ESHRE 2004 - Berlin (Germany)
Pre-Congress Course 2
"Testicular Stem Cells"
June 27, 2004

Transplantation of testicular stem cell suspensions and testicular grafting

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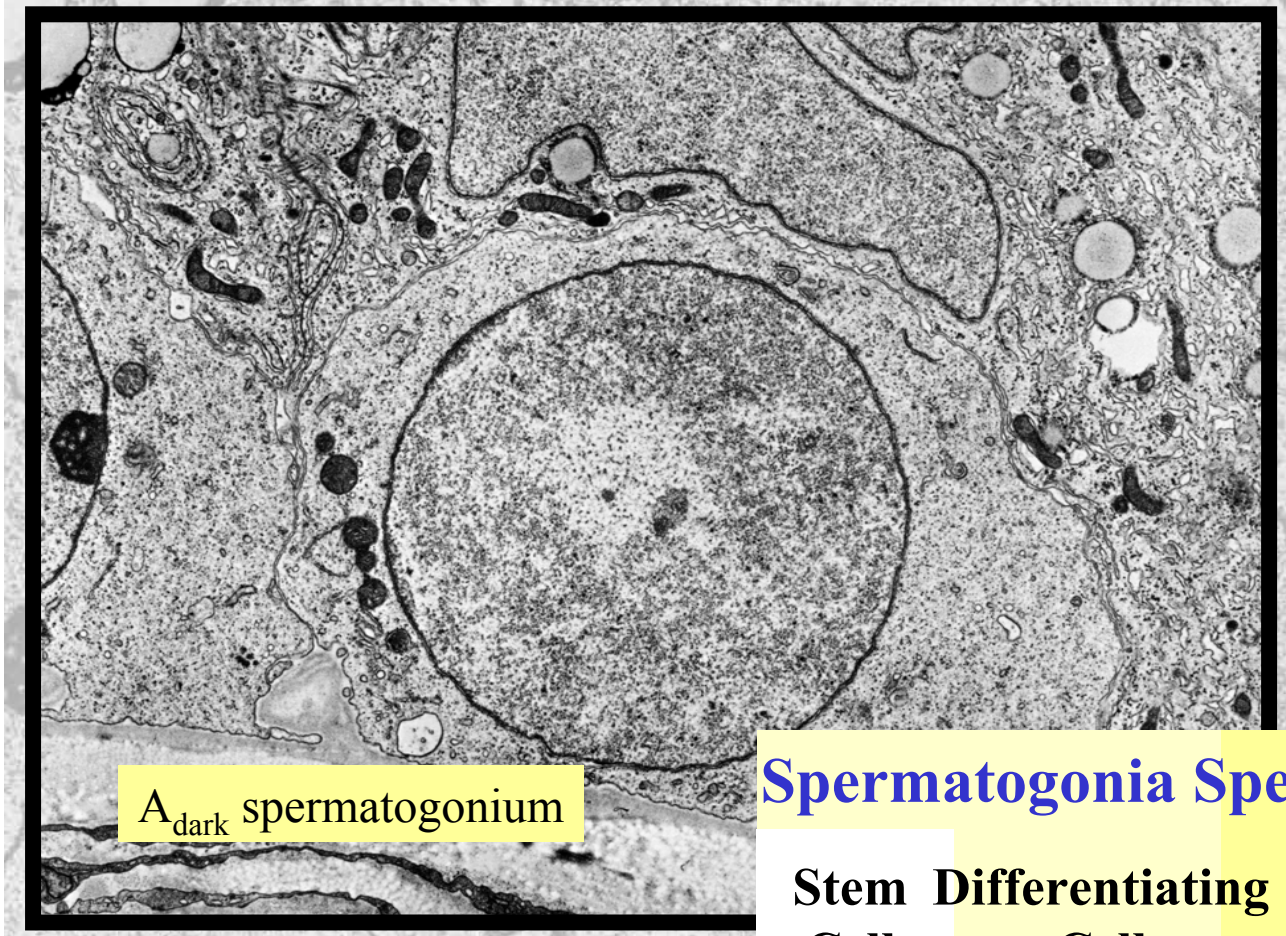
Department of Cell Biology and Physiology

The University of Pittsburgh School of Medicine

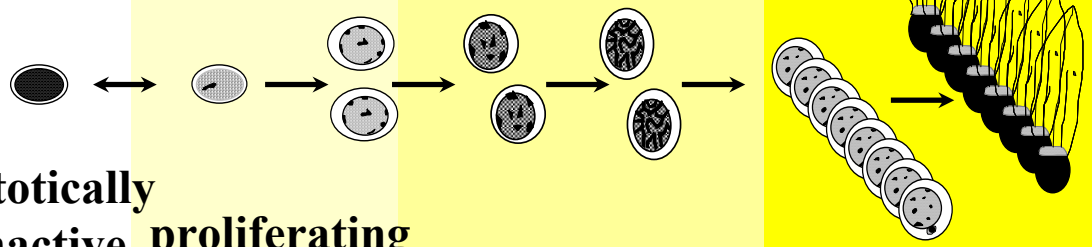
A different kind of medical school. A different kind of medicine.



A_{dark} spermatogonium

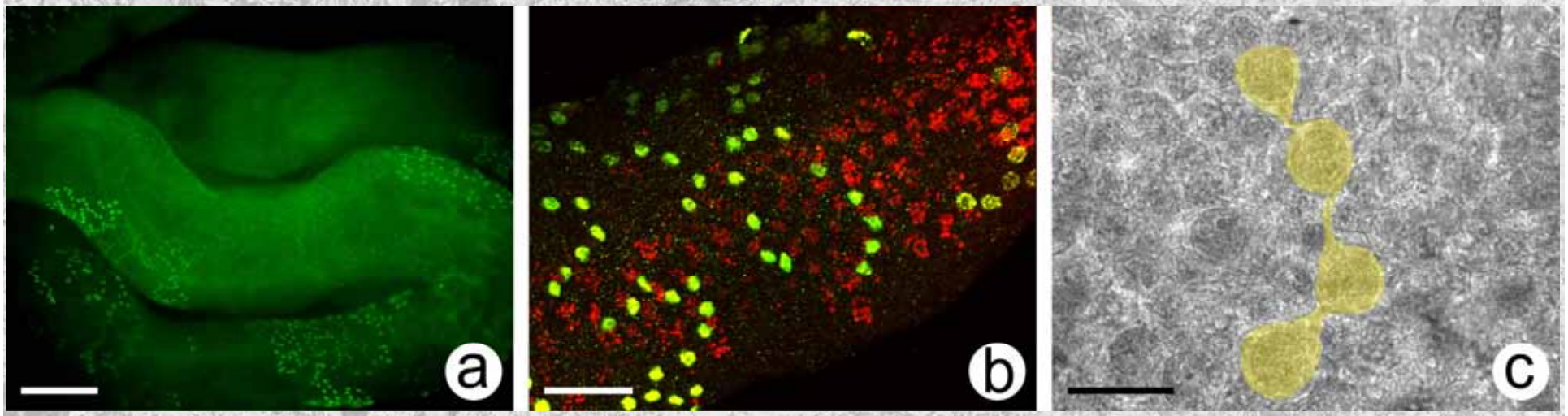
Spermatogonia Spermatocytes Spermatids

Stem Cell **Differentiating Cell** **Meiotic Cells** **Postmeiotic Cells**

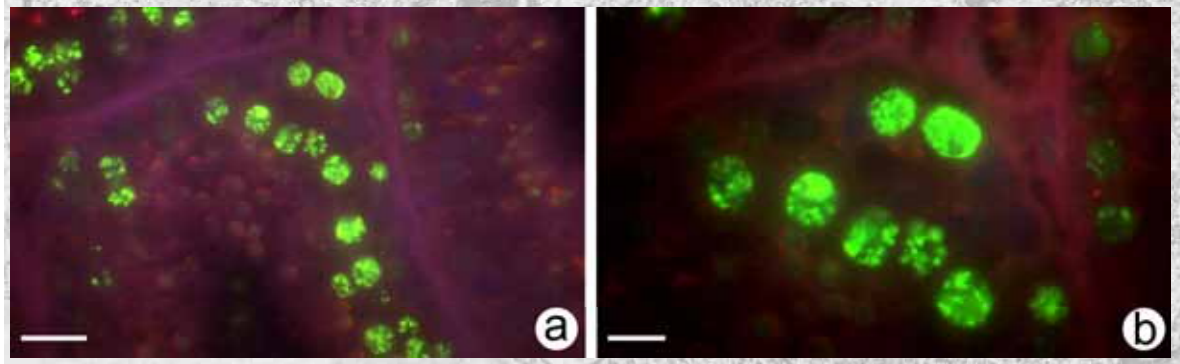


mitotically inactive **proliferating**

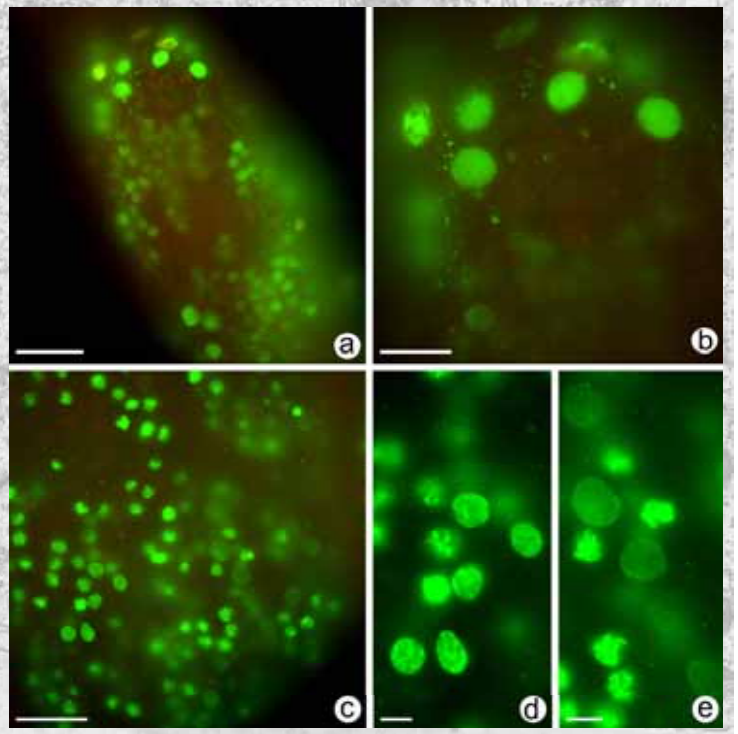
Monkey Spermatogonia: Clonal expansion in whole mounts



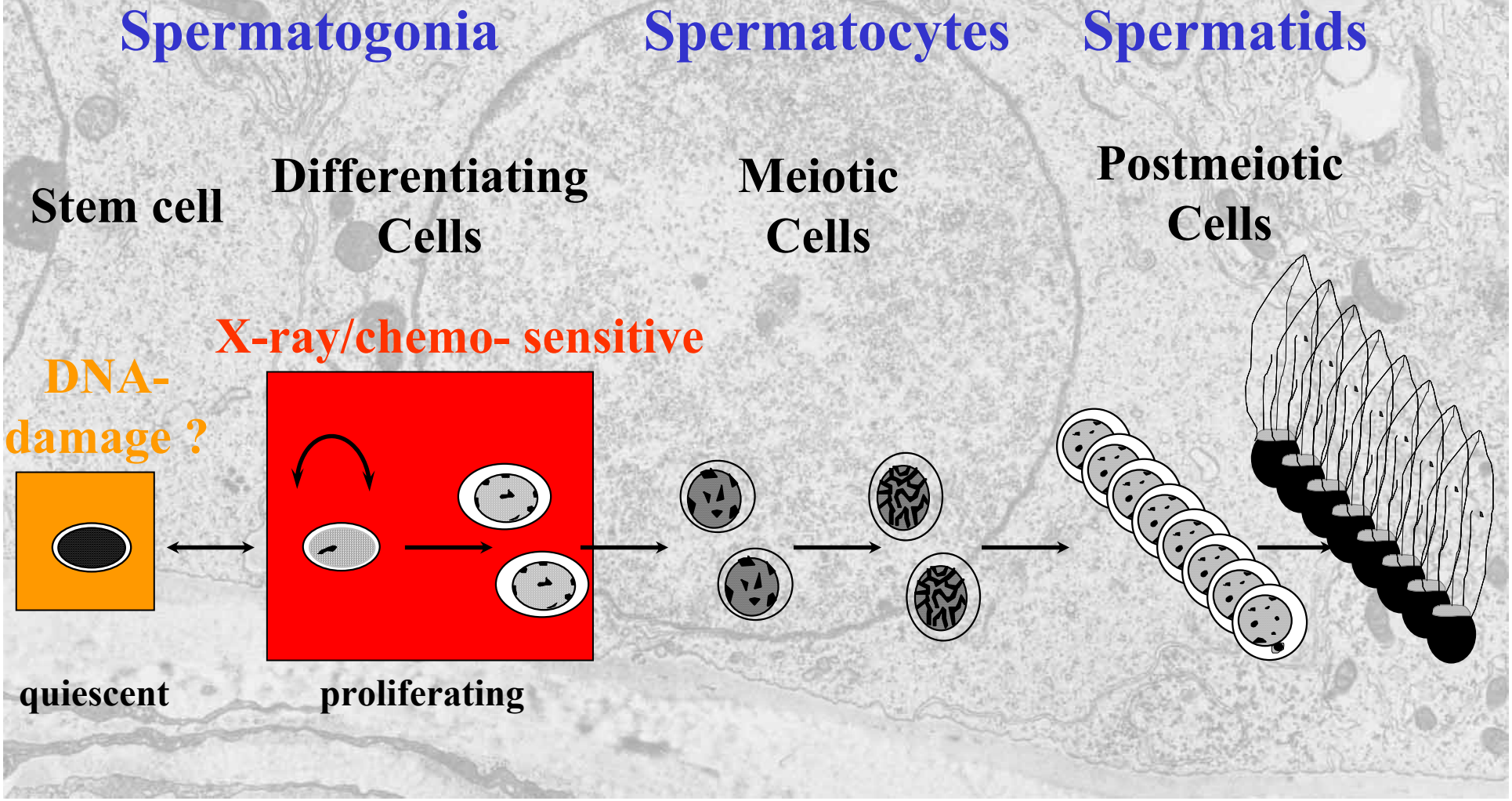
Confocal Microscopy: BrdU and acrosin IHC

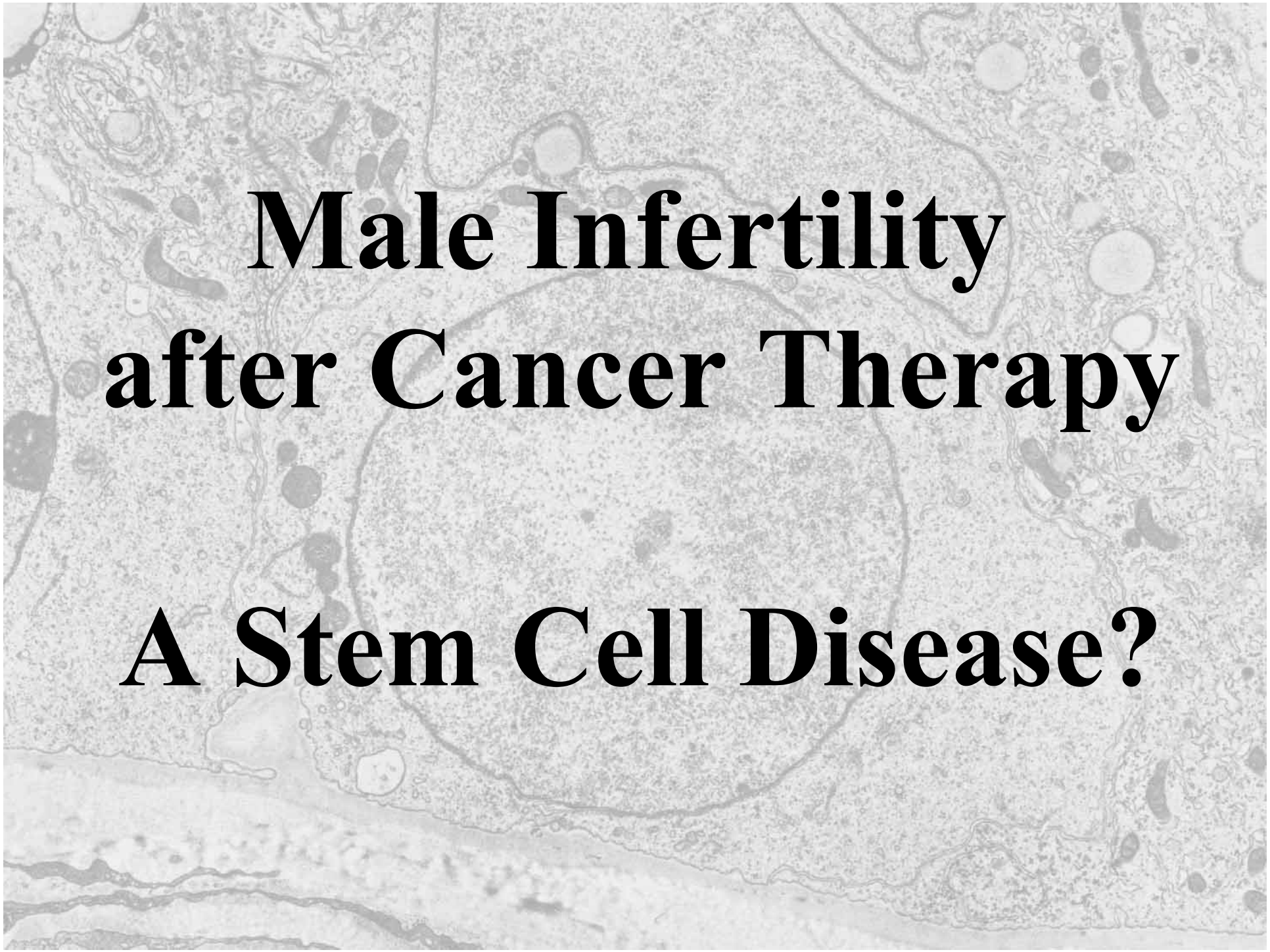


BrdU-Tracing



Schematic Representation of Defects on Human Germ Cell Development After Oncological Therapy





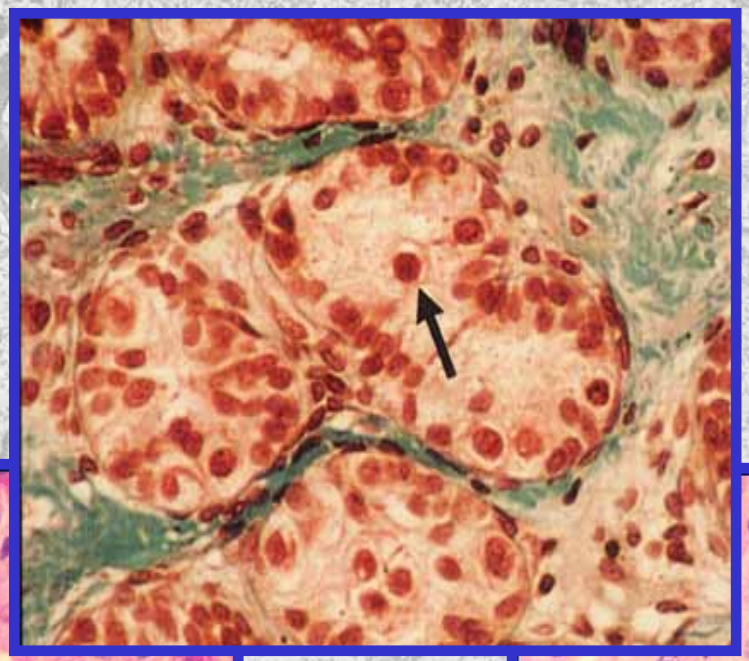
Male Infertility after Cancer Therapy

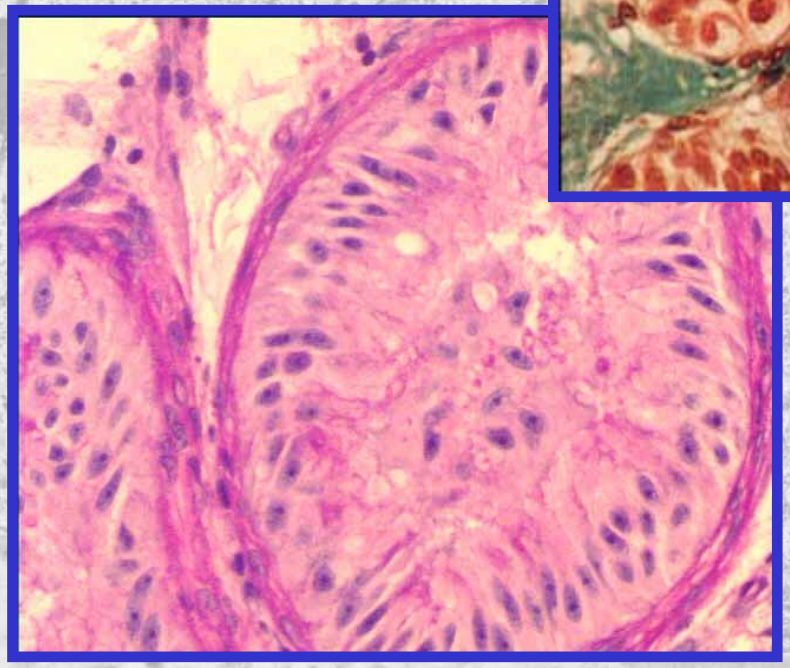
A Stem Cell Disease?

Testis development after cancer therapy during childhood

Immature Testis

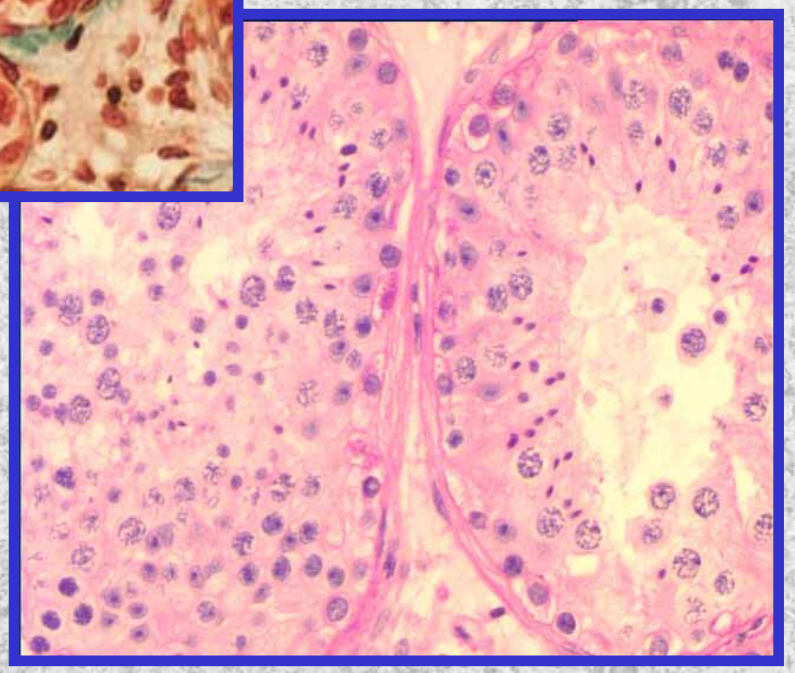
Period of treatment





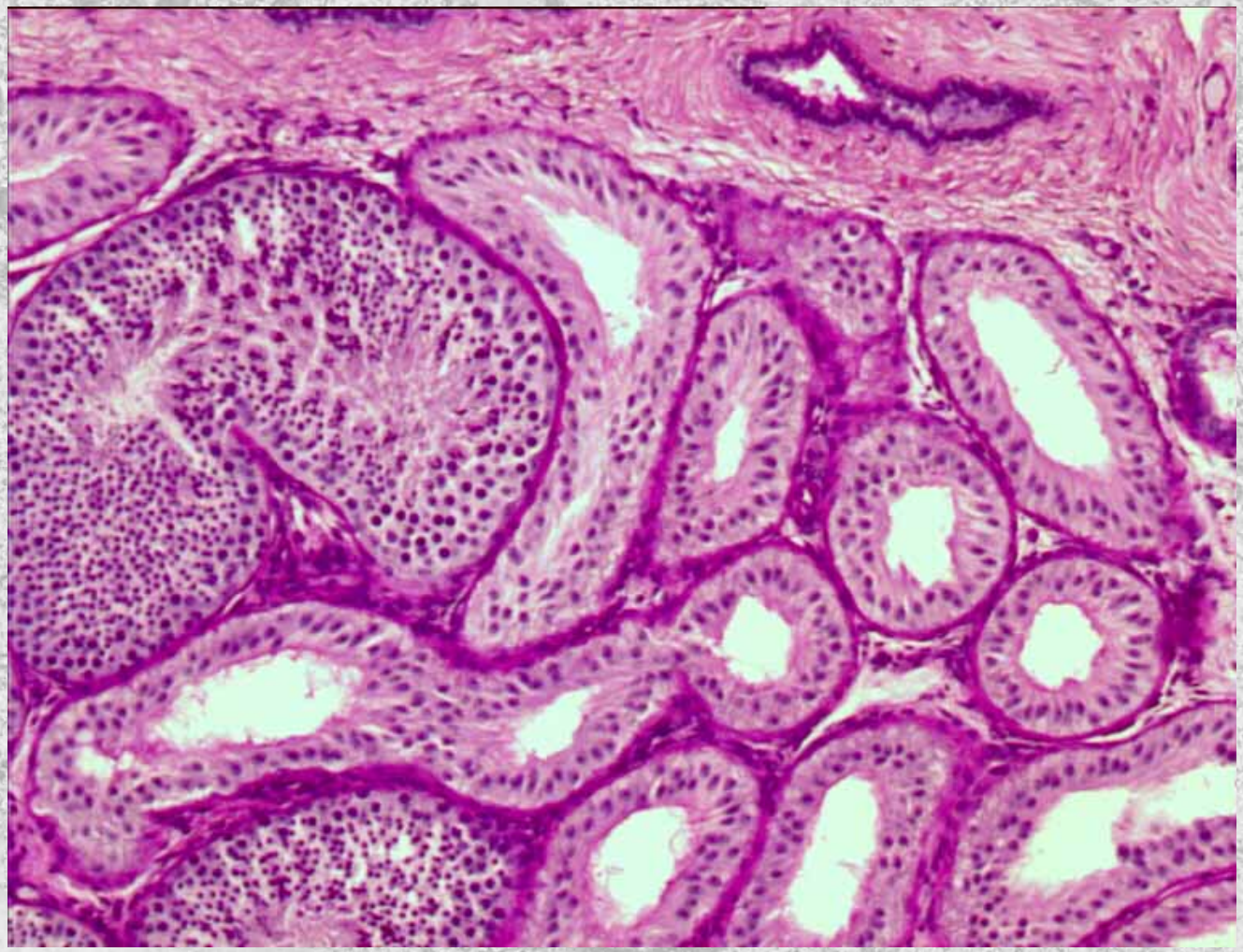
Sertoli-cell-only Syndrome

(Side effect after onological therapy)



Normal adult testis

Cynomolgus monkey testis after testicular X-irradiation



Focal Sertoli Cell Only: All or Nothing

Stem Cell Treatment Option I: Protection

1. Suppress stem cell turnover

2. Expand the stem cell population

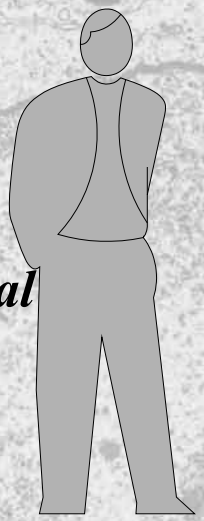
Van Alphen et al., Cancer Res 49: 533-536
Protection from radiation-induced damage in the rhesus monkey (Macaca mulatta) by FSH.
Kamischke et al., J Endocrinol 179: 183-194 (2003)
Gonadal protection from radiation by GnRH antagonist or recombinant human FSH: a controlled clinical trial in a male nonhuman primate (Macaca fascicularis).

3. Protect genetic integrity of spermatogonia

Walter CA et al., Proc Natl Acad Sci U S A 95: 10015-10019 (1998).
Mutation frequency declines during spermatogenesis in young mice but increases in old mice.
Goriely A et al., Science 301: 643-646 (2003).
Evidence for selective advantage of pathogenic FGFR2 mutations in the male germ line.

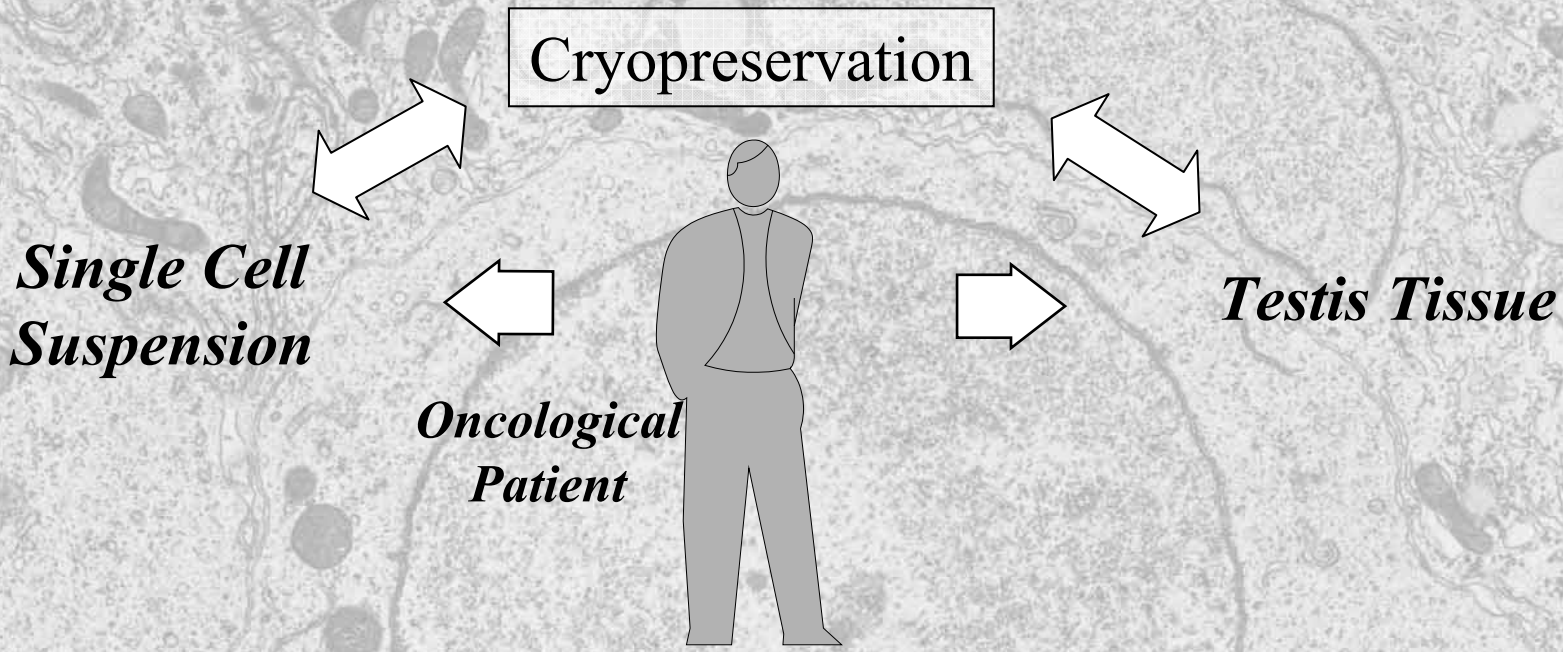
Future Gonadal Protection Strategies

Cryopreservation

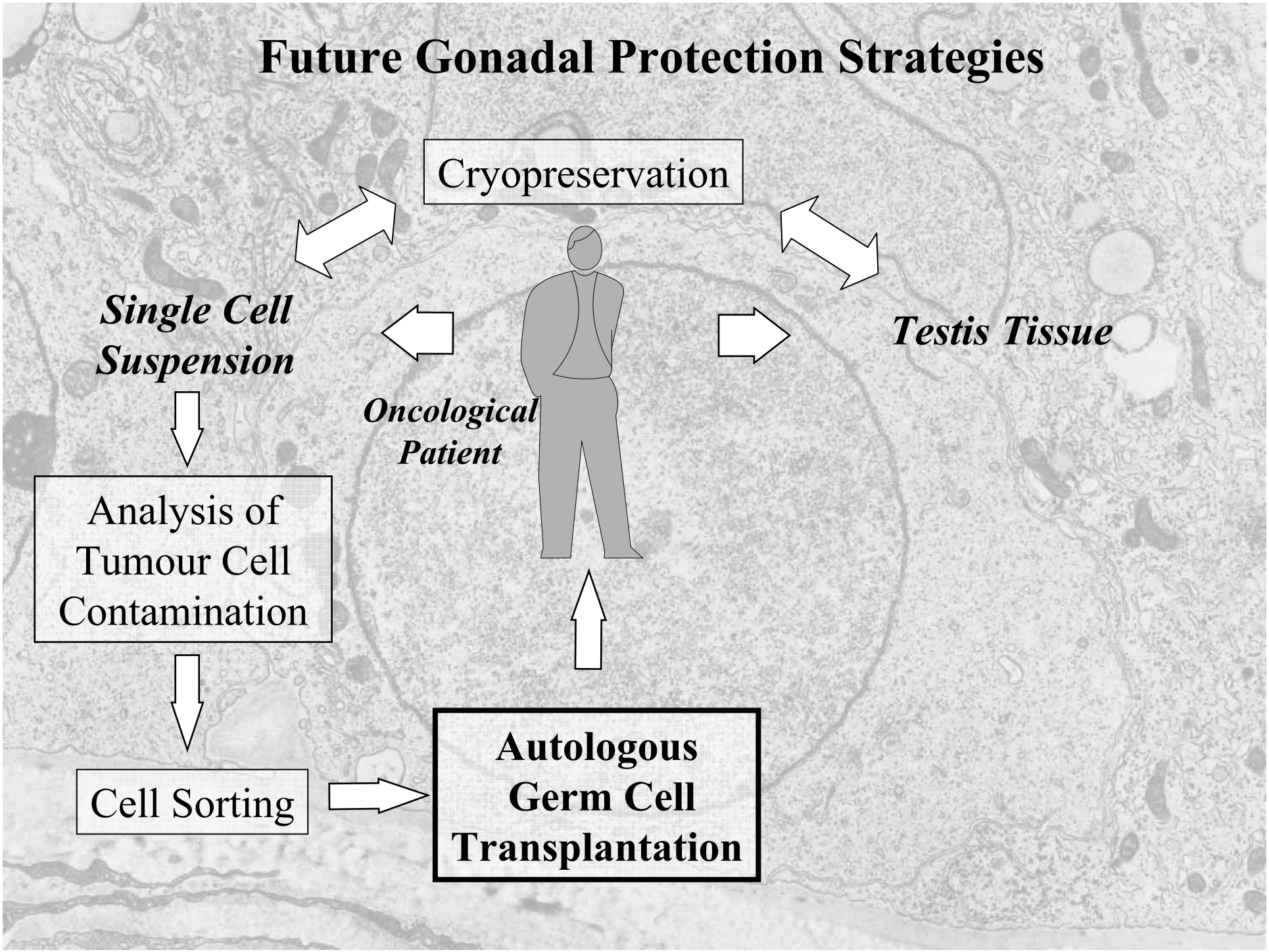


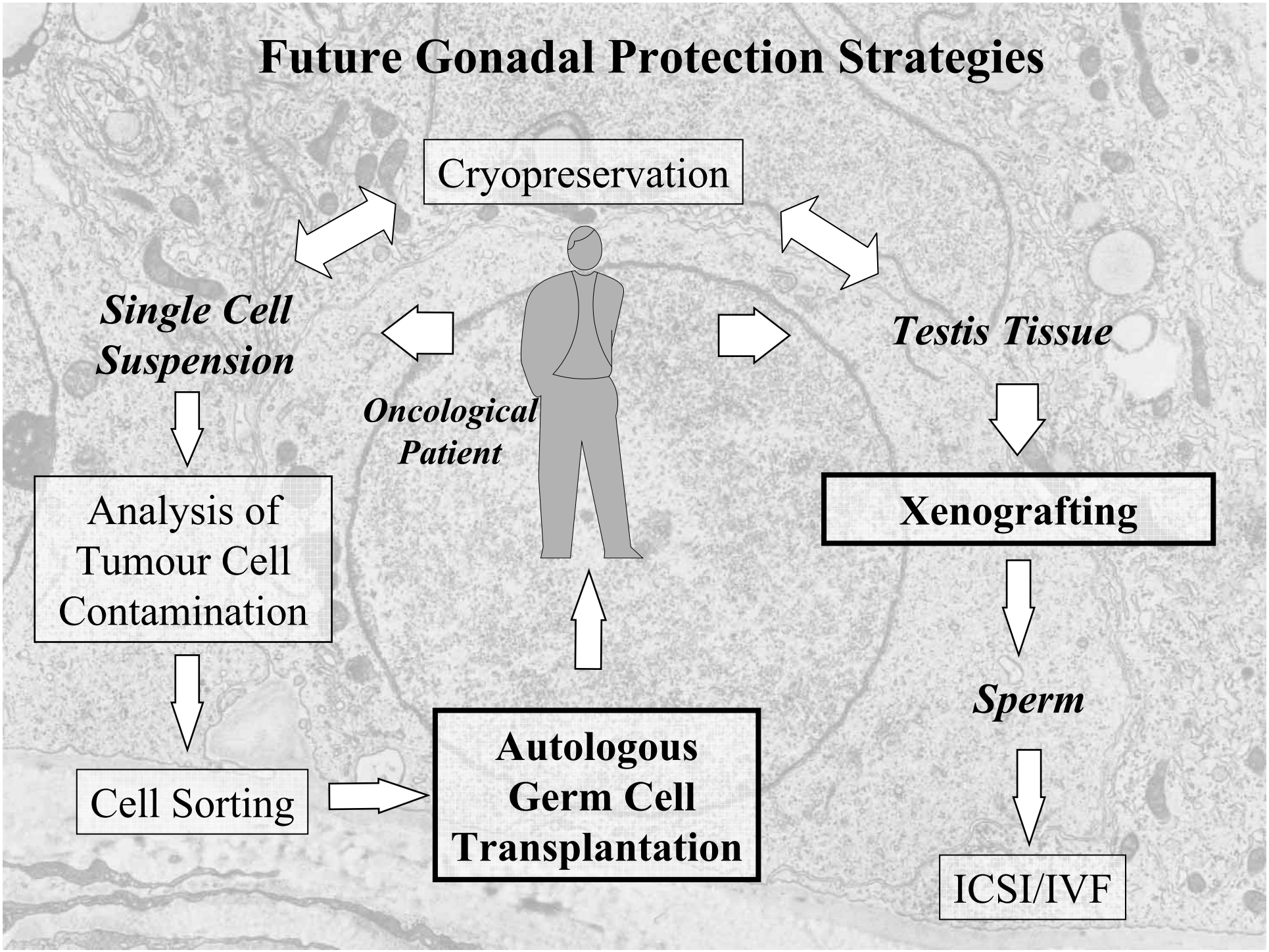
Oncological Patient

Future Gonadal Protection Strategies



Future Gonadal Protection Strategies




Future Gonadal Protection Strategies
Cryopreservation
Single Cell Suspension
Oncological Patient
Testis Tissue
Analysis of Tumour Cell Contamination
Xenografting
Sperm
Cell Sorting
Autologous Germ Cell Transplantation
ICSI/IVF

Undifferentiated Spermatogonia are stem cells: **Potential for (re)colonization and (re)population**

Germ Cell Transplantation

Transfer of the spermatogonium out of the donors stem cell niche to the recipients stem cell niche.

Ectopic Testicular Grafting

Transfer of the spermatogonium with its niche from the donor to an ectopic site in the recipient

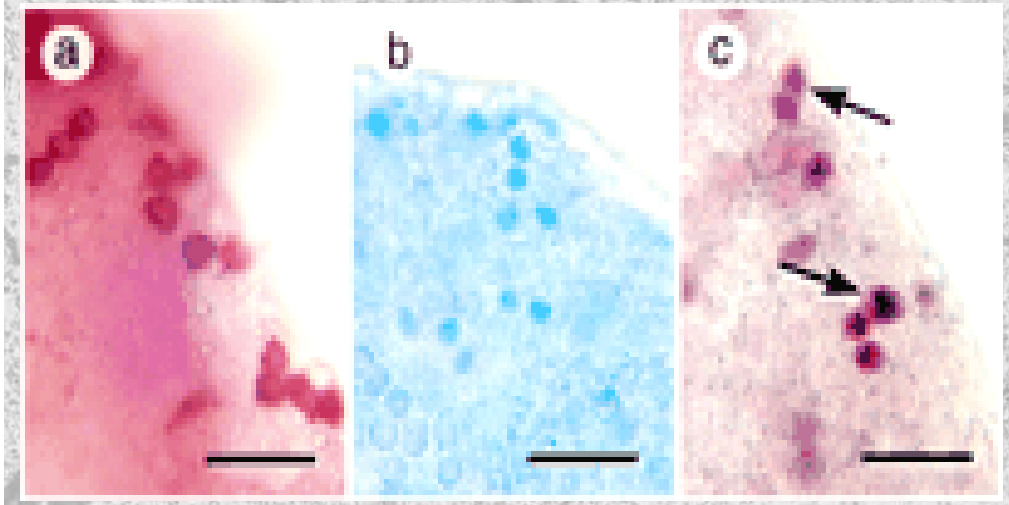
Stem Cell Treatment Option II: Germ Cell Transplantation

Aim: Extracorporeal storage and reinjection of target cells

Development of Germ Cell Transplantation

- **1994** **Spermatogenesis following male germ cell transplantation.** (Brinster and Zimmermann, PNAS 91: 11298)
- **1996** **Rat spermatogenesis in mouse testis**. (Clouthier et al., Nature 381: 418)
- **1996** **Reconstitution of spermatogenesis from frozen spermatogonial stem cells**. (Avarbock et al., Nat Med 2: 693)
- **1998** **Culture of mouse spermatogonial stem cells**. (Nagano et al., Tissue & Cell 30: 389)
- **1999** **Germ cell transfer into rat, bovine, monkey and human testes.** (Schlatt et al., Hum Reprod 14: 144)
- **2001** **Primate spermatogonial stem cells colonize mouse testes**. (Nagano et al., Biol Reprod 64: 1409)
- **2001** **Transgenic mice produced by retroviral transduction of male germ-line stem cells.** (Nagano et al. PNAS 98:13090)
- **2002** **Germ cell transplantation into X-irradiated monkey testes.** (Schlatt et al. Hum Reprod (17: 55)

Xenologous transplantation of primate spermatogonia

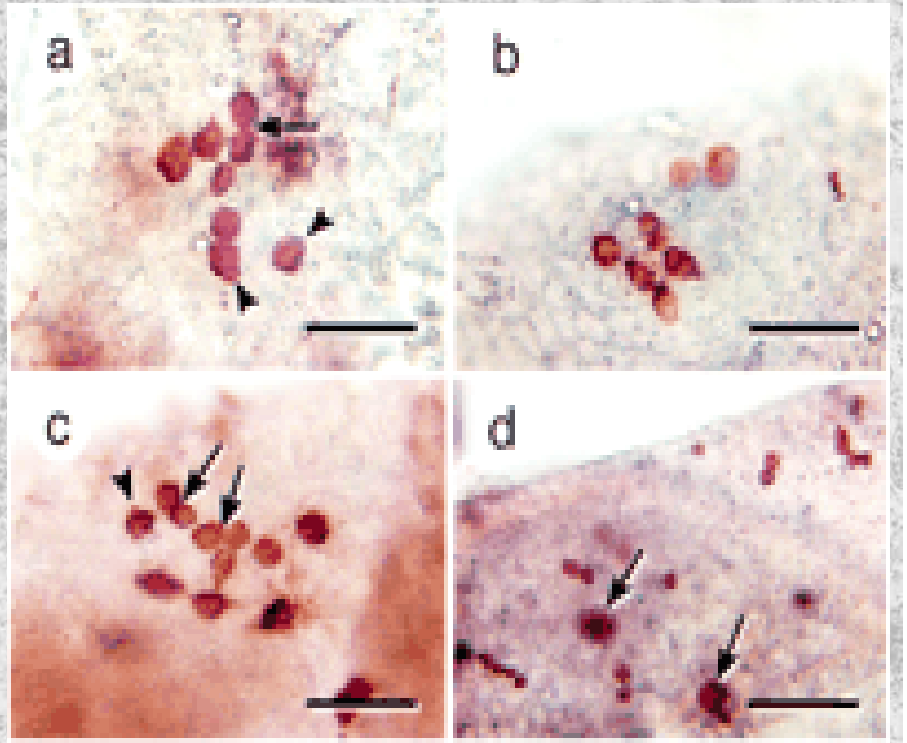


Baboon germ cell colonising mouse testes

Nagano et al., 2001 Primate spermatogonial stem cells colonise mouse testes. Biol Reprod 64: 1409-1416

Reis et al., 2000 Xenogeneic transplantation of human spermatogonia. Zygote 8: 97-105

Nagano et al., 2002 Long-term survival of human spermatogonial stem cells in mouse testes. Fertil Steril 78: 1225-33



Baboon germ cell colonising mouse testes after cryopreservation

→ **No Colonisation**

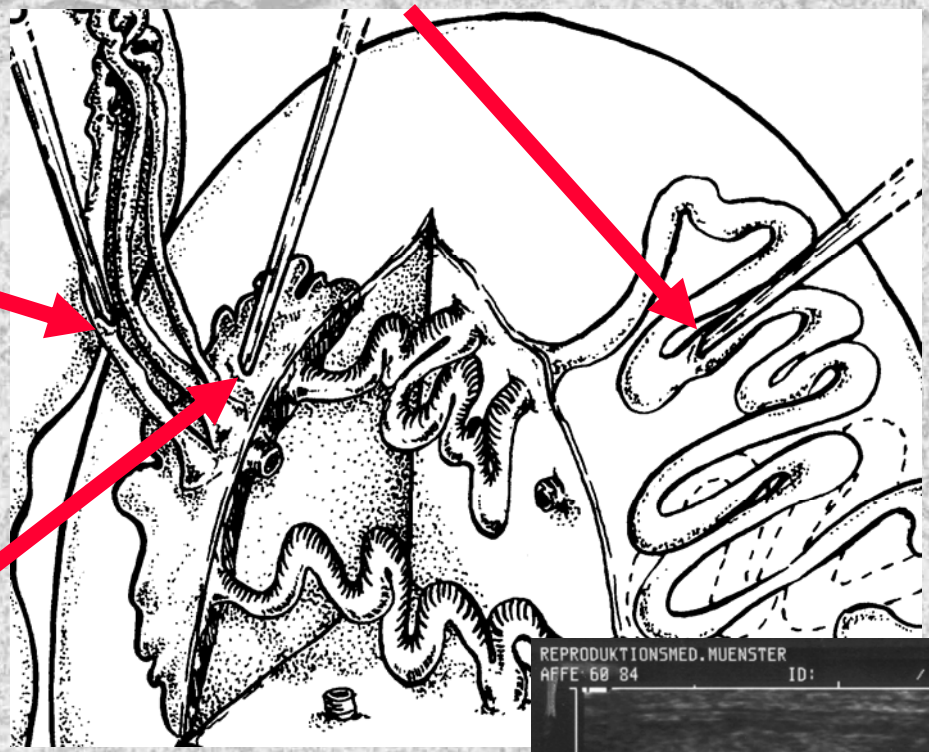
→ **Colonisation**

Germ Cell Infusion into the Monkey Testis

Microinjection of seminiferous tubules:
difficult and inefficient

Injections into efferent ducts:
surgically demanding and inefficient

Injections into the rete testis:
Easy, efficient and reproducible

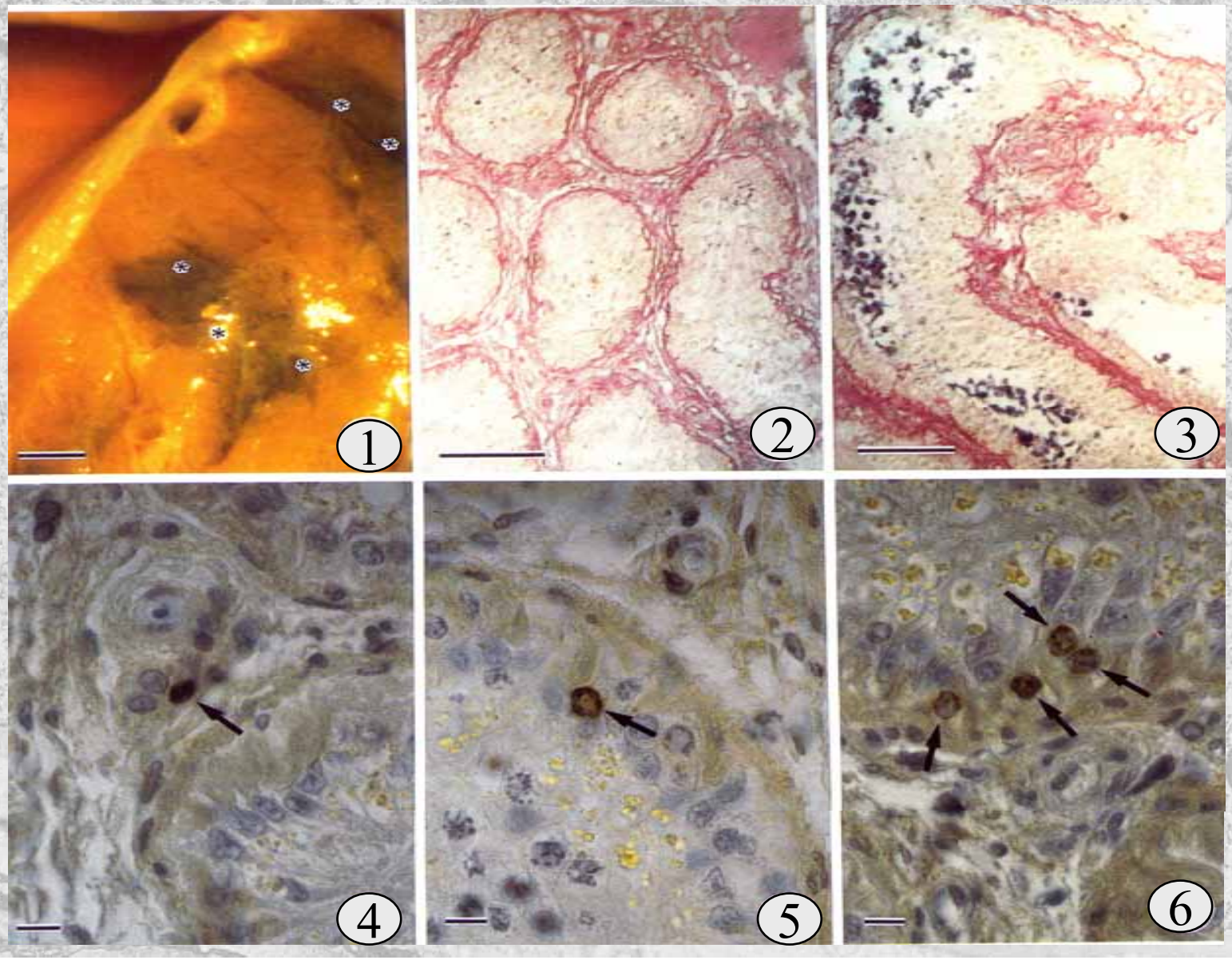


involuted recipient testis

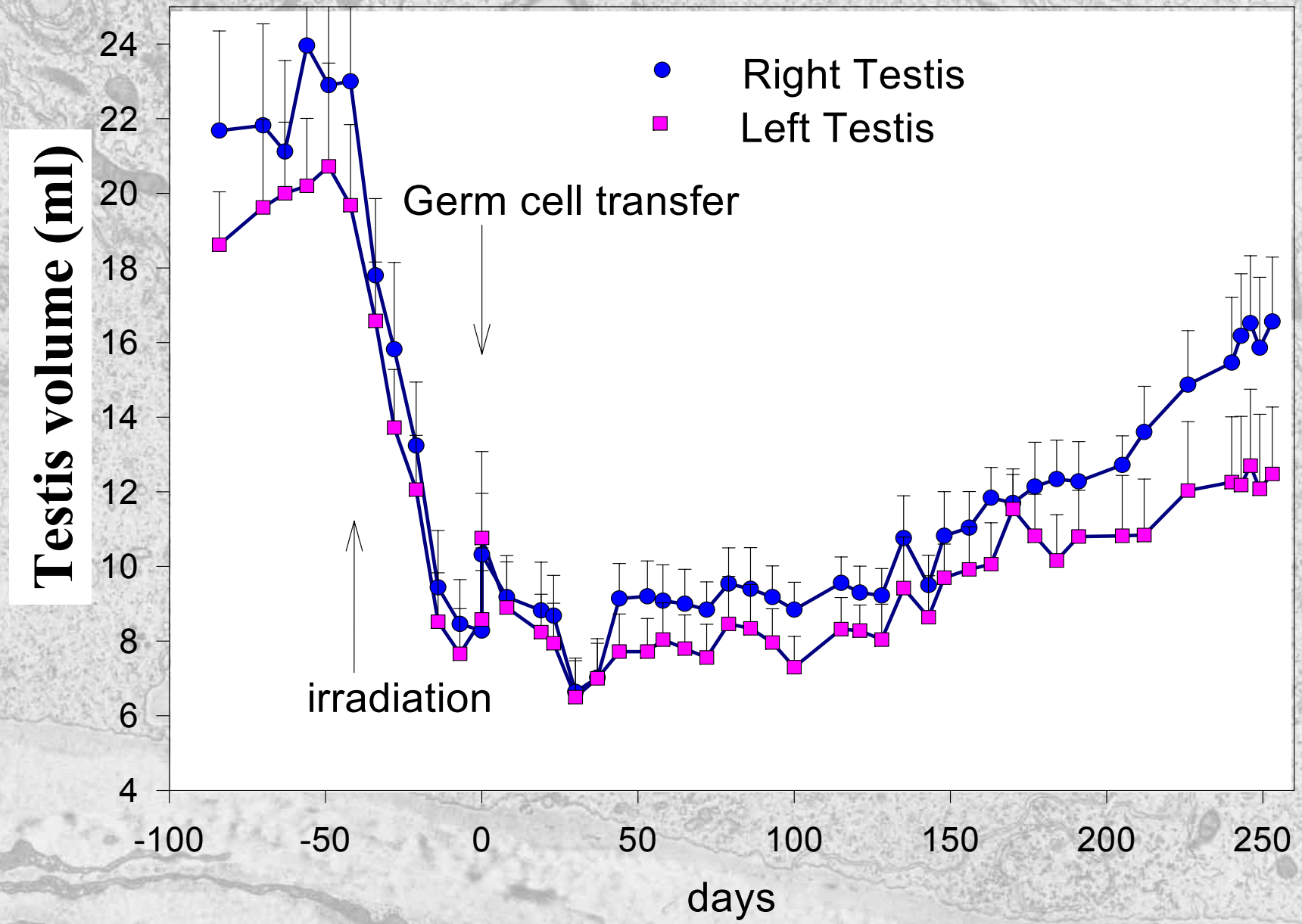
ultrasound guidance

Germ Cell Transfer into Rat, Bovine, Monkey and Human Testes

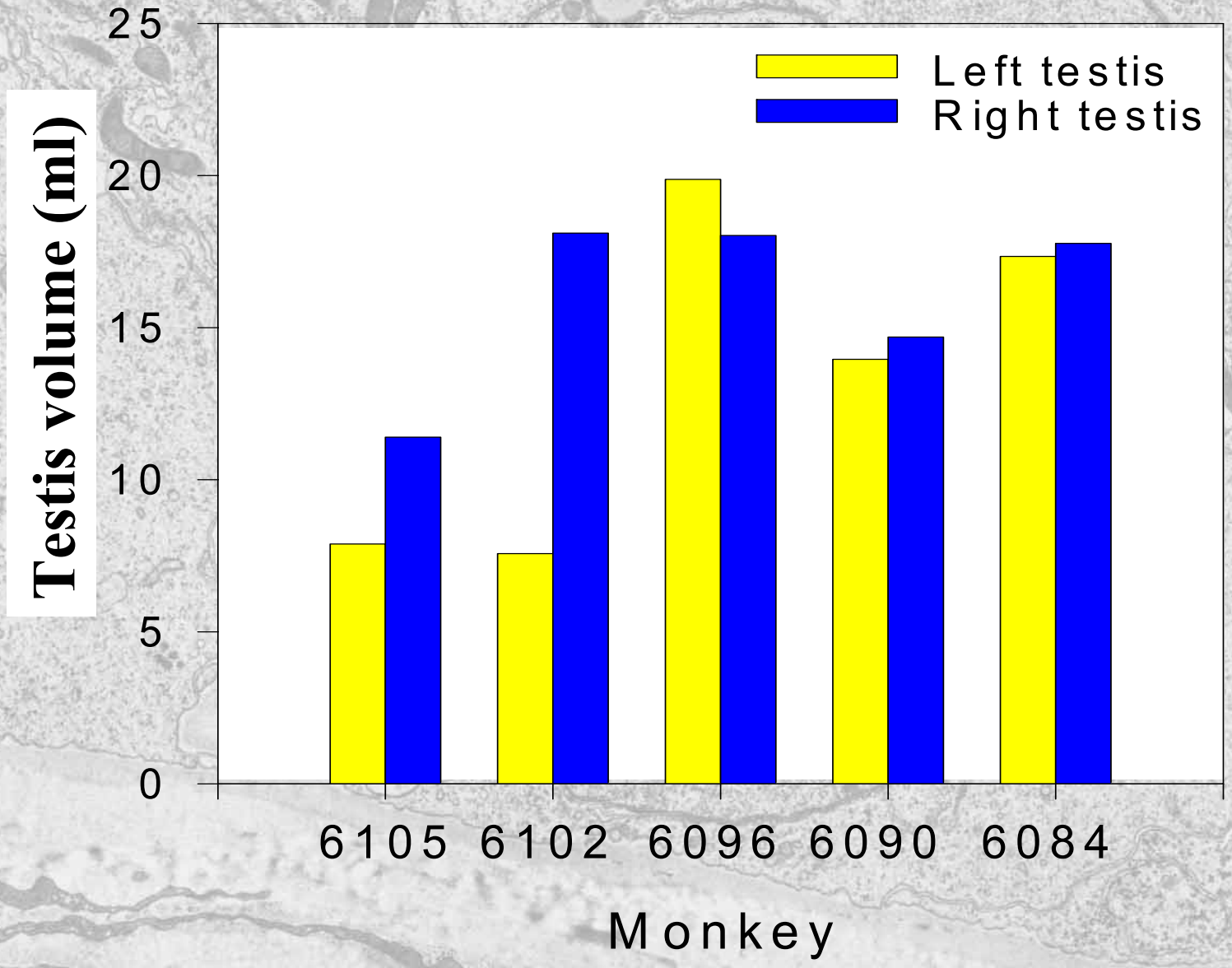
Schlatt et al., Hum Reprod 14: 144-150 (1999)



Testis Volume Following Irradiation and Germ Cell Transfer

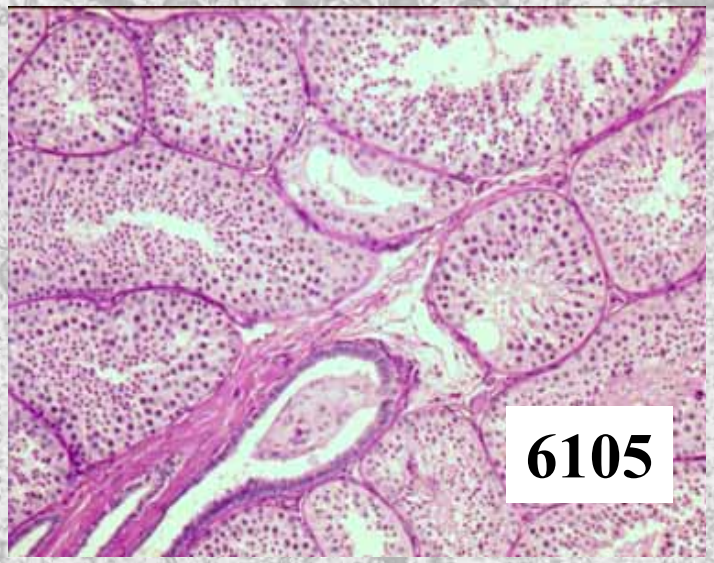


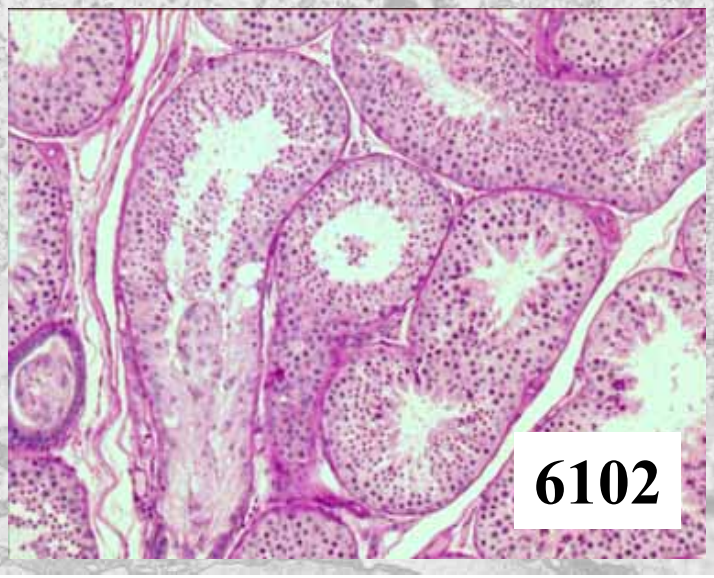
Individual testis volume 35 weeks after germ cell transfer



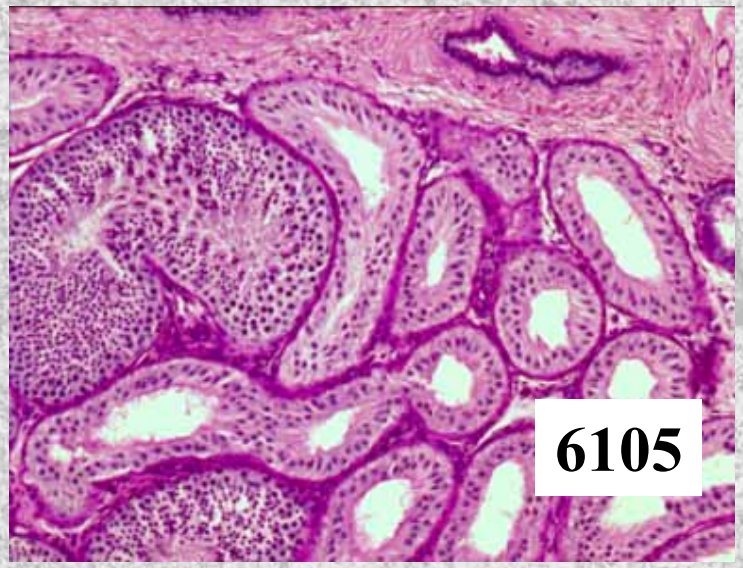
Histology of monkey testes 35 weeks after germ cell transfer

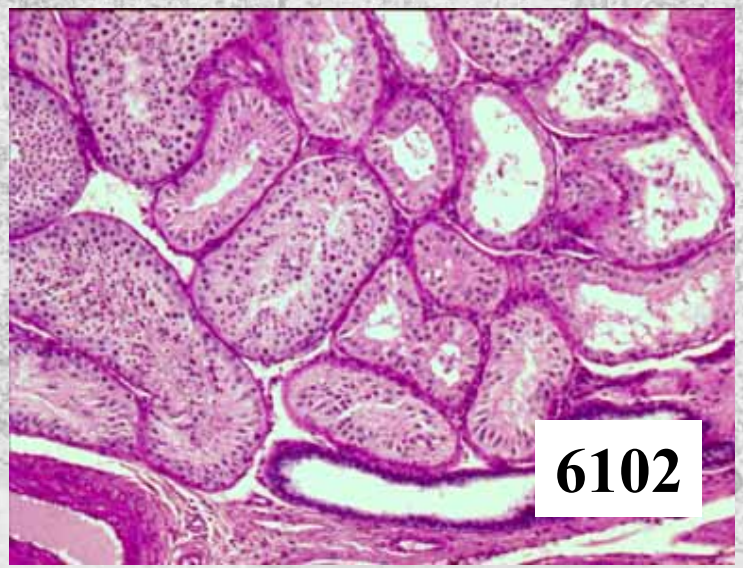
Right testis
Germ cell transfer





Left Testis
Saline Injection





Warning!!!

Jahnukainen K, Hou M, Petersen C, Setchell B, Soder O

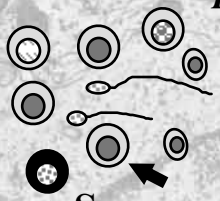
Intratesticular transplantation of testicular cells from leukemic rats causes transmission of leukemia.

Cancer Research 61: 706-710 (2001)

Procedure for Enrichment of Differentiating Spermatogonia

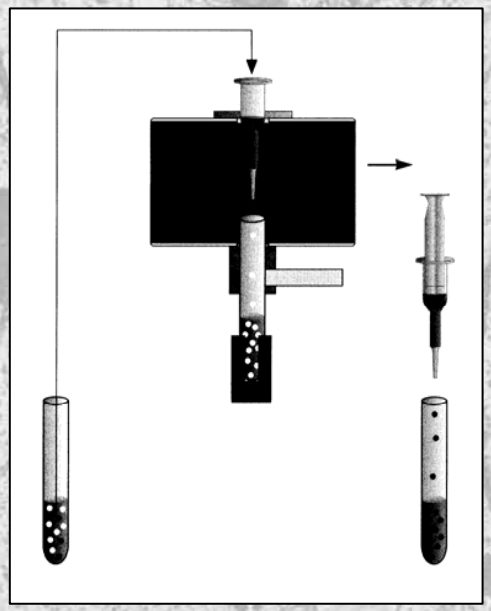
Enzymatic digestion of testis tissue

Testicular cell suspension



Spermatogonium

Tumour cell



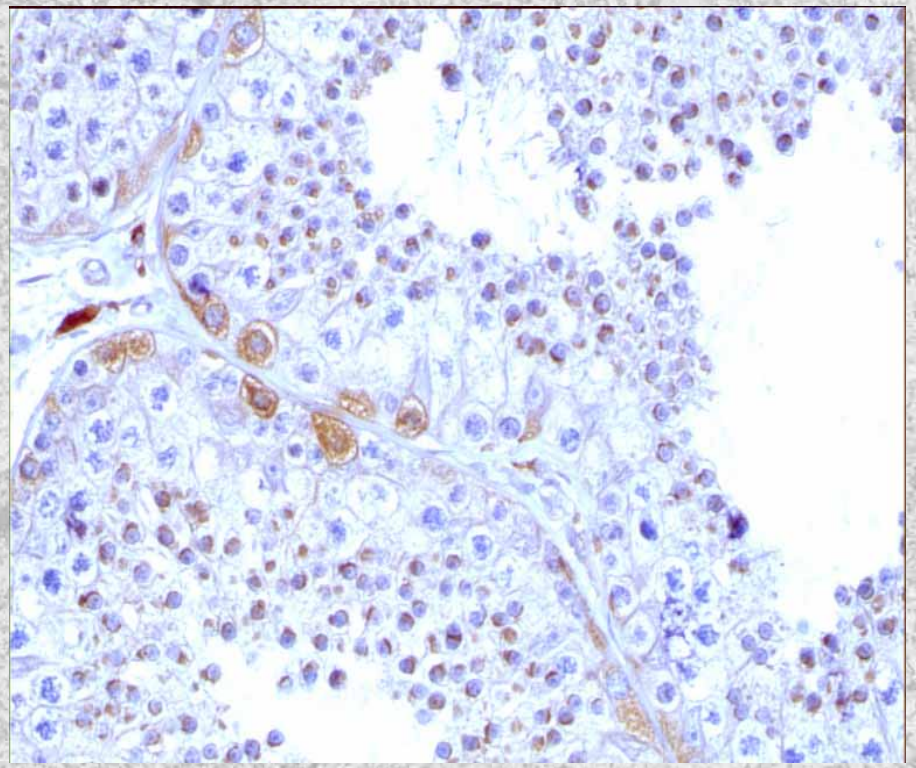
Magnetic cell Separation

Positive Selection

Negative Selection

Enrichment of spermatogonia

Depletion of tumour cells



C-kit Immunoreactivity in Marmoset Testes

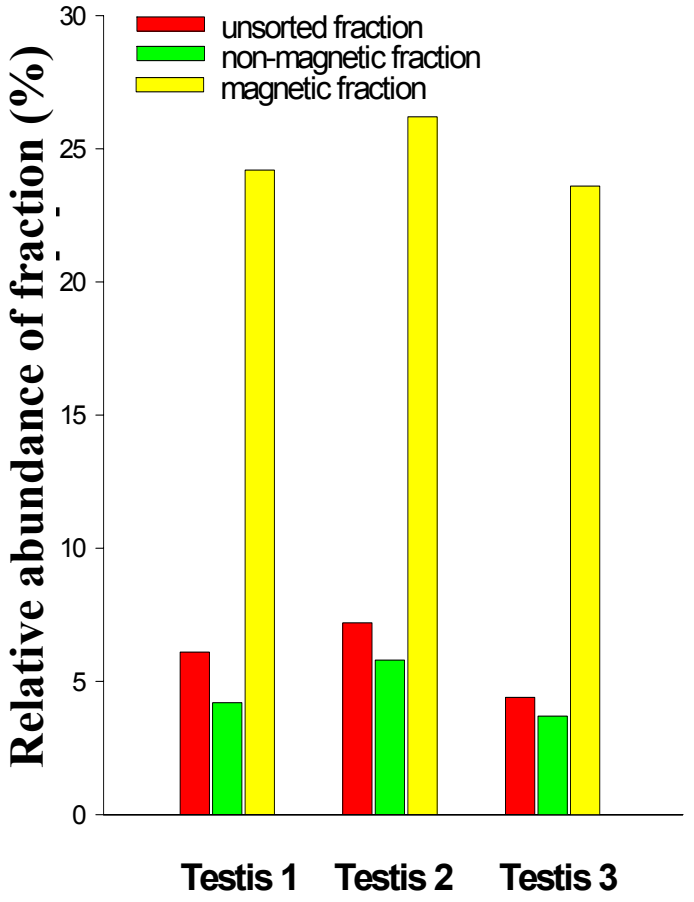
Magnetic Cell Sorting of Primate Spermatogonia

Marmoset Testes

Experiment	MACS separation fraction	FITC-positive cells (%) in 1C population	2C population	S-phase population	4C population
I	Unsorted	0.0	6.7	11.7	0.7
I	Nonmagnetic	0.0	2.5	2.0	0.3
I	Magnetic	0.8	54.5	10.9	30.5
II	Unsorted	0.0	1.5	4.0	0.2
II	Nonmagnetic	0.0	0.9	0.4	0.1
II	Magnetic	1.1	24.4	31.0	2.0
III	Unsorted	0.0	2.8	7.5	0.5
III	Nonmagnetic	0.0	1.3	1.4	0.1
III	Magnetic	0.5	30.5	23.1	2.6

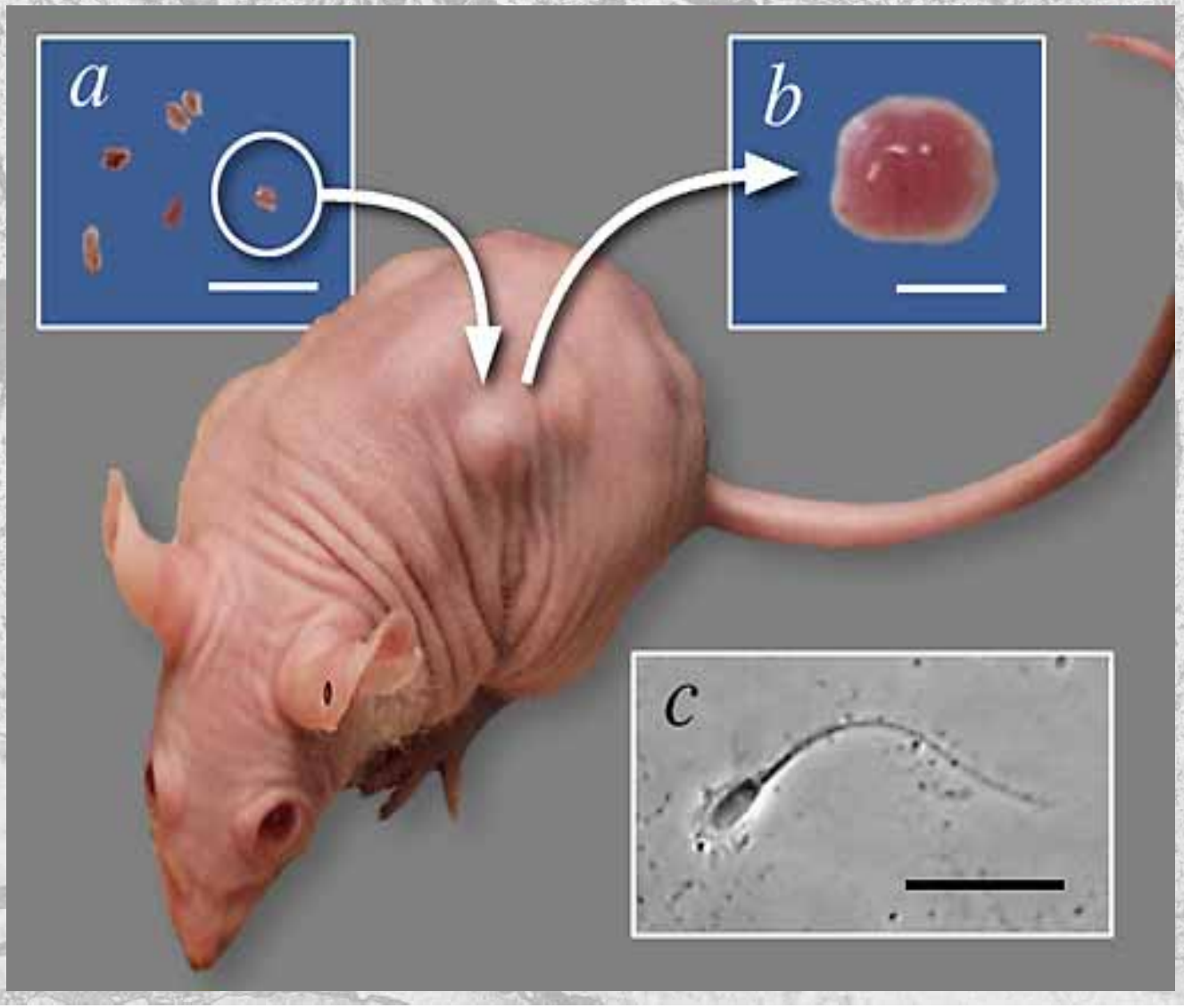
* Data represent relative numbers of FITC-positive cells among the populations of 1C, 2C, S-phase and 4C cells in the unsorted, nonmagnetic and magnetic fractions of 6 adult marmoset monkey testes; each experiment was performed independently on pooled testicular cells from 2 monkeys.

Human Testes



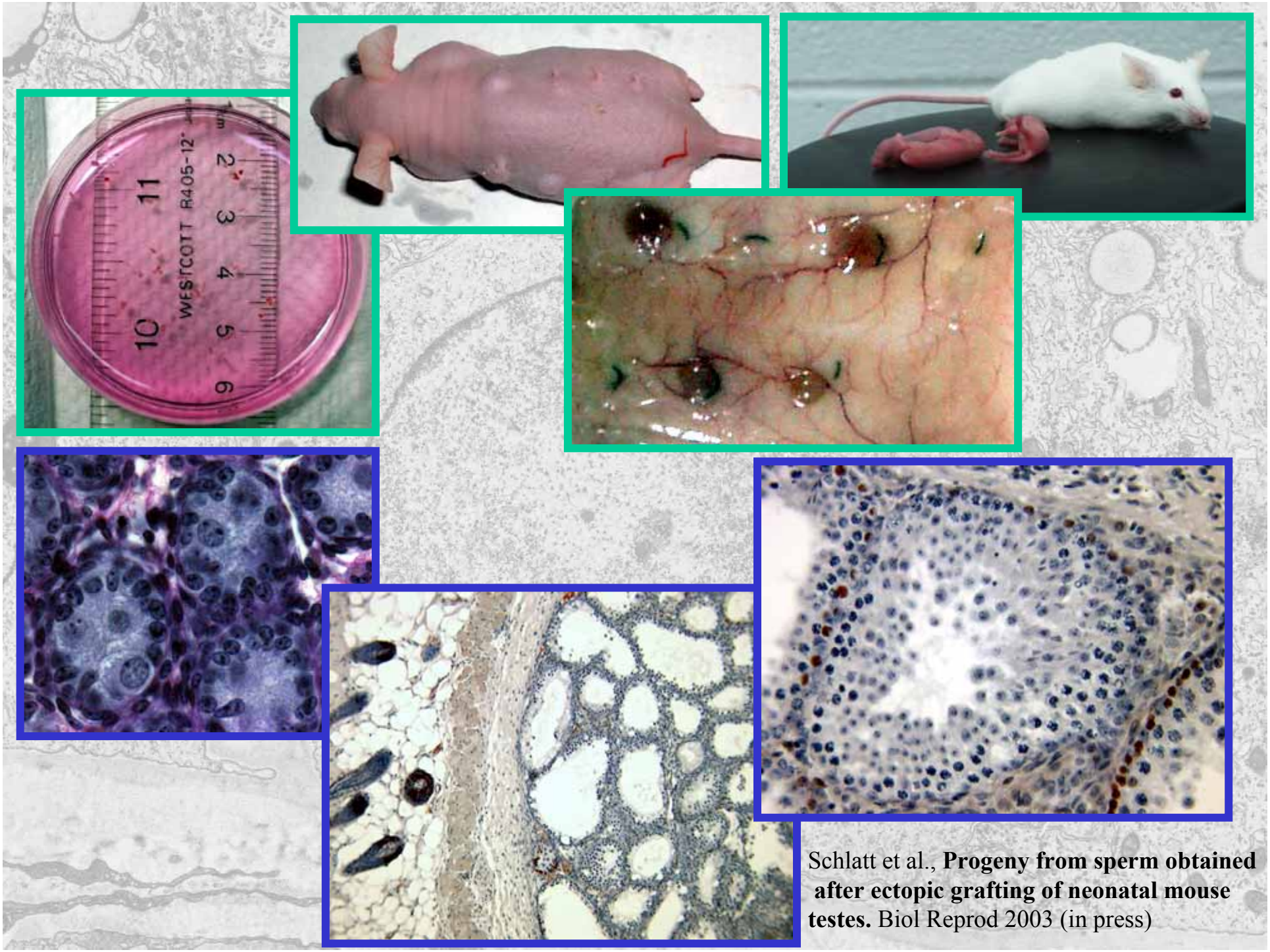
Stem Cell Treatment Option III: Testicular Grafting

Aim: Extracorporeal storage and activation of testicular tissue

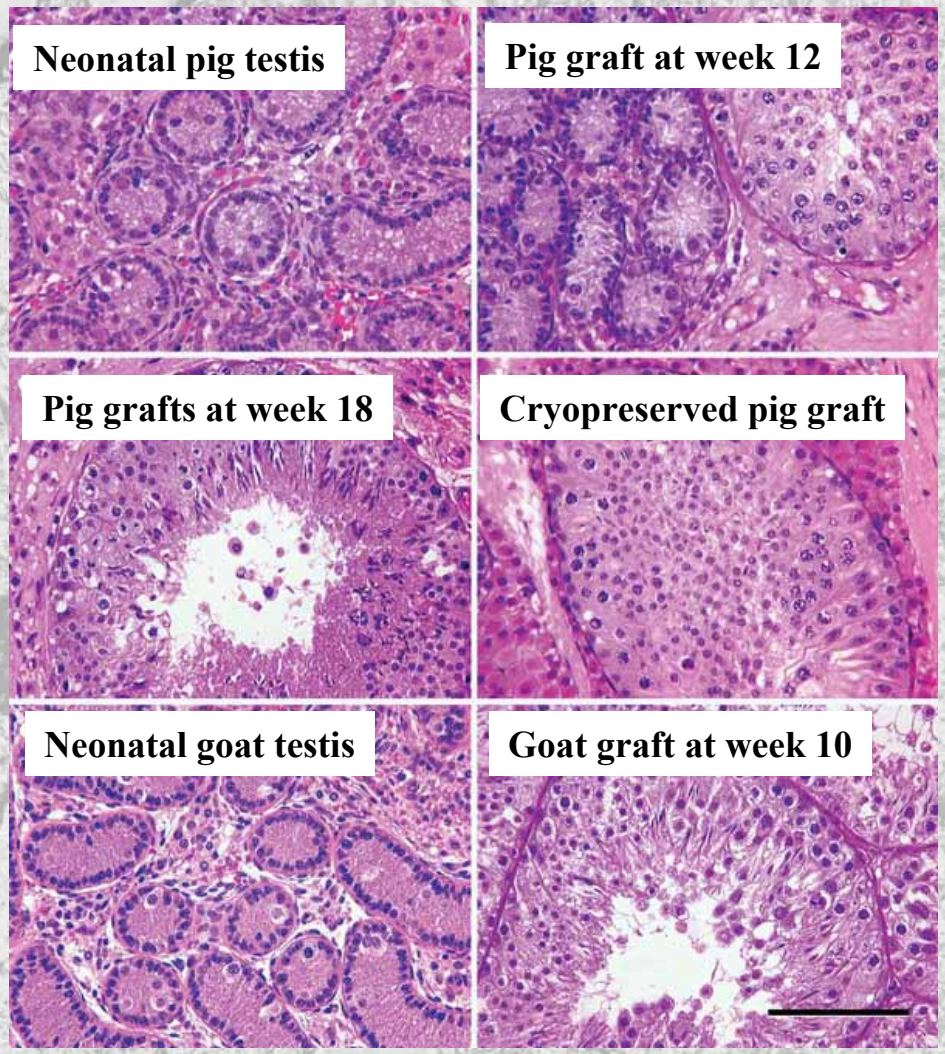


Stem cell plus niche
Xeno/Auto-grafting

Honaramooz et al.,
Nature 418: 778-781
(2002)
Sperm from neonatal mammalian testes grafted in mice.

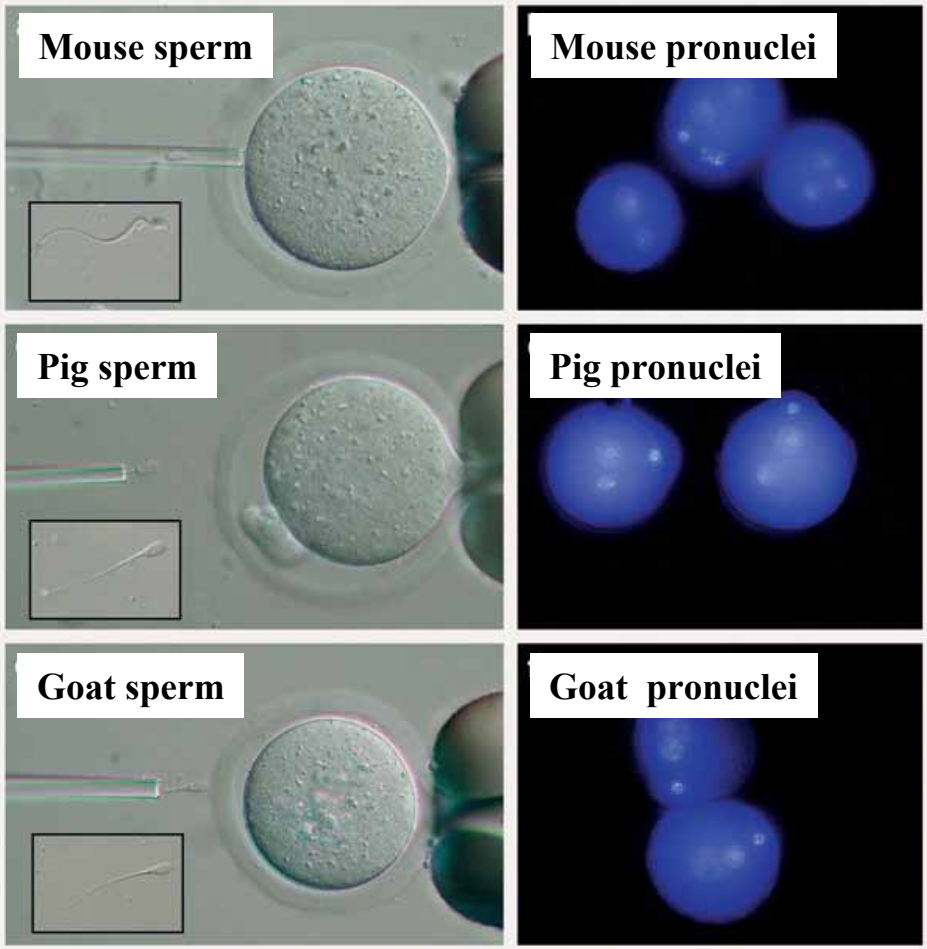


Schlatt et al., **Progeny from sperm obtained after ectopic grafting of neonatal mouse testes.** Biol Reprod 2003 (in press)



Histology of grafts from pig and goat testis

Sperm morphology and oocyte-activating competence of sperm from testicular grafts

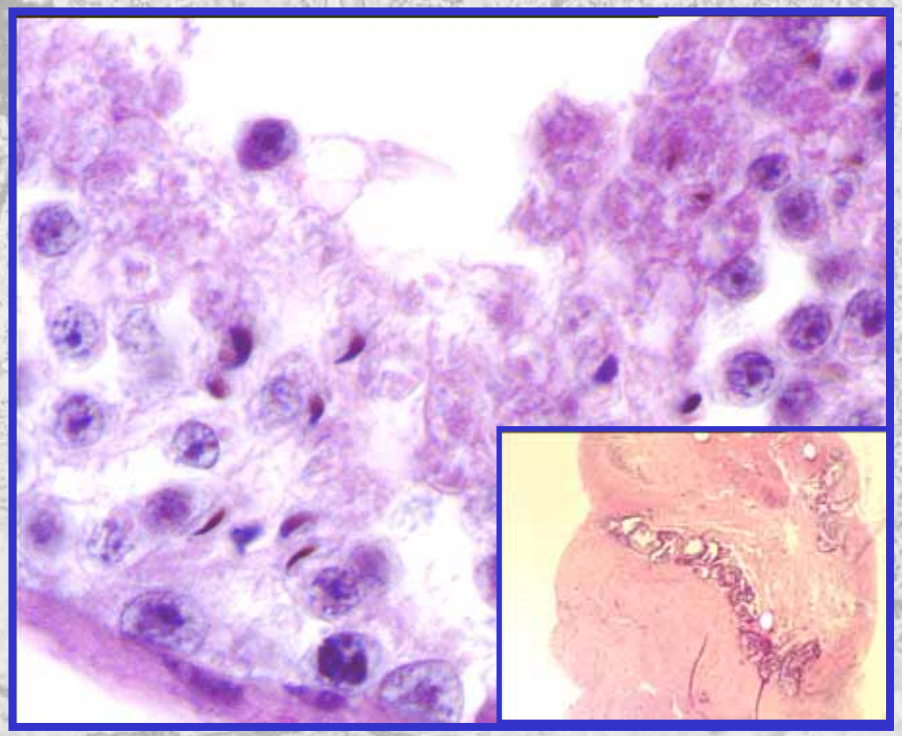


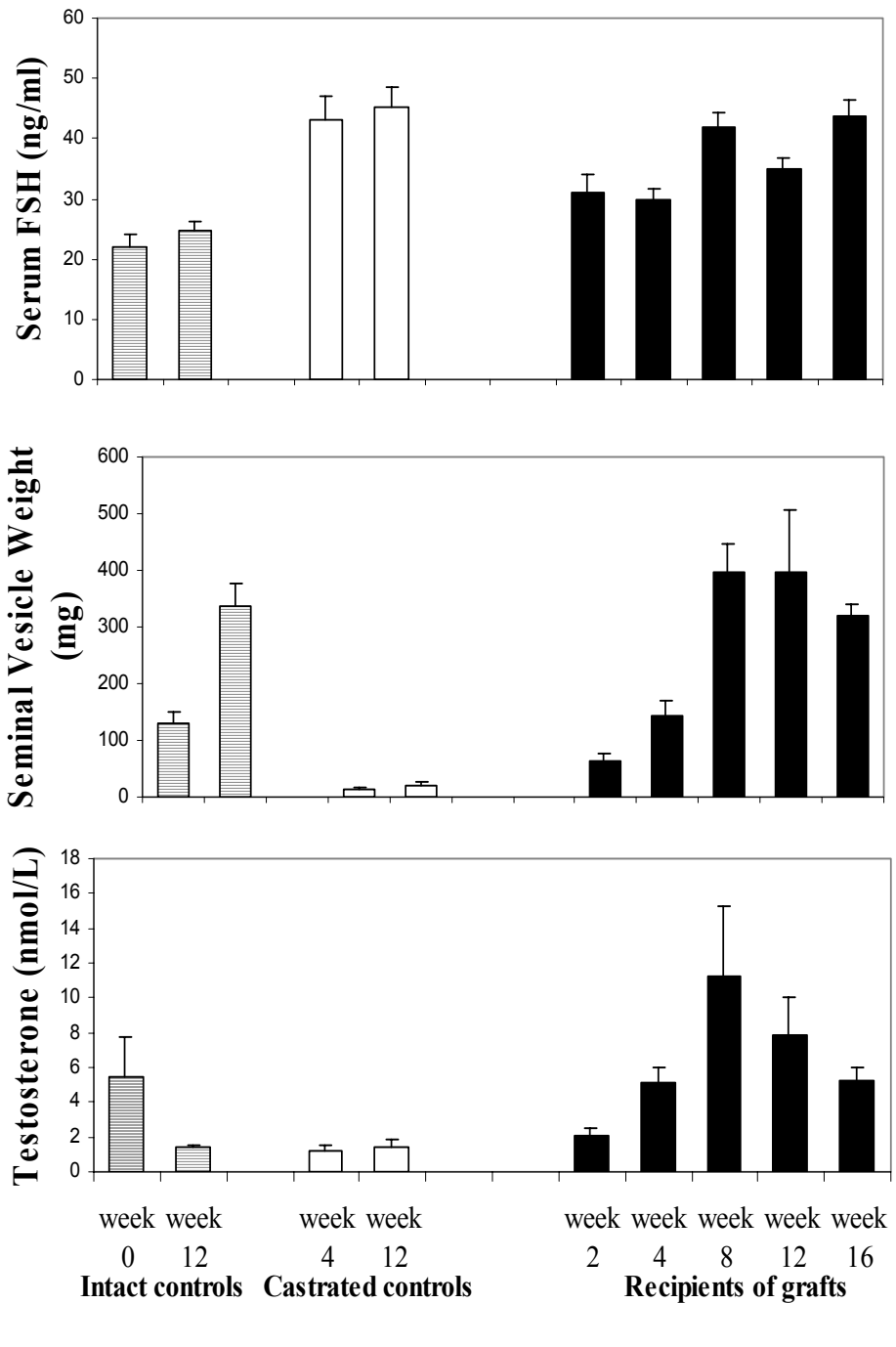
(All oocytes are from mice)

Schlatt S, Kim SS, Gosden R.

Spermatogensis and steroidogenesis in mouse, hamster and monkey testicular tissue after cryopreservation and heterotopic grafting to castrated hosts.

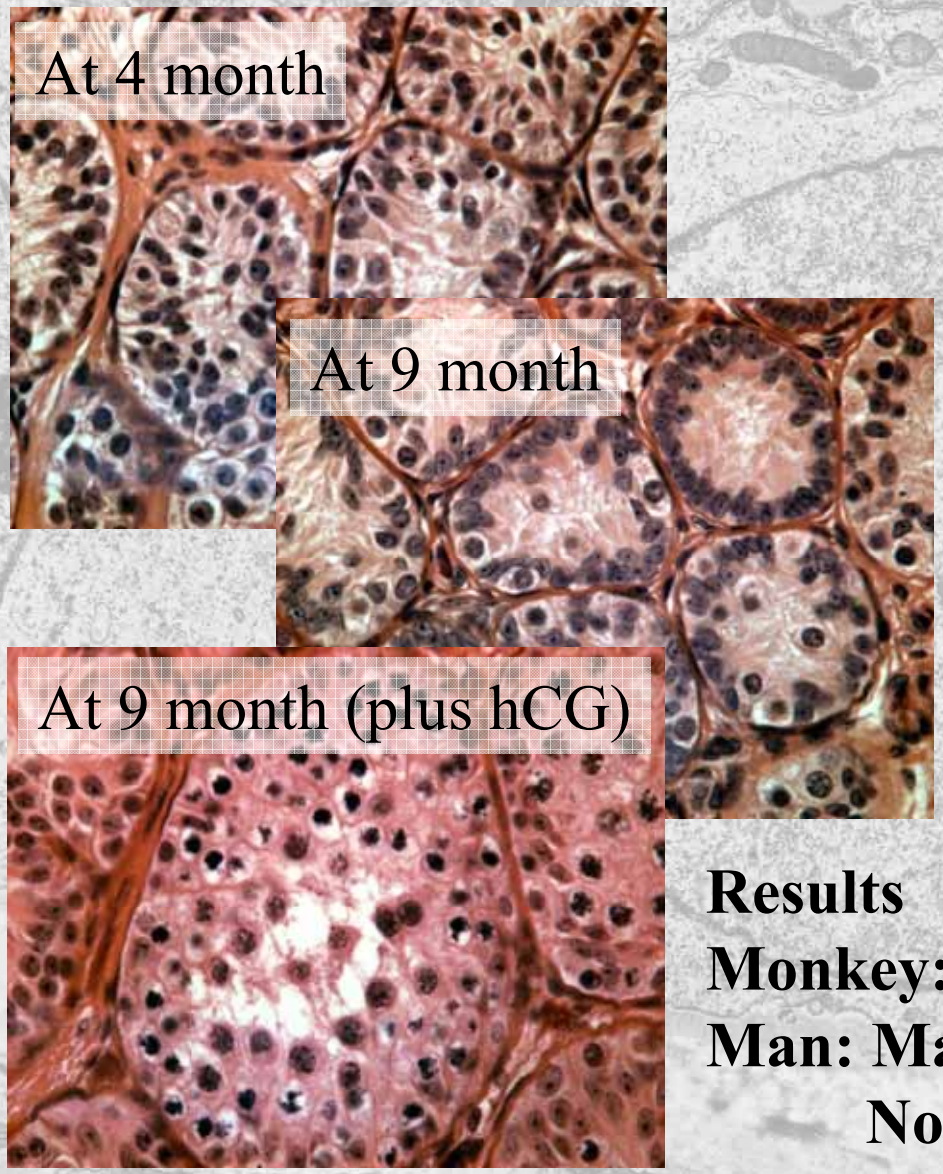
Reproduction 124: 339-346 (2002)



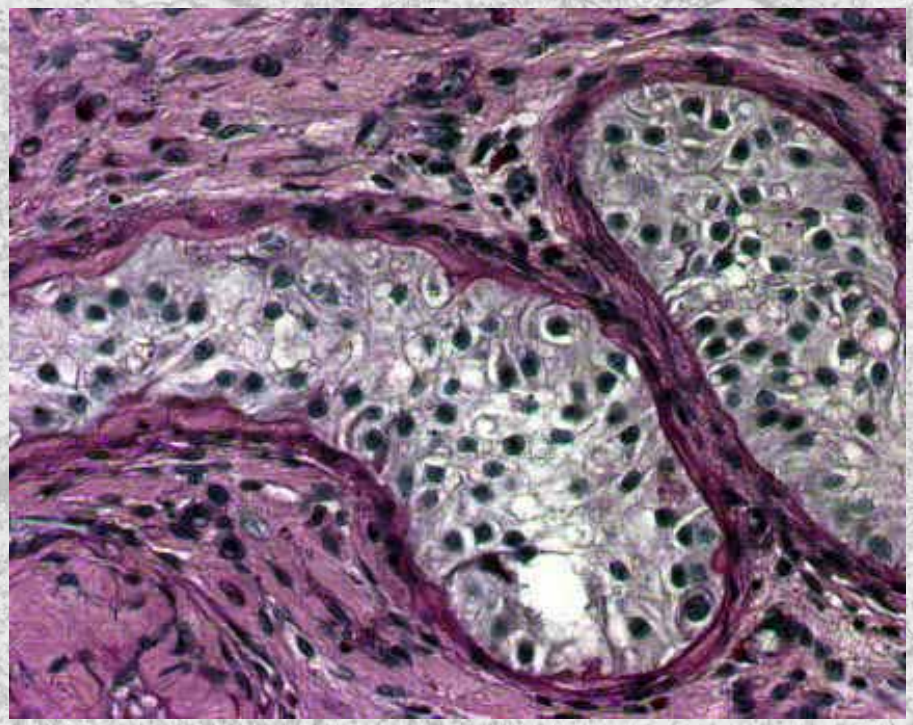


Xenografting of primate testicular tissue

Monkey Graft Donor: Juvenile macaque
(age: 15 month, tissue was cryopreserved)



Human:
Source of testes: Transsexual patients
Spermatogenic status: Complete regression



Results
Monkey: Initiation of testis function
Man: Maintenance of tubular structure
No restoration of spermatogenesis

Stem Cell Treatment Option IV: Artificial Gametes

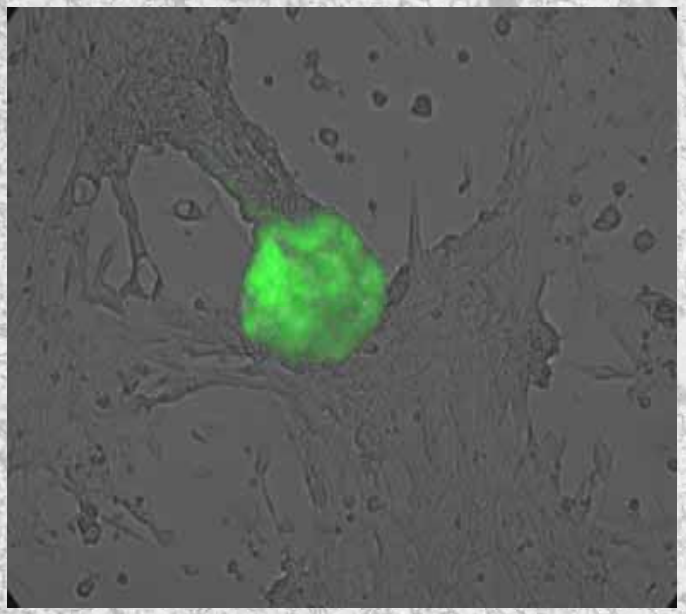
Toyooka et al., **Embryonic stem cells can form germ cells in vitro.** PNAS 100: 11457-114562 (2003)

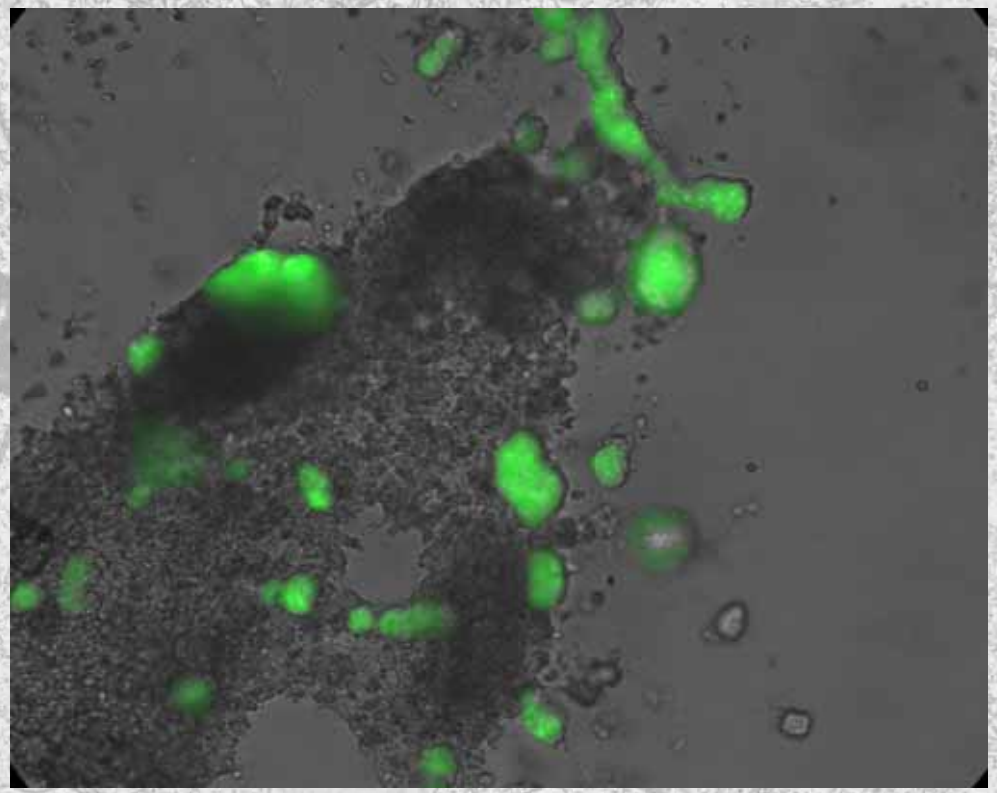
Hubner et al., **Derivation of oocytes from mouse embryonic stem cells.** Science 300: 1251-1256 (2003)

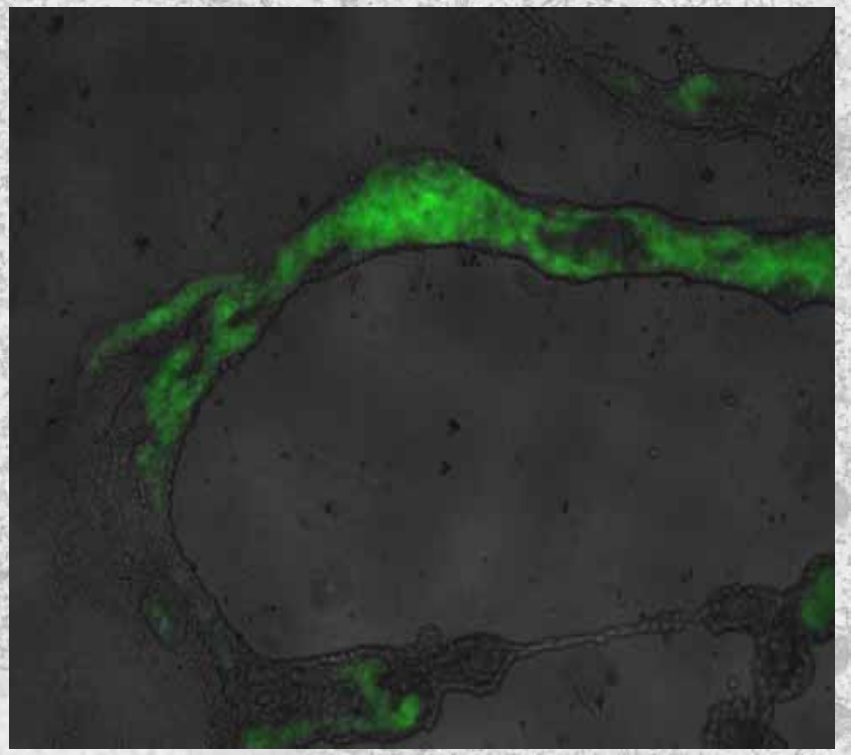
Geijsen et al., **Derivation of embryonic germ cells and male gametes from embryonic stem cells.** Nature 427: 148-154 (2004)

Feng et al., **Generation and in vitro differentiation of a spermatogonial cell line.** Science 297: 392-395 (2002)

Embryonic Germ Cells in Testicular Cell Cultures

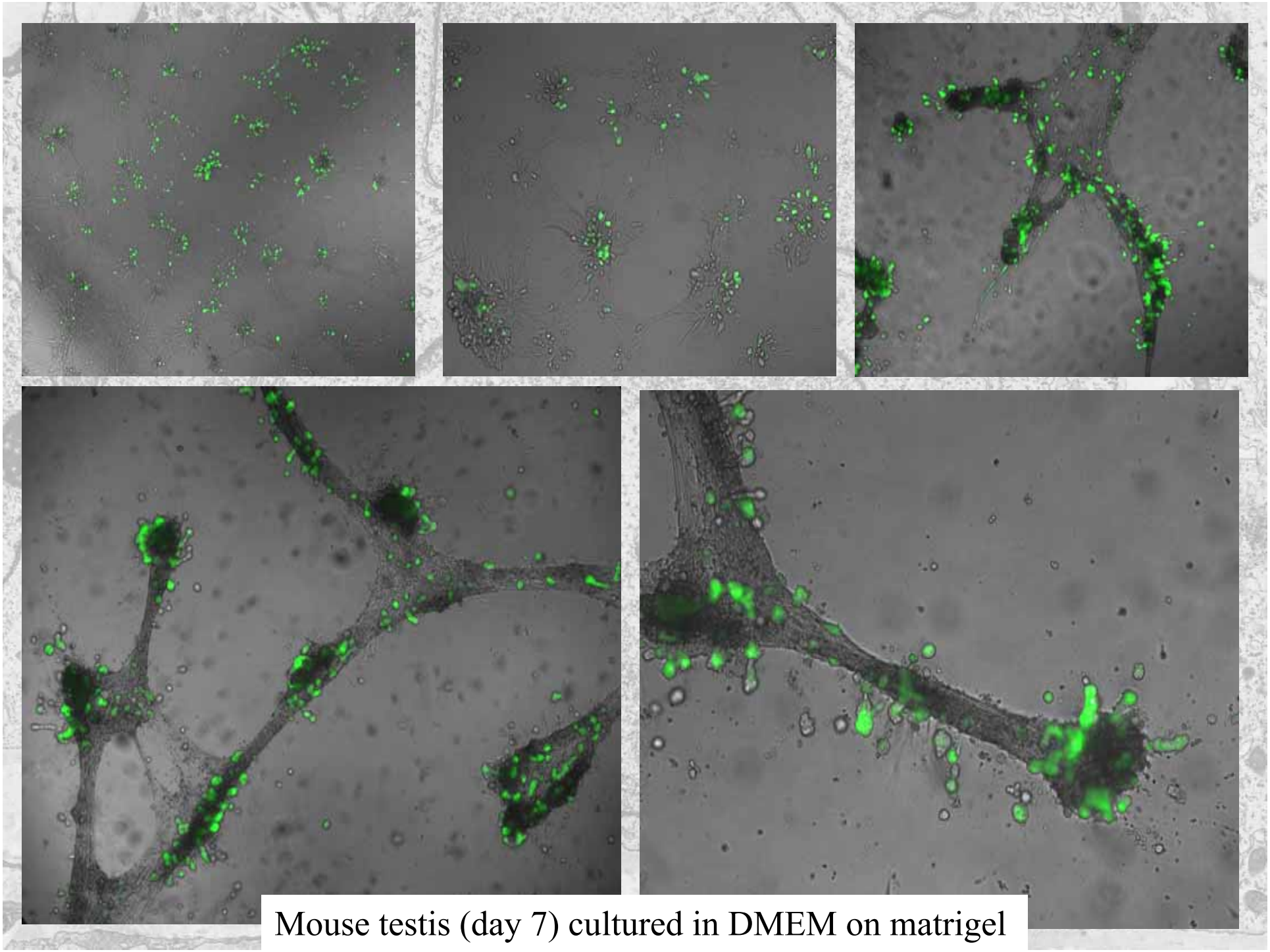




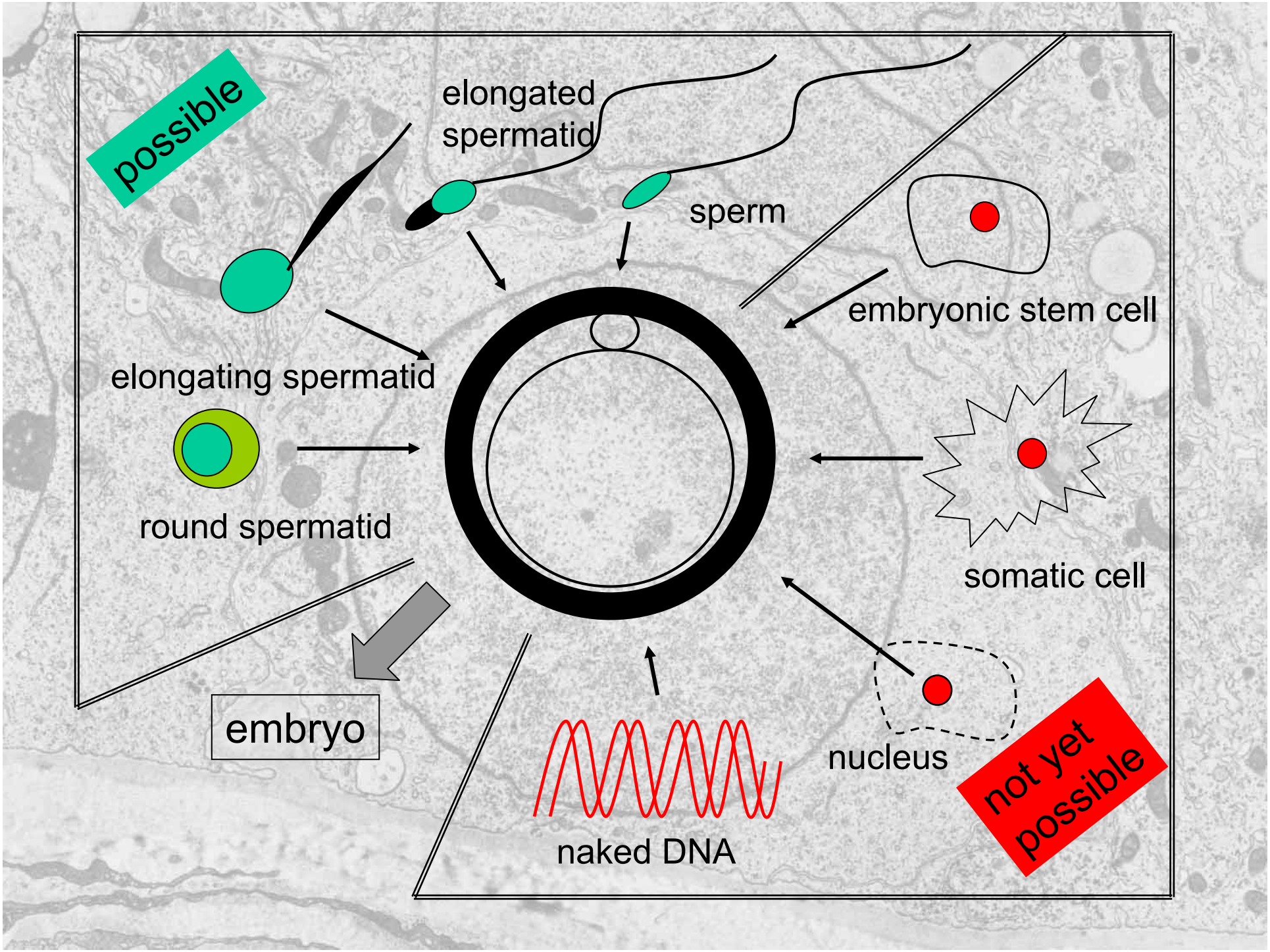


GFP-expression in mouse embryonic germ cells

Mouse testis (day 7) cultured in DMEM
No matrix substance



Mouse testis (day 7) cultured in DMEM on matrigel


possible
elongated spermatid
sperm
embryonic stem cell
elongating spermatid
round spermatid
somatic cell
embryo
naked DNA
nucleus
not yet possible

Acknowledgement

Institute of Reproductive Medicine, Münster, Germany

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G. Rosiepen
A. Schepers
V. v. Schönfeldt
G.F. Weinbauer
J. Wistuba

Centre for Reproduction, Growth and Development LGI, Leeds, UK

P.F. Brook
R.G. Gosden

Institute of Reproduction and Development, Monash University Clayton, Australia

D. deKretser
K.L. Loveland
T. Meehan
S. Meachem

Center for Animal Transgenesis and Germ Cell Research Pennsylvania University Kennett Square, USA

H. Schöler
I. Dobrinski
A. Honaramooz