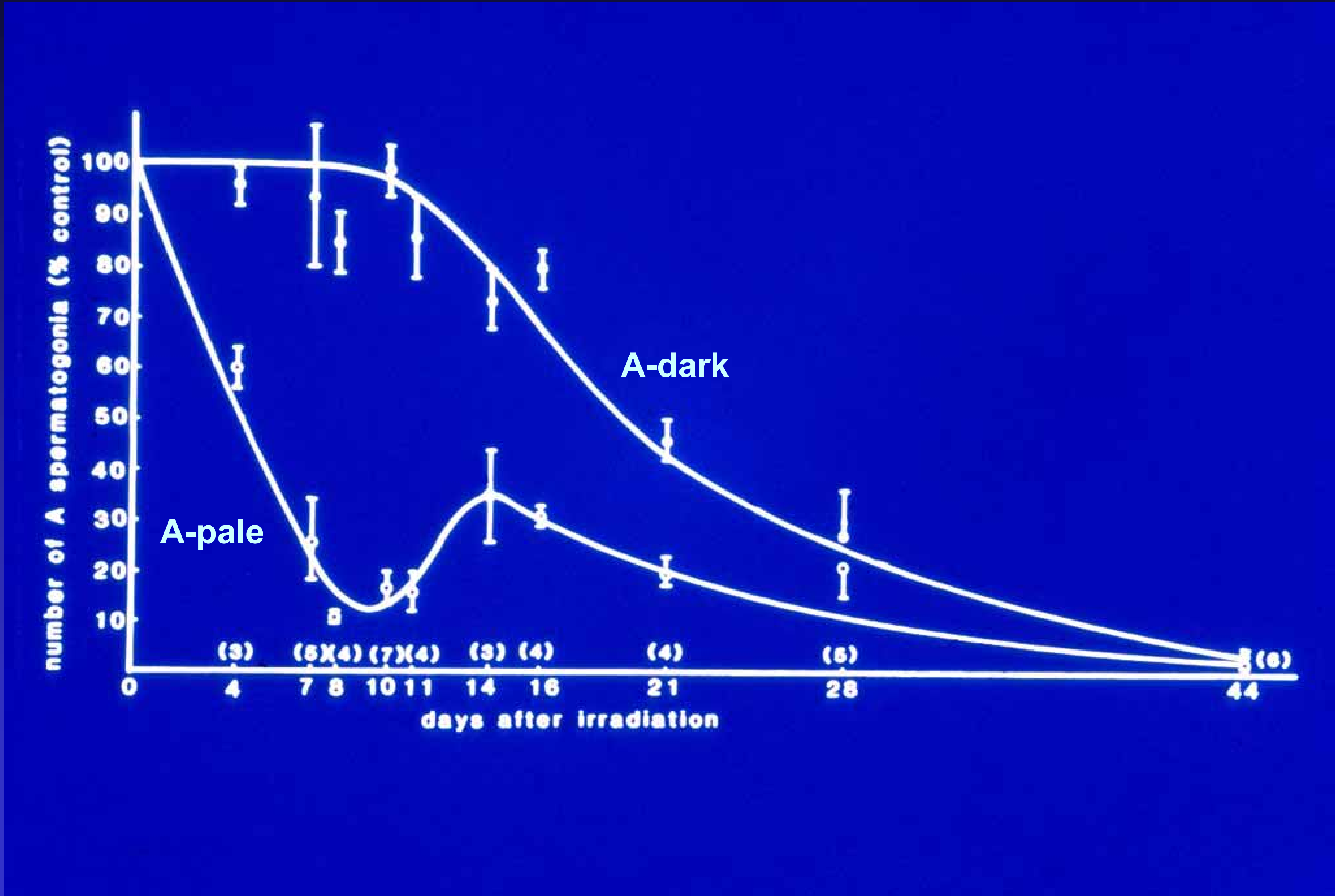
Seminiferous epithelium in the human



Seminiferous epithelium in the human

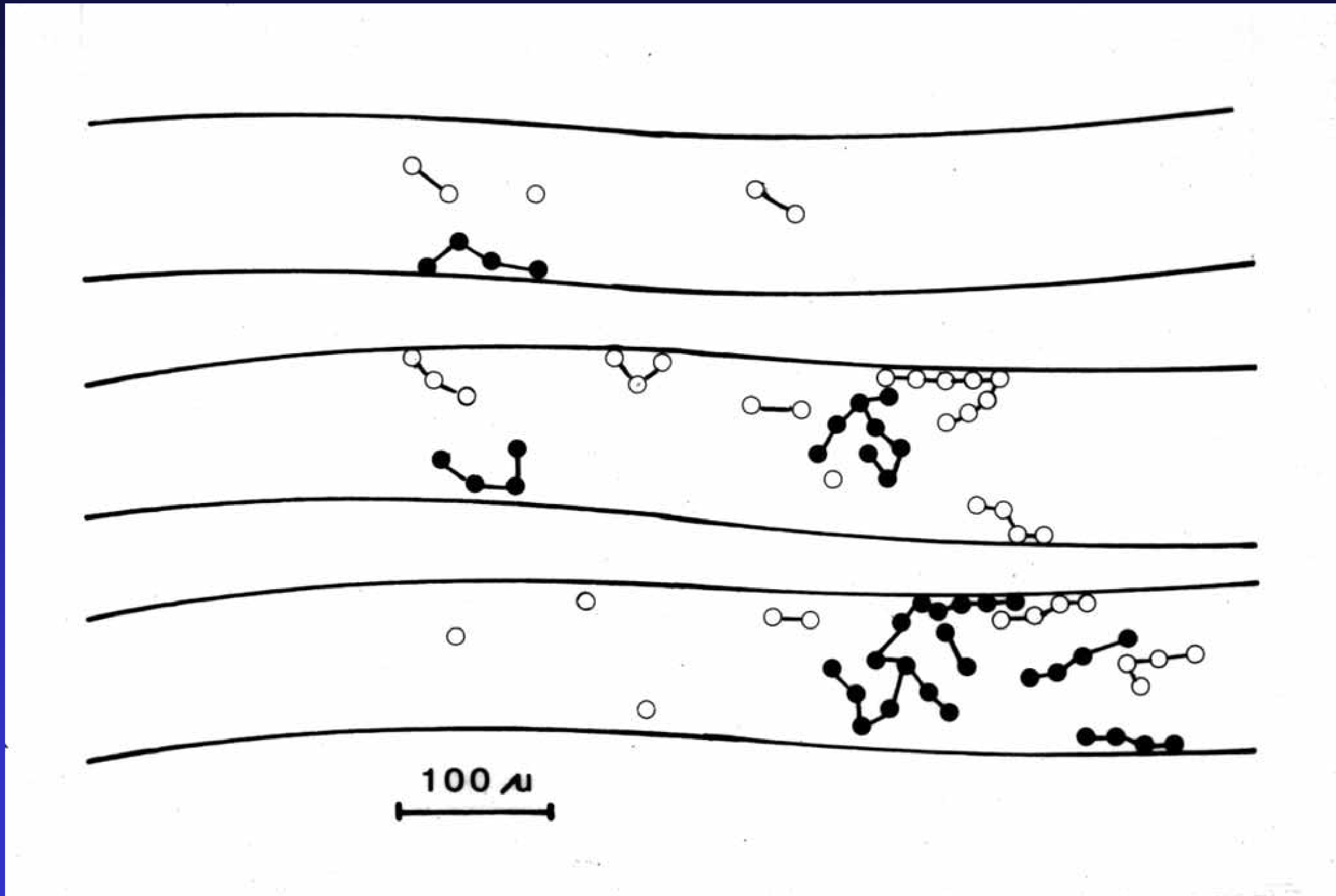


Depletion of monkey A-pale and A-dark spermatogonia after irradiation



van Alphen et al., 1988

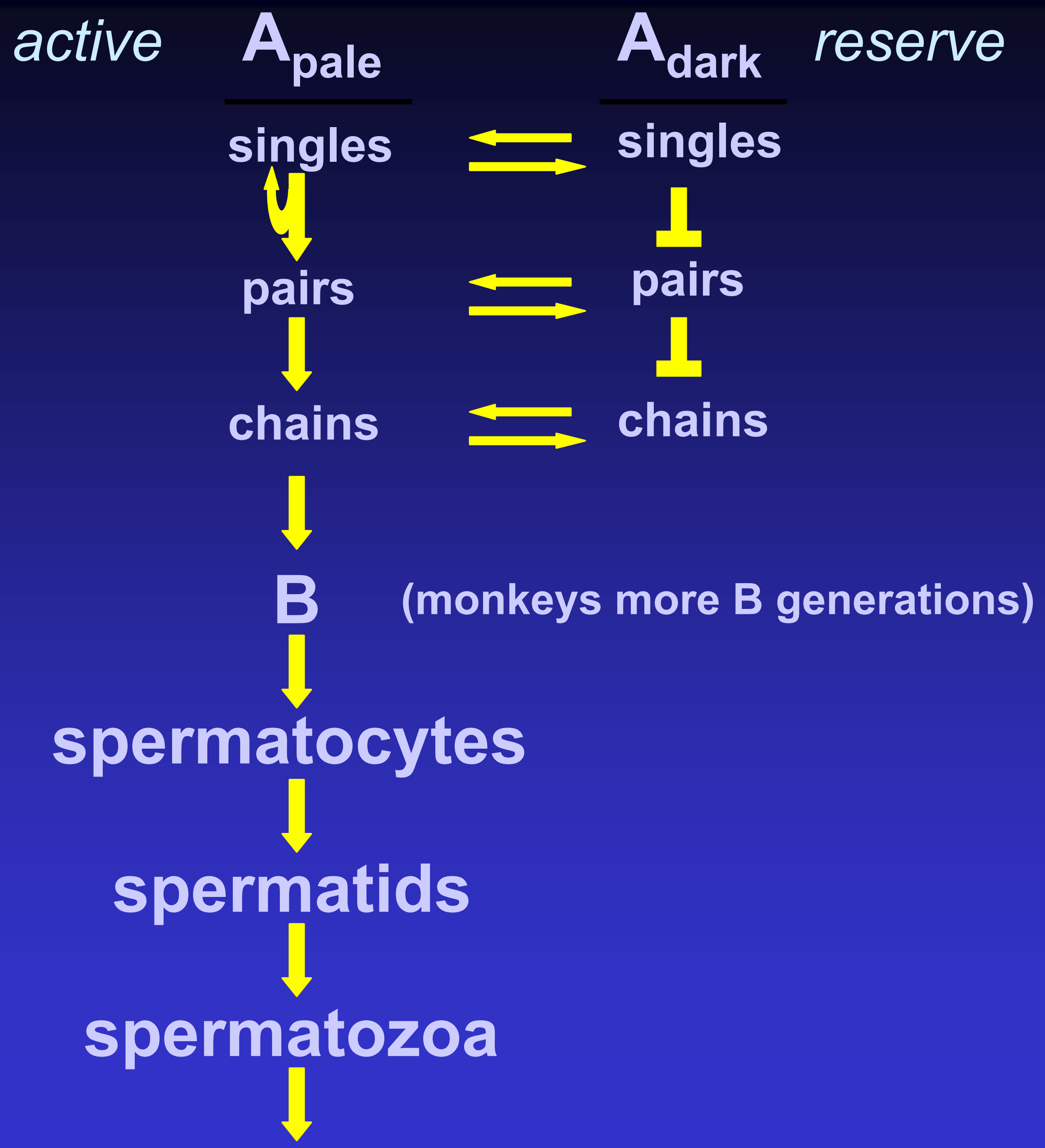
Topographical arrangement of A-pale and A-dark spermatogonia in the rhesus monkey 90 days after 2 Gy of X-rays

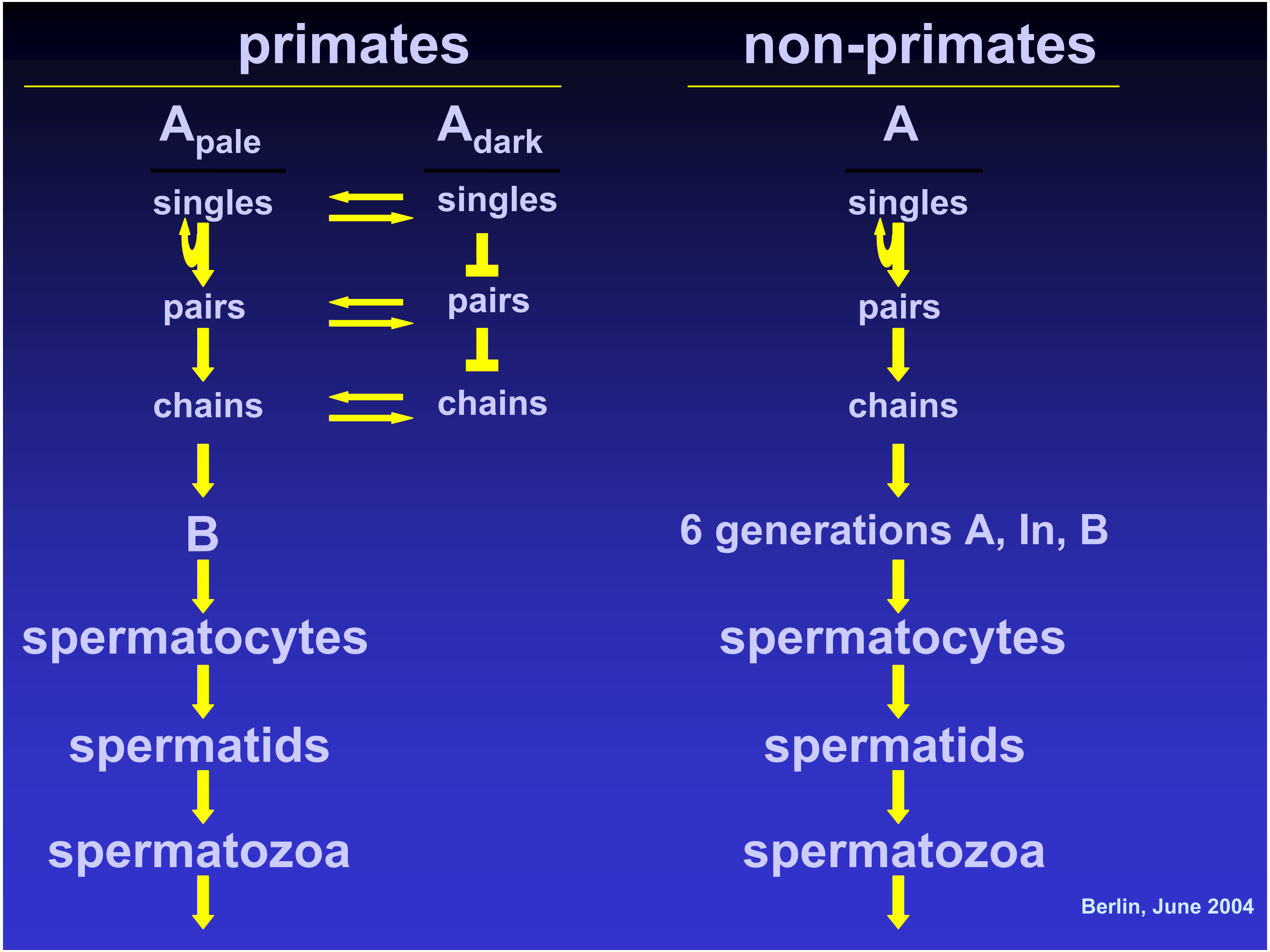


Van Alphen et al., 1988a

- A-pale proliferate, A-dark are quiescent
- A-dark transform into A-pale after cell loss
- A-pale form new A-dark during recovery
- Both A-pale and A-dark consist of singles and clones of 2^n cells
- There are B spermatogonia

Human


active
A_{pale}
A_{dark}
reserve
singles
singles
pairs
pairs
chains
chains
B
(monkeys more B generations)
spermatocytes
spermatids
spermatozoa


primates
non-primates
A_{pale}
A_{dark}
A
singles
singles
singles
pairs
pairs
pairs
chains
chains
chains
B
6 generations A, In, B
spermatocytes
spermatocytes
spermatids
spermatids
spermatozoa
spermatozoa
Berlin, June 2004

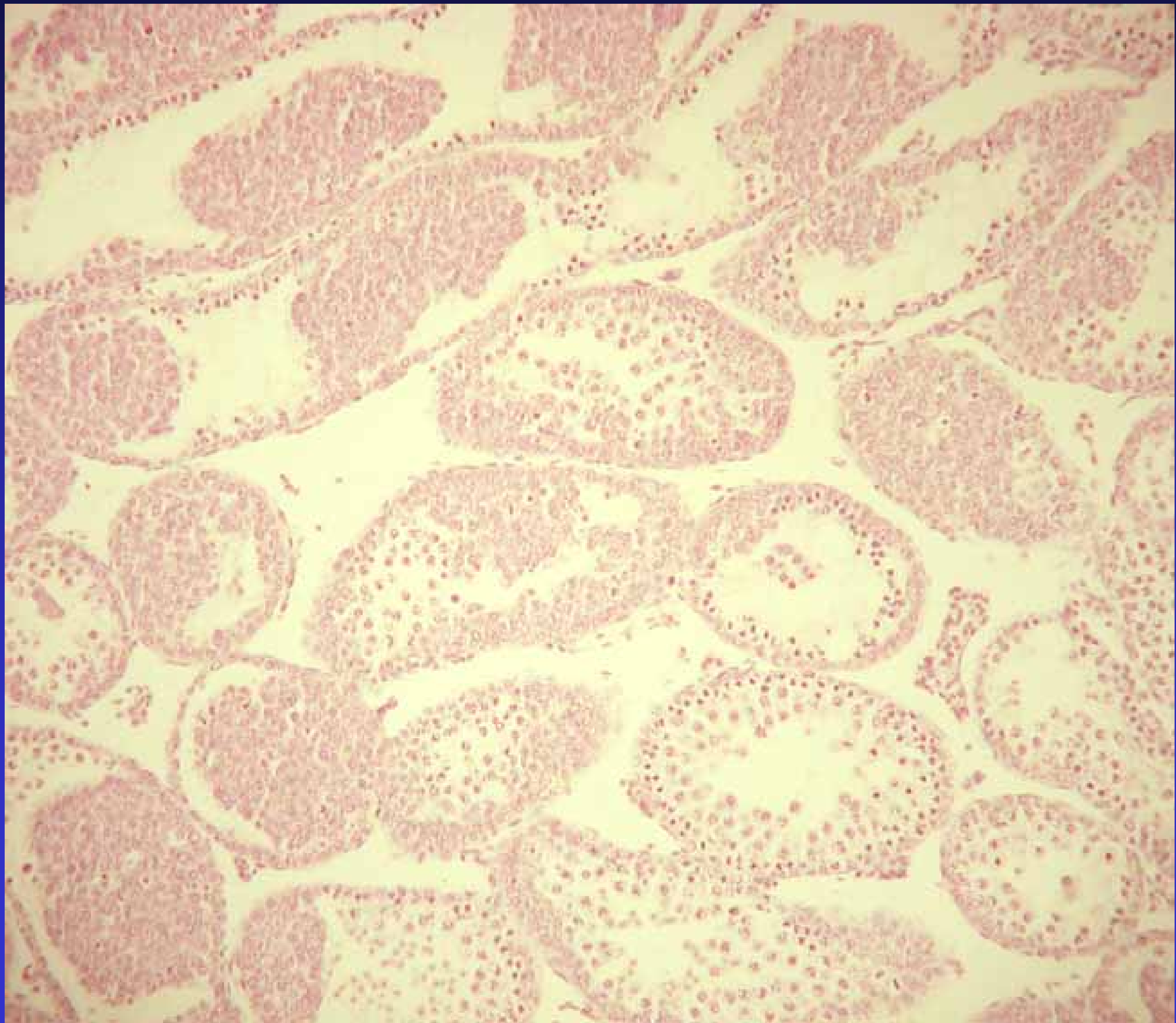
1. Knowledge on the development of the spermatogenic stem cell lineage
2. Principals of the nature of spermatogonial stem cells in primates and non-primates
3. Understand the principles of the regulation of differentiation and self-renewal
4. Possible important new development - Side population of stem cells

A_s

A_{pr}

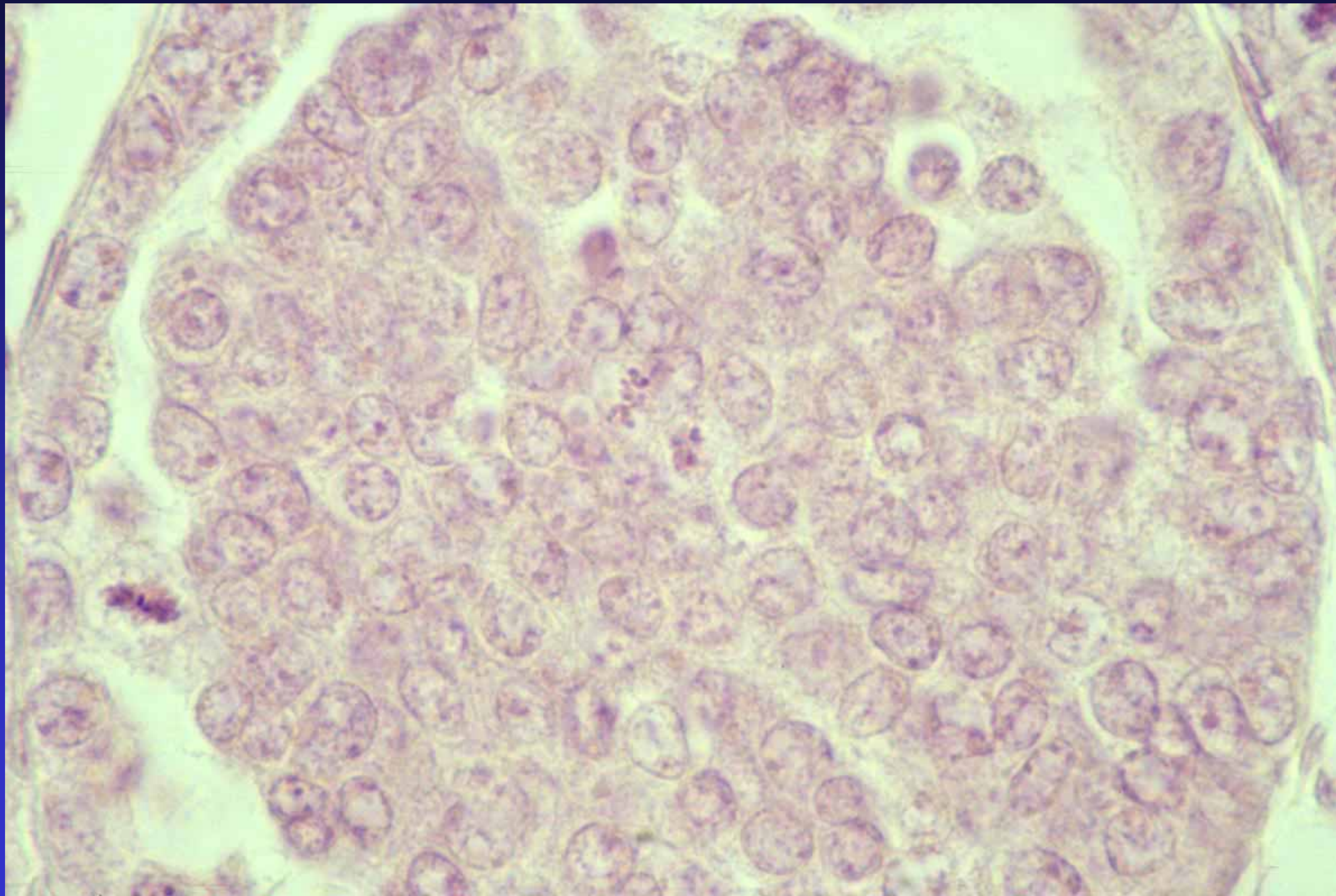
Glial cell line derived neurotrophic factor (GDNF) overexpression

Clusters of stem cells at 3 weeks p.p.



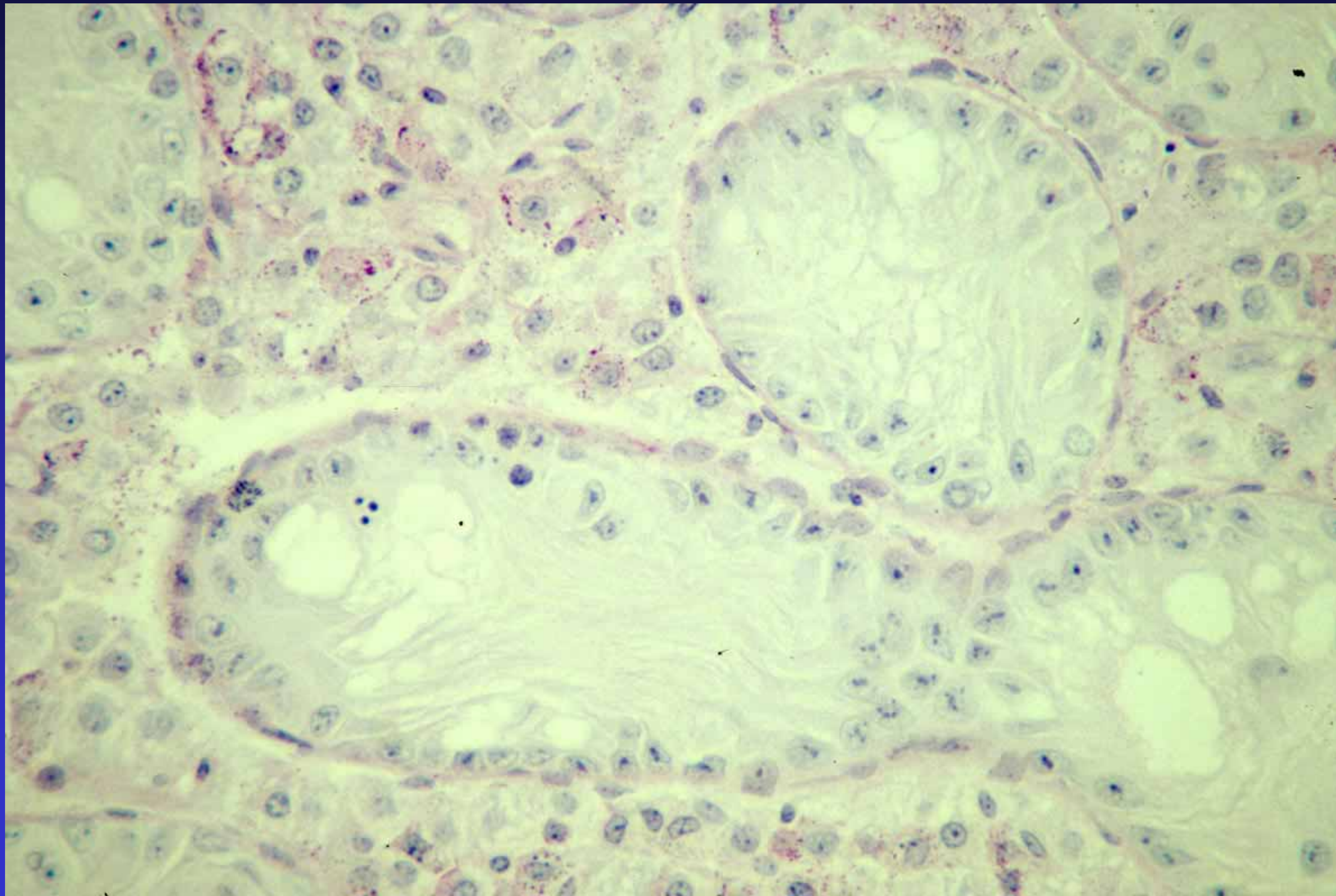
Meng et al., 2000

GDNF overexpression
Cluster of stem cells



Meng et al., 2000

GDNF +/- mouse
Depletion of seminiferous epithelium



Meng et al., 2000

Sertoli cells

FSH +

GDNF

c-Ret
GFR

(receptors on stem cells)

+

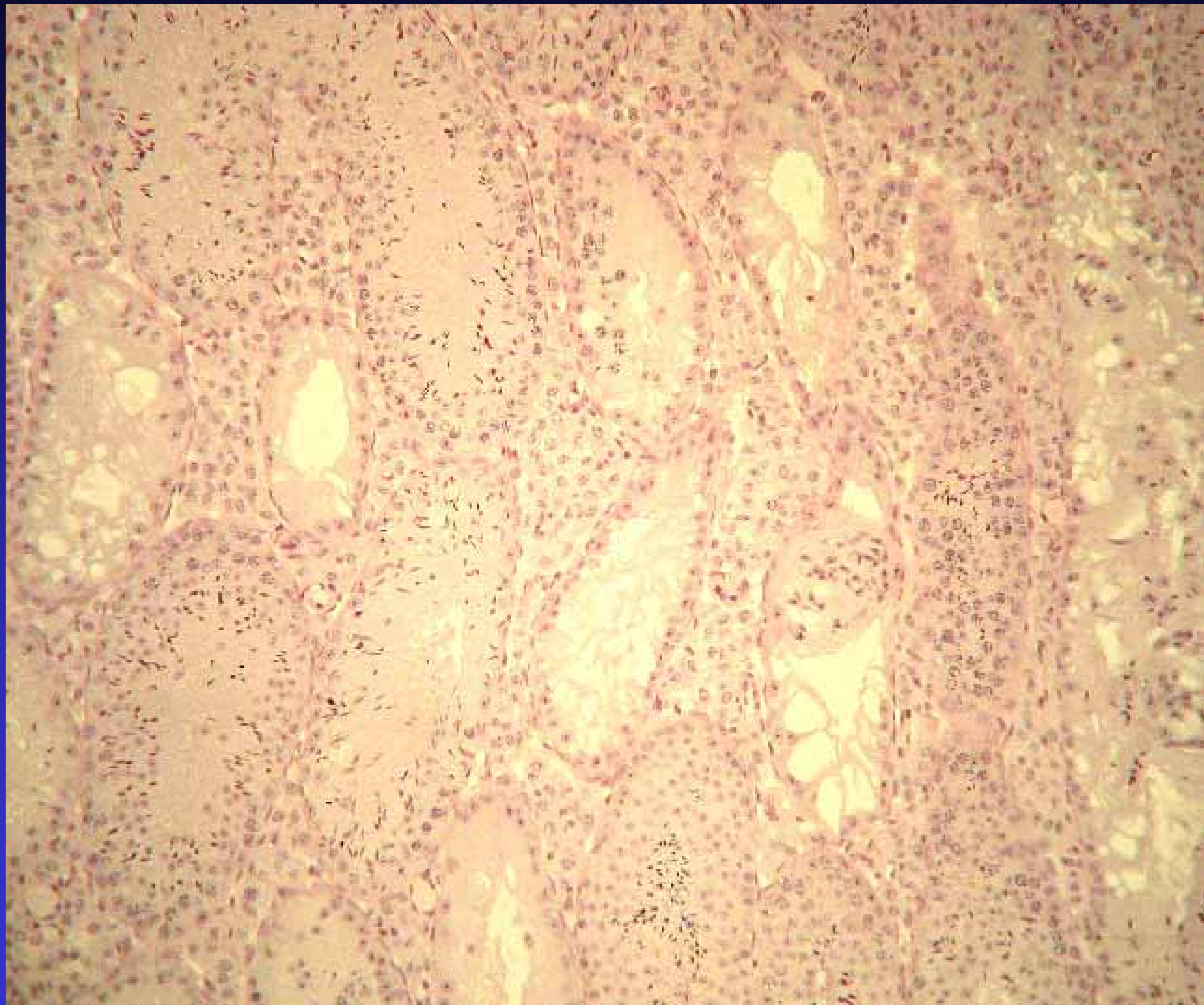
A_s

A_{pr}

Meng et al., 2000

Berlin, June 2004

lu/lu (luxoid) mouse testis at 6 months



Berlin, June 2004

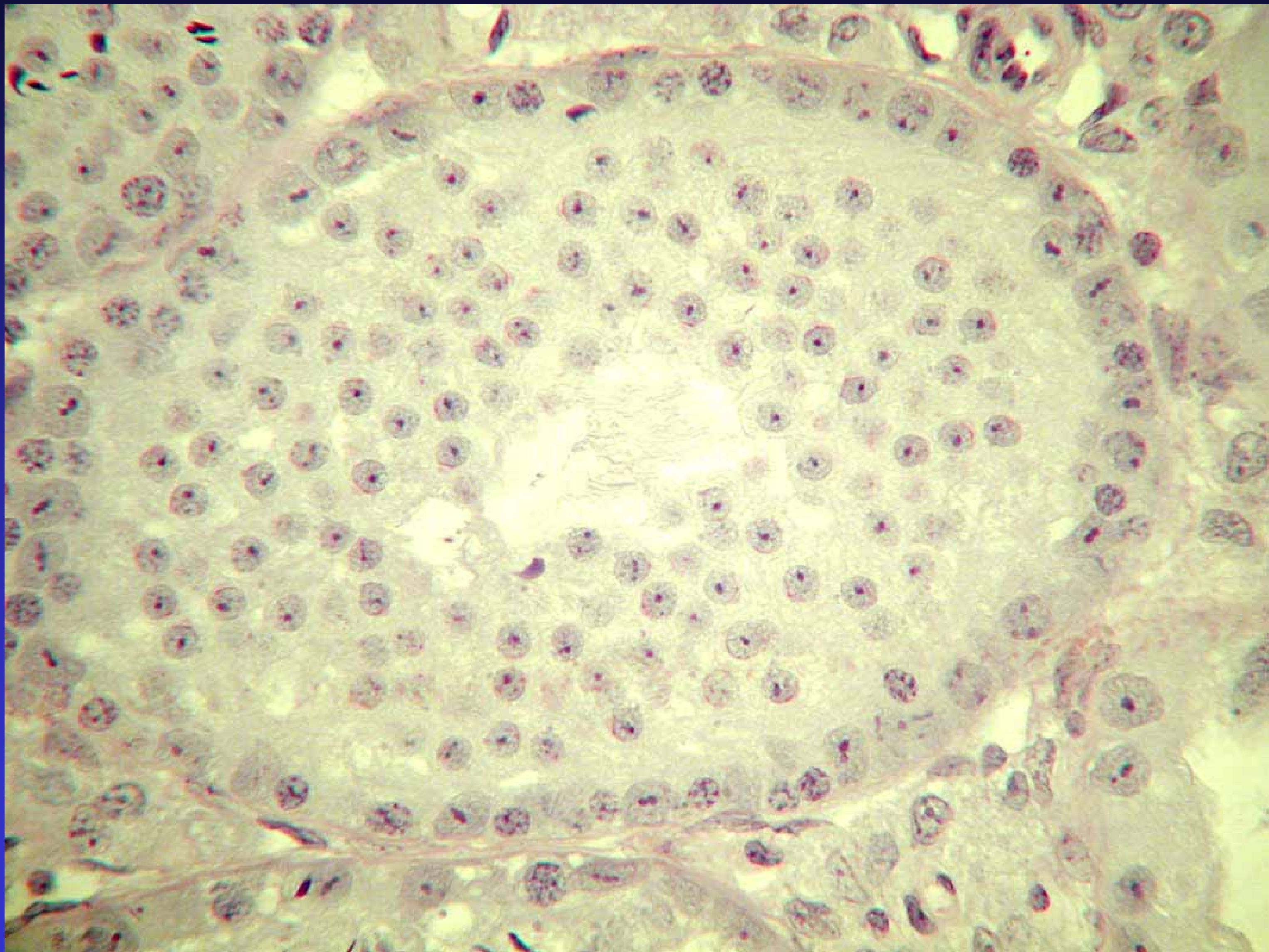
Buaas et al., 2004

lu/lu (luxoid) mouse testis

Berlin, June 2004

Buaas et al., 2004

lu/lu mouse testis



Buaas et al., 2004

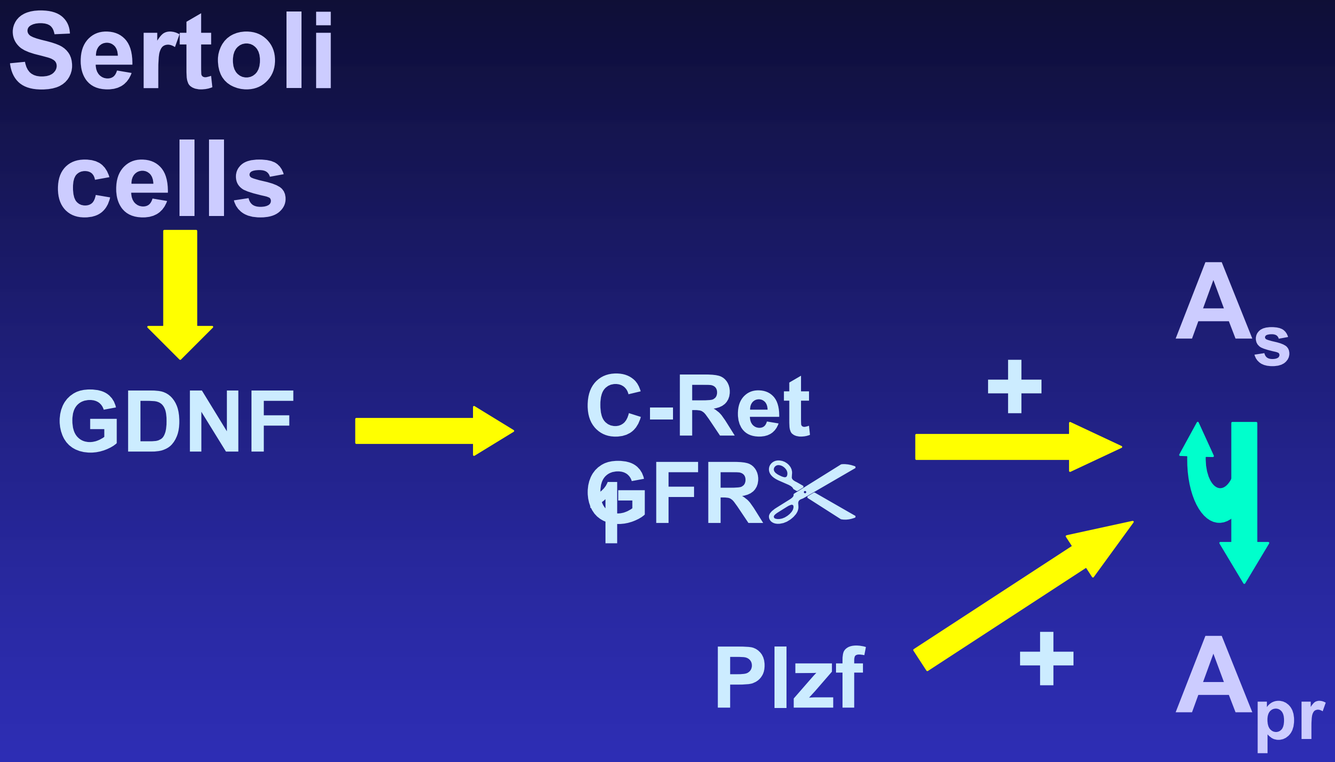
lu/lu mouse has mutation in Plzf

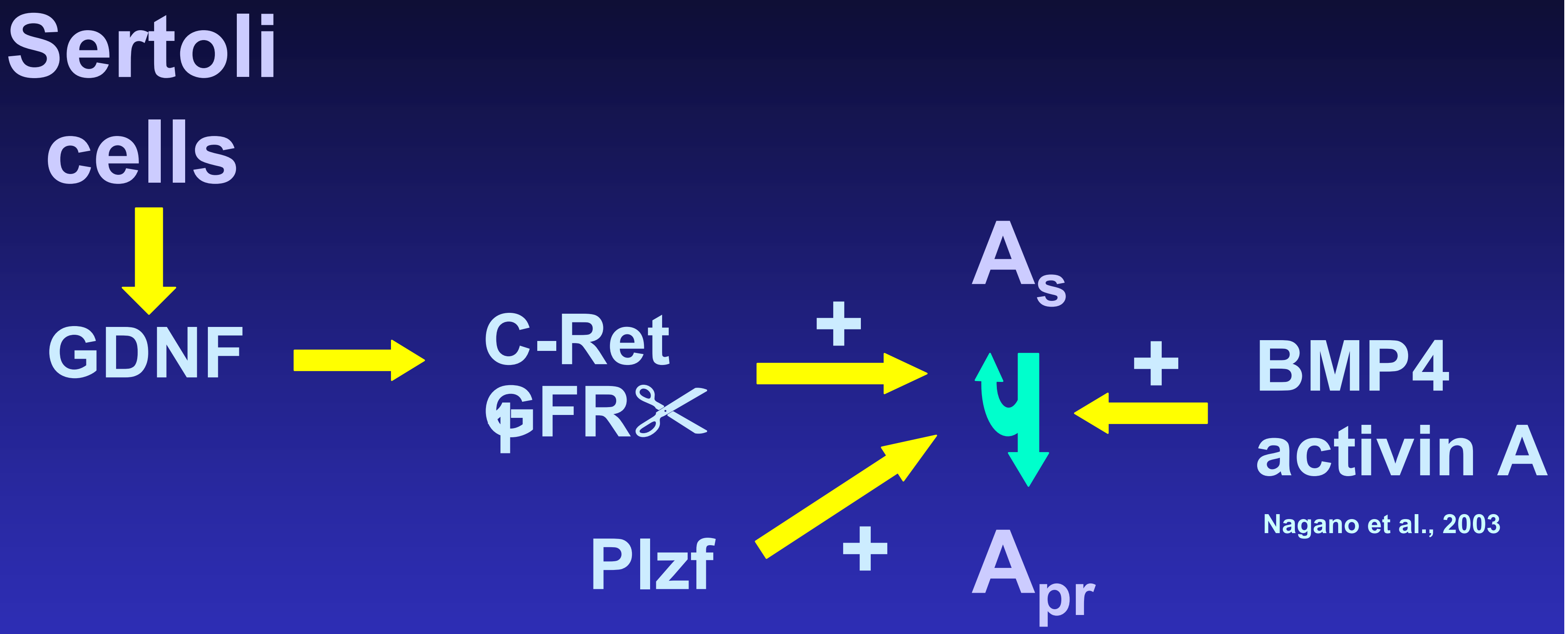
(*promyelocytic leukemia zinc-finger*)

Transcriptional repressor that regulates the epigenetic repression of chromatin domains necessary for cell differentiation

Recruits members of the Polycomb family (e.g. BMI1

Buaas et al., 2004


Sertoli cells
GDNF
C-Ret
GFR
Plzf
+
+
A_s
A_{pr}

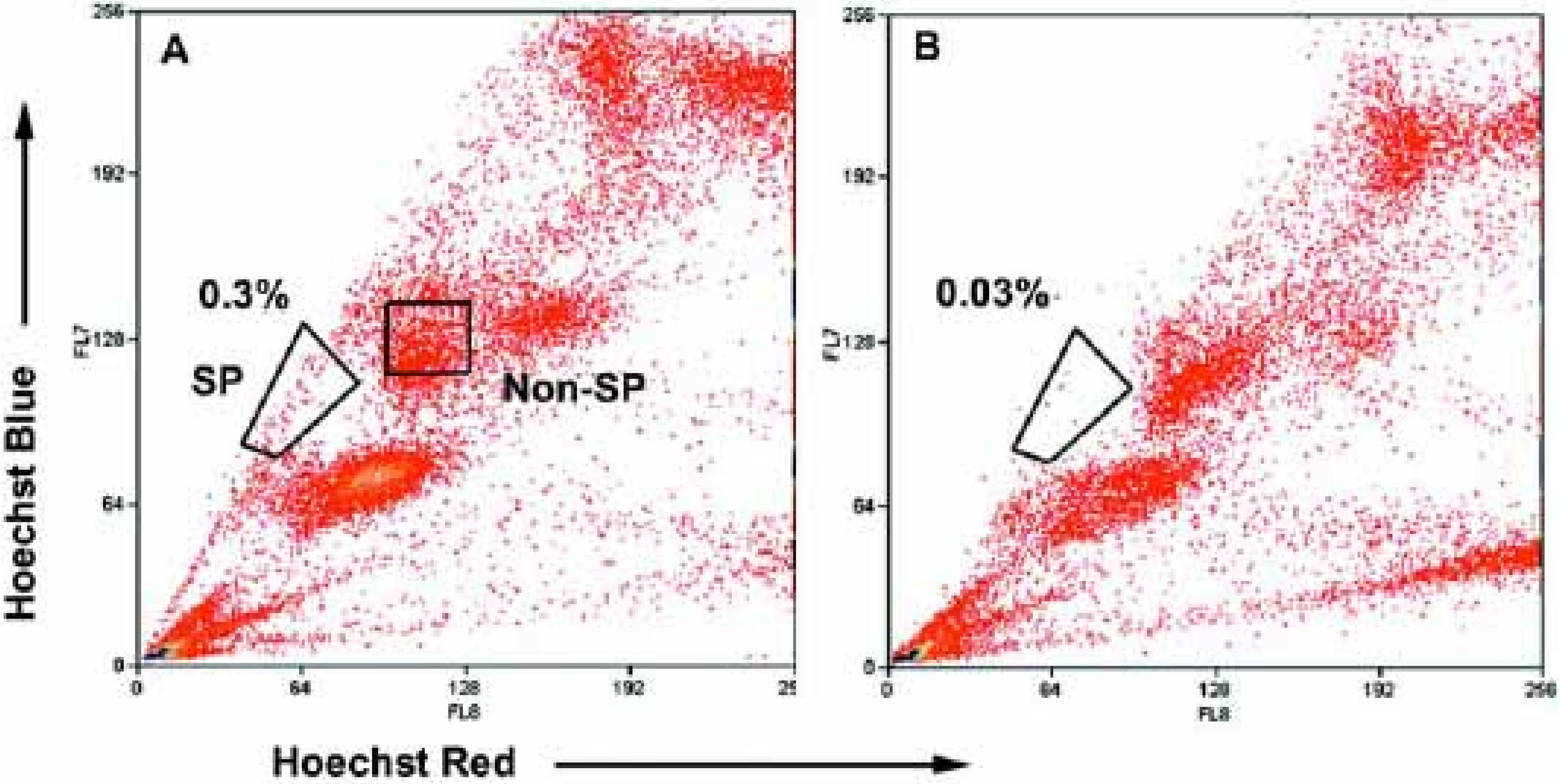

Sertoli cells
GDNF
C-Ret
GFR
+
Plzf
+
A_s
A_{pr}
+
BMP4
activin A
Nagano et al., 2003

1. Knowledge on the development of the spermatogenic stem cell lineage
2. Principals of the nature of spermatogonial stem cells in primates and non-primates
3. Understand the principles of the regulation of differentiation and self-renewal
4. Possible important new development - Side population of stem cells

Side population stem cells

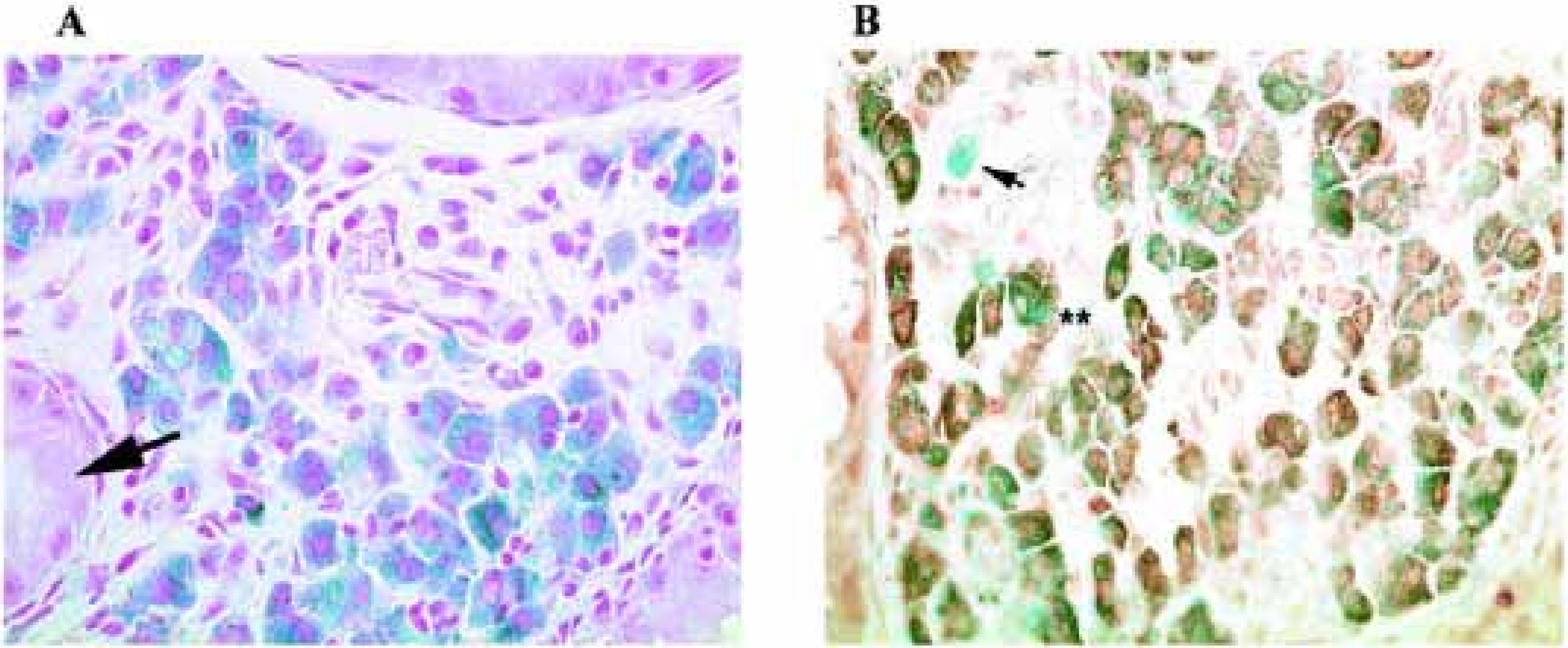
- rapidly efflux the fluorescent DNA-binding dye Hoechst 33342
- side population cells are found in many tissues and are stem cells in several tissues (hemopoiesis, mammary gland etc.)
- Kubota *et al.* 2003 – Testicular side population does not contain spermatogonial stem cells
- Falciatori *et al.* 2004 and Lassale *et al.* 2004 – There are spermatogonial side population stem cells
- Lo *et al.* 2004 – There are side population Leydig stem cells!

+ verapamil



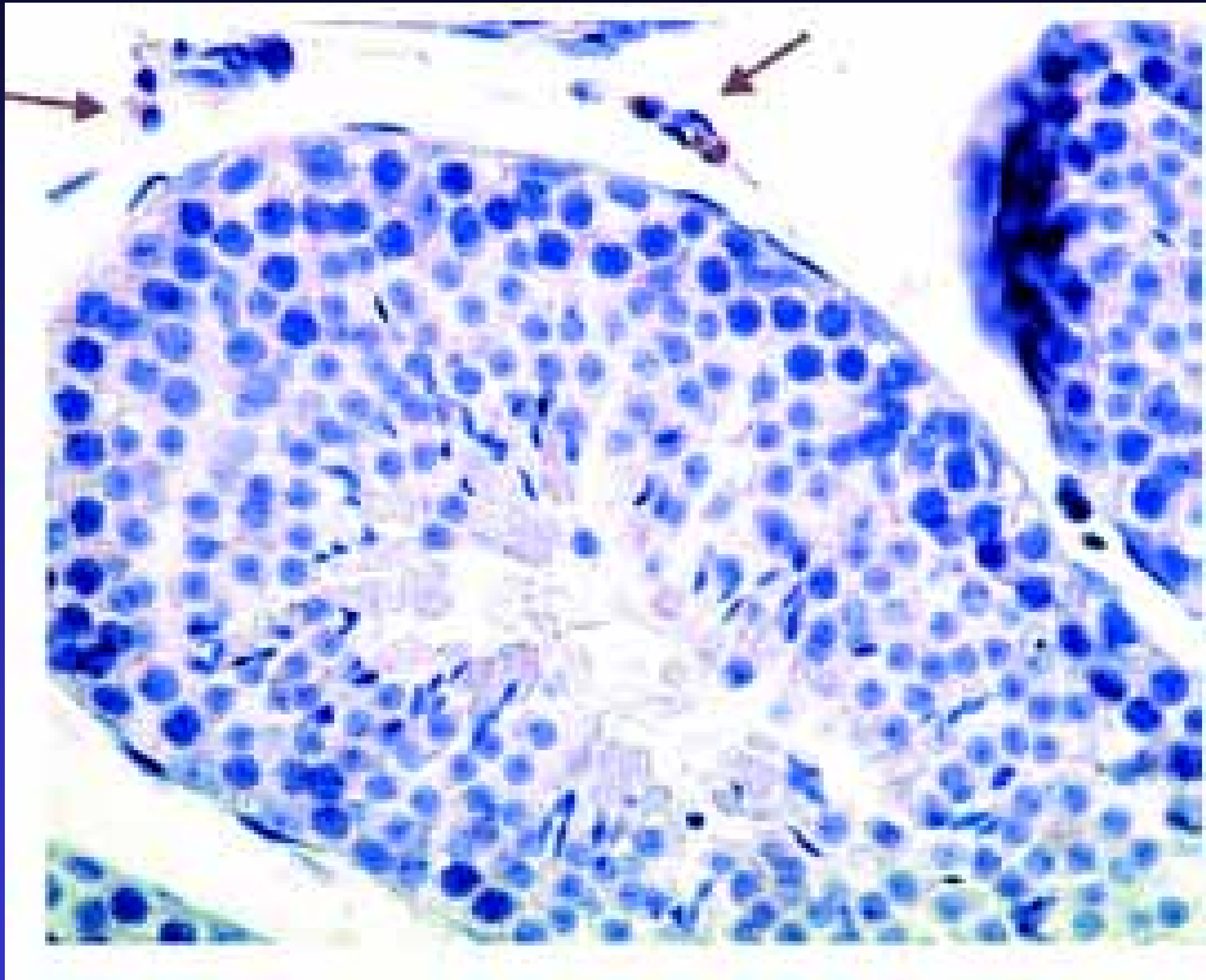
Lo et al., 2004

X-gal   p450scc (brown)

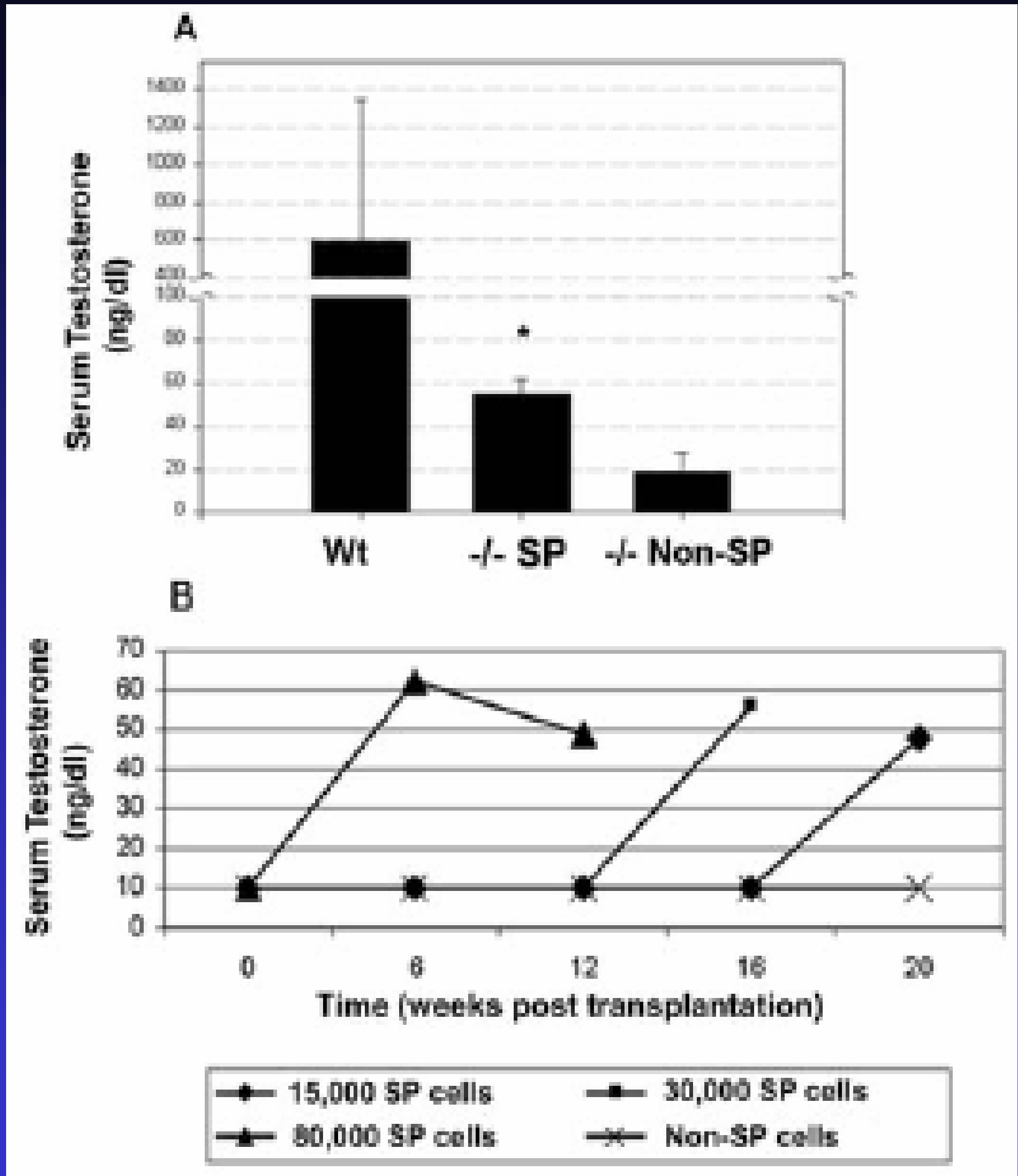


Lo et al., 2004

LHRKO mice



Lo et al., 2004



Lo et al., 2004

Berlin, June 2004

Perspectives

- Leydig stem cell assay
- Purification and characterization of Leydig stem cells
- Transplantation to ectopic side when castration is unavoidable?

1. Knowledge on the development of the spermatogenic stem cell lineage
2. Principals of the nature of spermatogonial stem cells in primates and non-primates
3. Understand the principles of the regulation of differentiation and self-renewal
4. Important new development - Side population contains Leydig stem cells