Potential use of stem cells in male fertility preservation

ESHRE Campus symposium
Thessaloniki, Greece
1-3 October 2009

Herman Tournaye, M.D. Ph.D.
Centre for Reproductive Medicine Brussels

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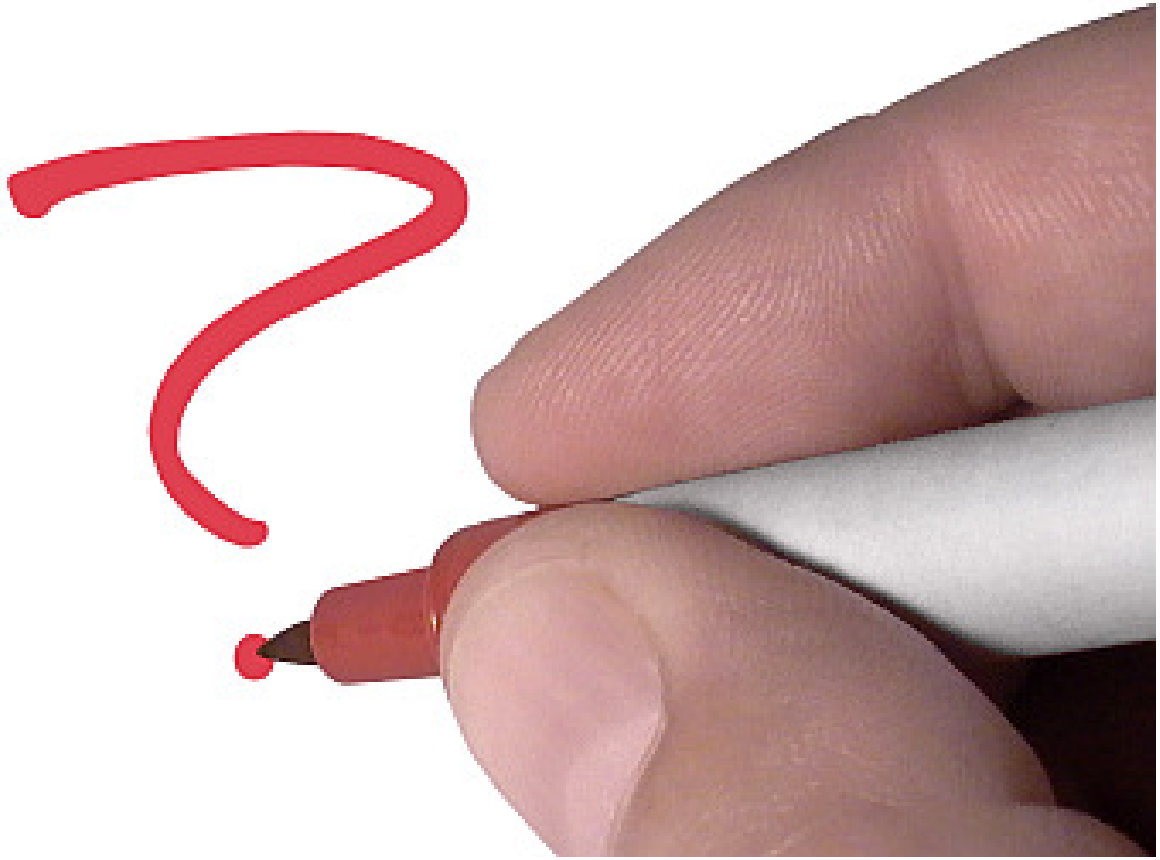
bite biology of the testis

Potential use of stem cells in male fertility preservation

What ?

How ?

When ?



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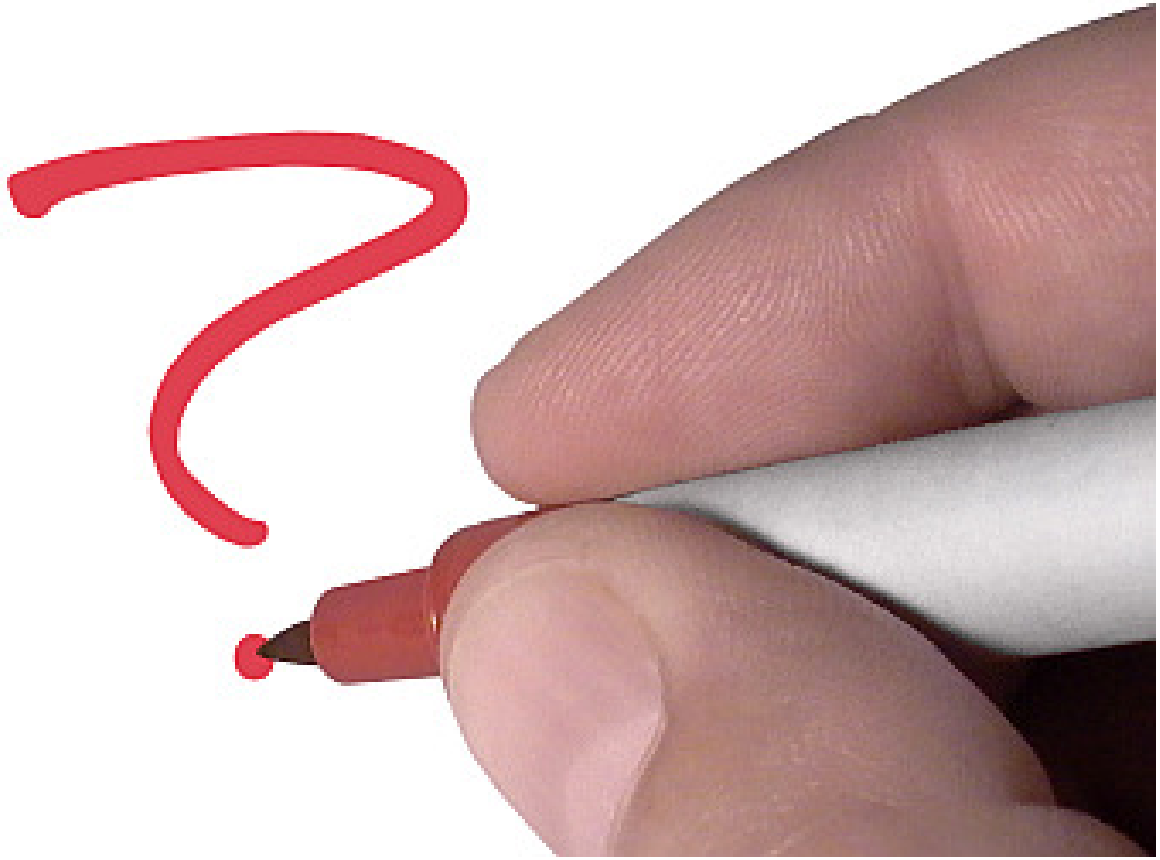

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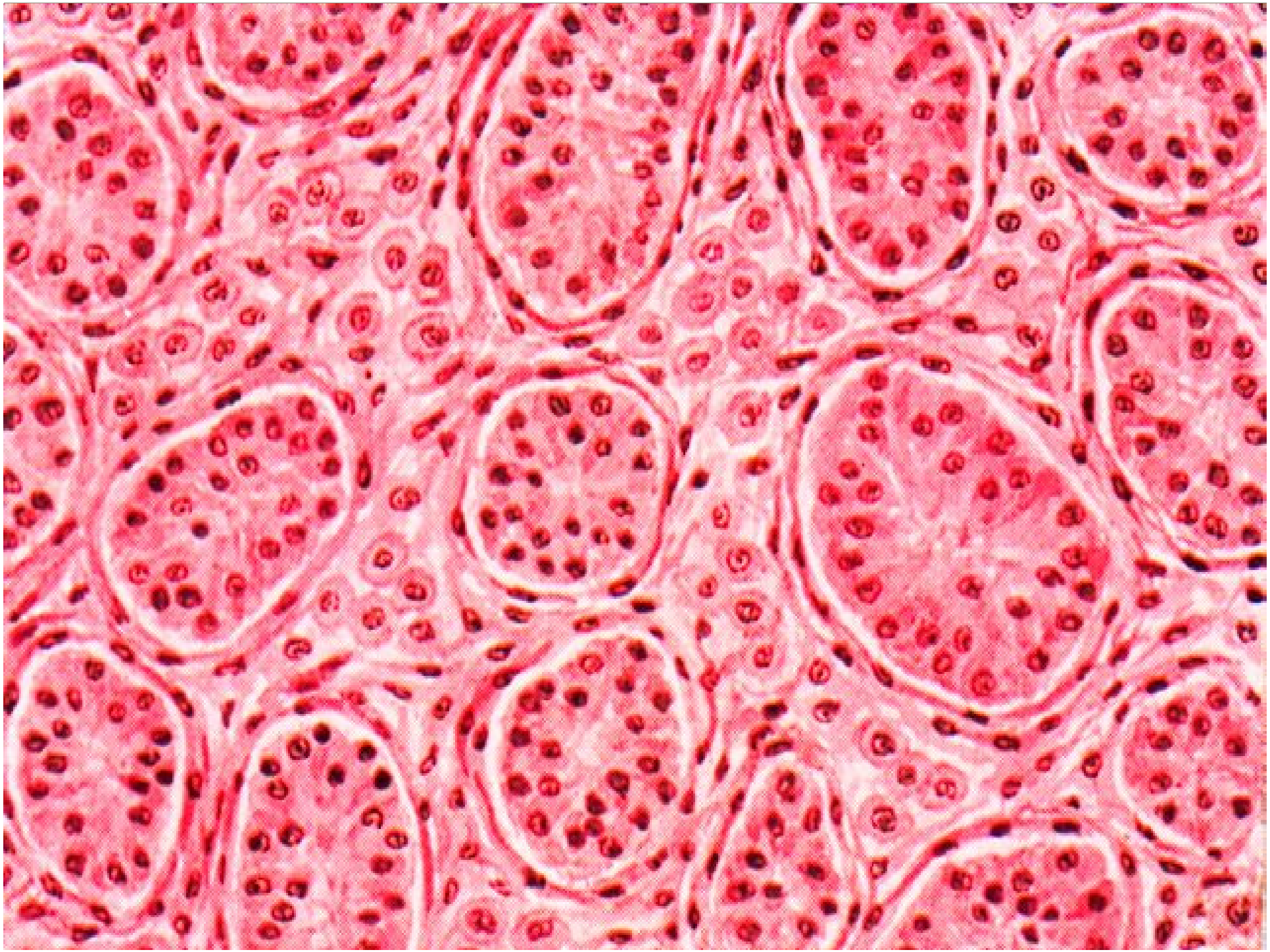
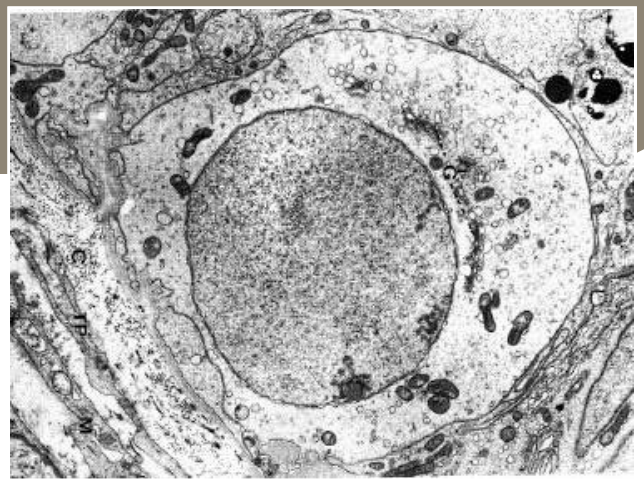


Testicular Stem Cell



- ✓ undifferentiated

  pluripotent (?) stem cell
- ✓ spermatogenic cell lineage
- ✓ cell fate • self-renewal
  - apoptosis
  - differentiation

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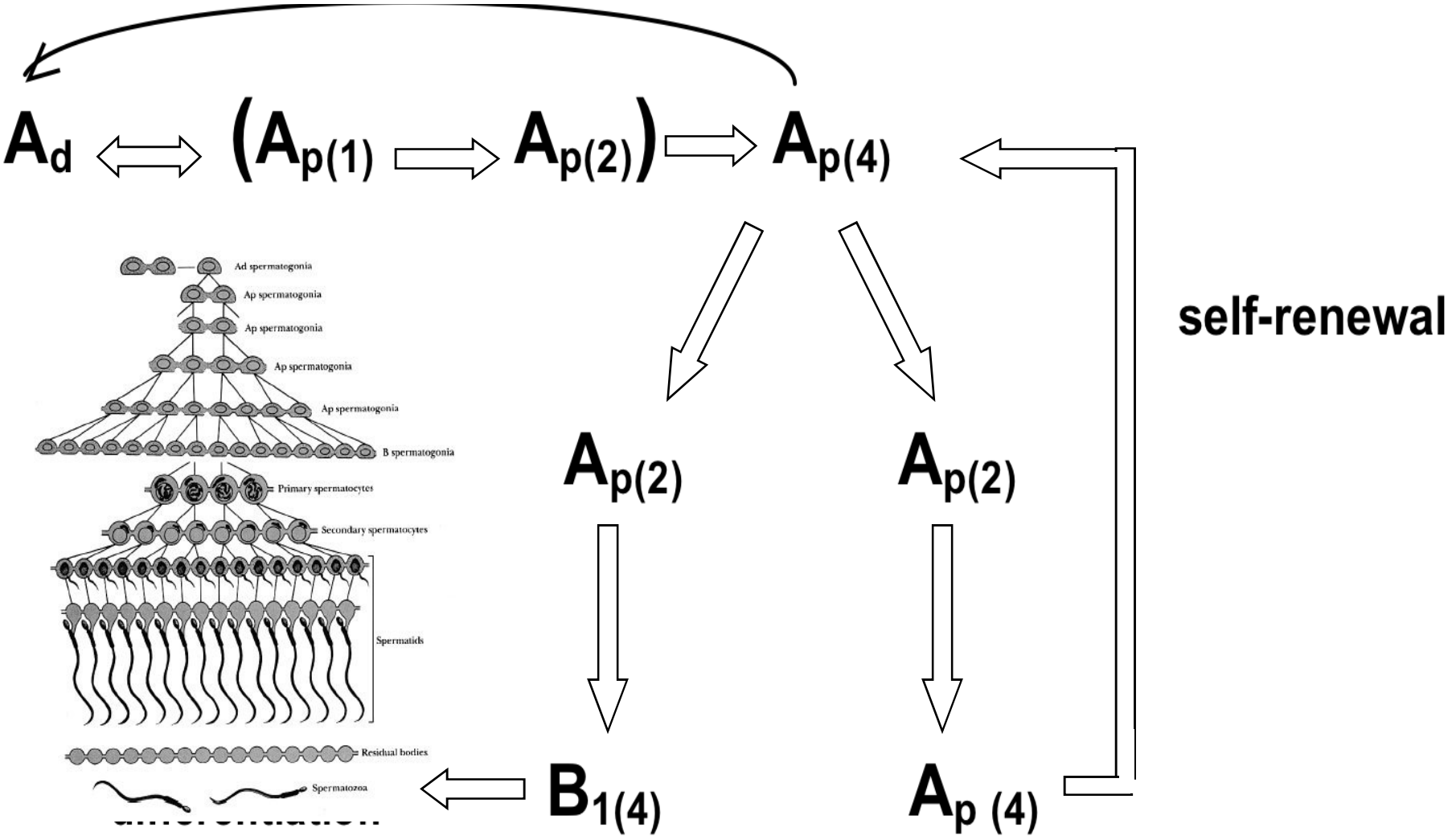
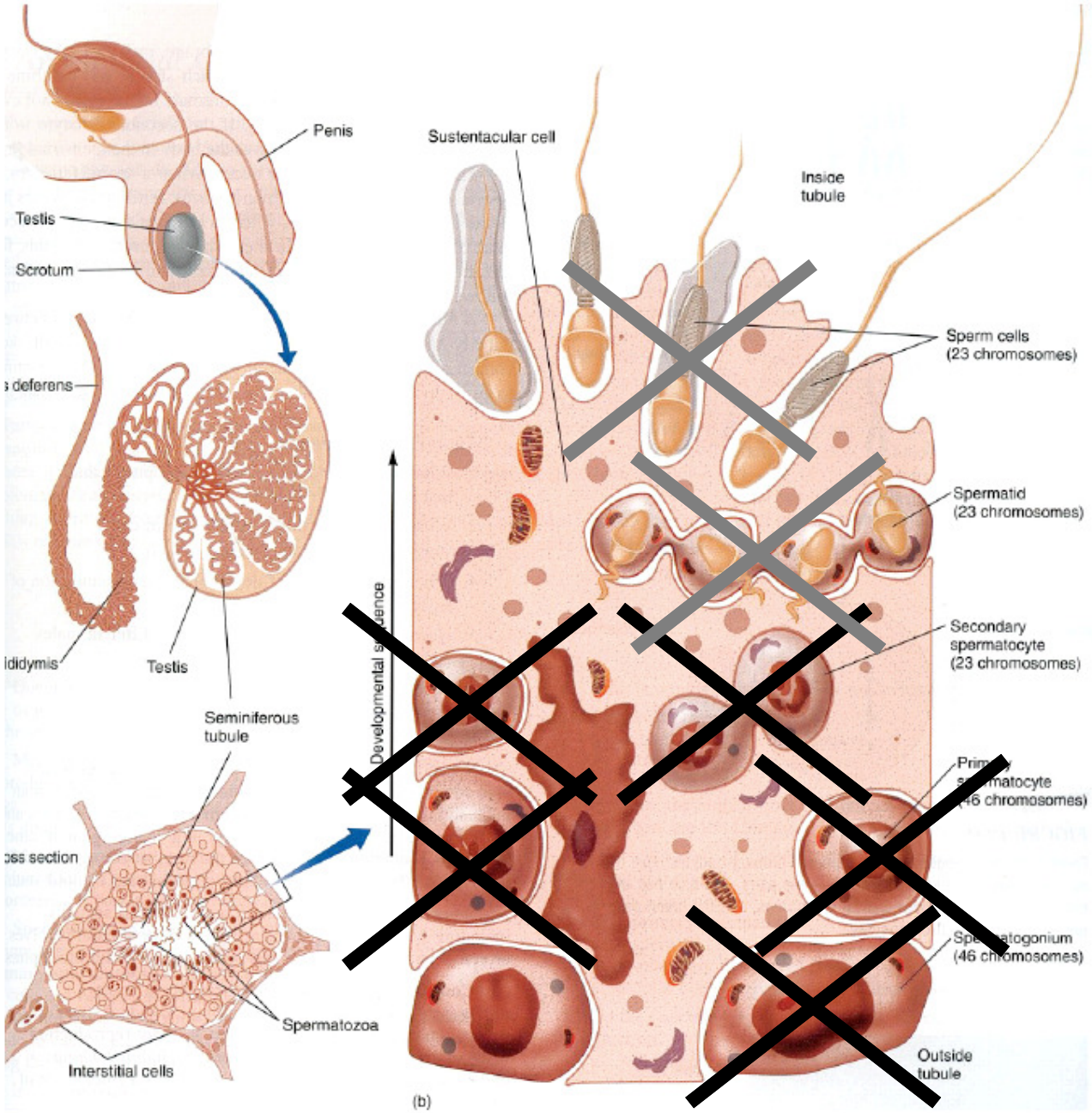
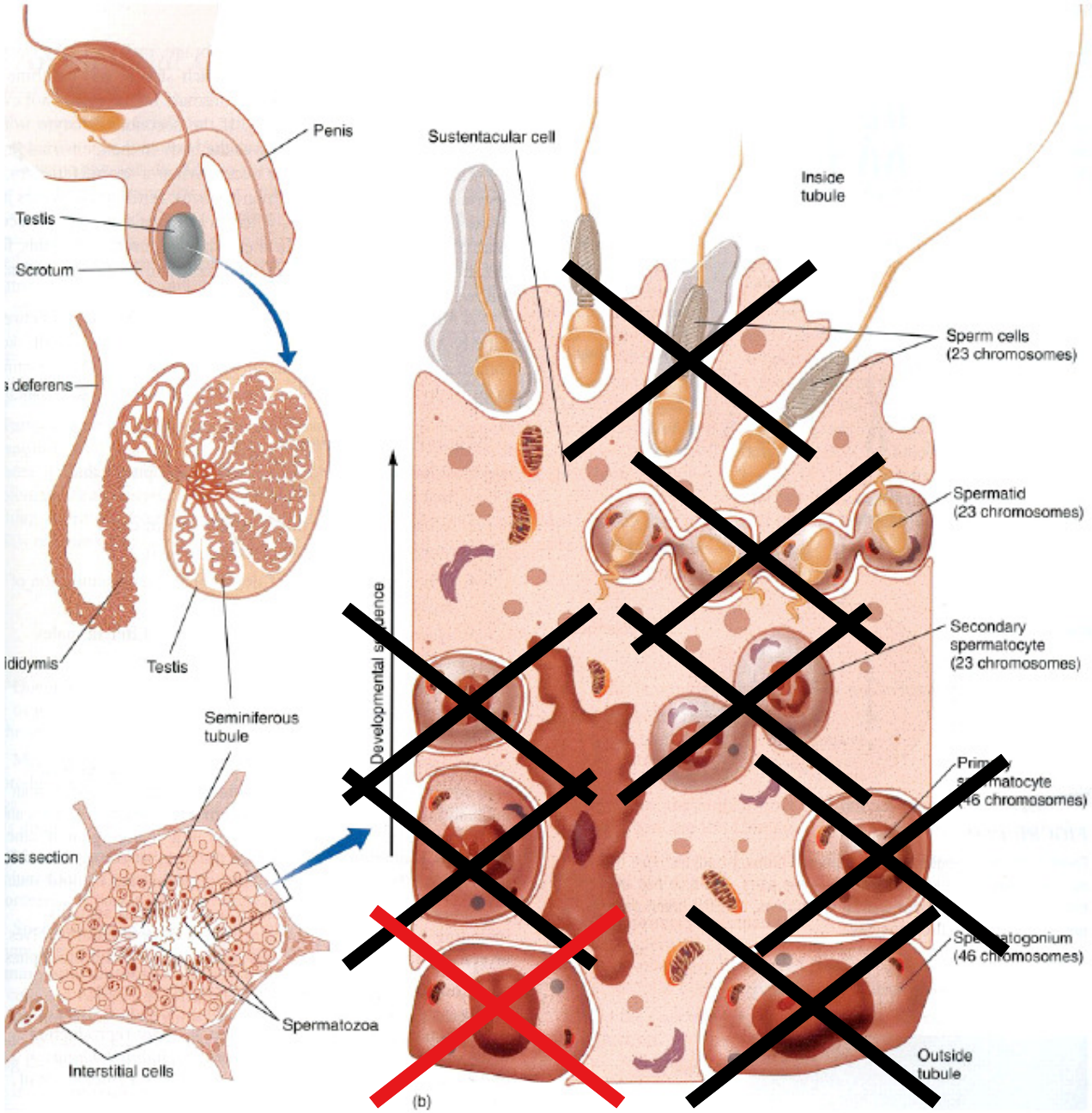
A_d/A_p-model of stem cell renewal




Penis
Testis
Scrotum
deferens
Testis
Seminiferous tubule
Spermatozoa
Interstitial cells
Sustentacular cell
Inside tubule
Sperm cells (23 chromosomes)
Spermatid (23 chromosomes)
Secondary spermatocyte (23 chromosomes)
Outside tubule
(b)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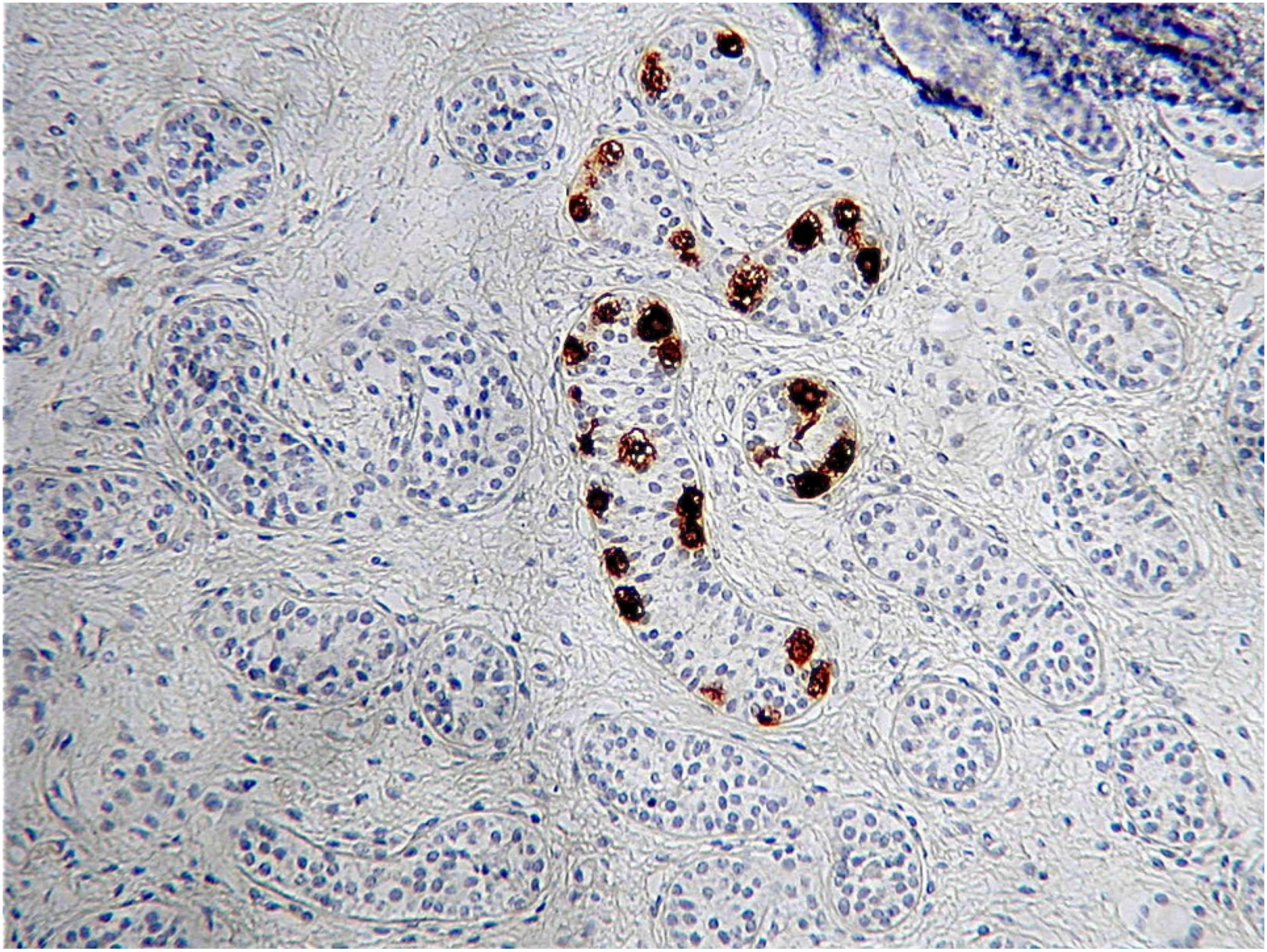


VOLUME 24 · NUMBER 18 · JUNE 20 2006

JOURNAL OF CLINICAL ONCOLOGY

ASCO SPECIAL ARTICLE

American Society of Clinical Oncology Recommendations on Fertility Preservation in Cancer Patients

Stephanie J. Lee, Leslie R. Schover, Ann H. Partridge, Pasquale Patrizio, W. Hamish Wallace, Karen Hagerty, Lindsay N. Beck, Lawrence V. Brennan, and Kutluk Oktay

From the Dana-Farber Cancer Institute, Boston, MA; Fertility Preservation Program, Center for Reproductive Medicine and Fertility, Weill Medical College, Cornell University; Fertile Hope, New York, NY; Oncology/Hematology Care, Crestview Hills, KY; American Society of Clinical Oncology, Alexandria, VA; Yale University Fertility Center, New Haven, CT; M.D. Anderson Cancer Center, Houston, TX; and the Royal Hospital for Sick Children, Edinburgh, Scotland, UK.

Submitted March 14, 2006; accepted March 24, 2006.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Address reprint requests to Kutluk Oktay, MD, The Center for Reproductive Medicine and Infertility, Cornell University, 505 E 70th St, HT-340, New York, NY 10021; e-mail: guidelines@asco.org.

0732-183X/06/2418-2917/$20.00

DOI: 10.1200/JCO.2006.06.5888

ABSTRACT

Purpose
To develop guidance to practicing oncologists about available fertility preservation methods and related issues in people treated for cancer.

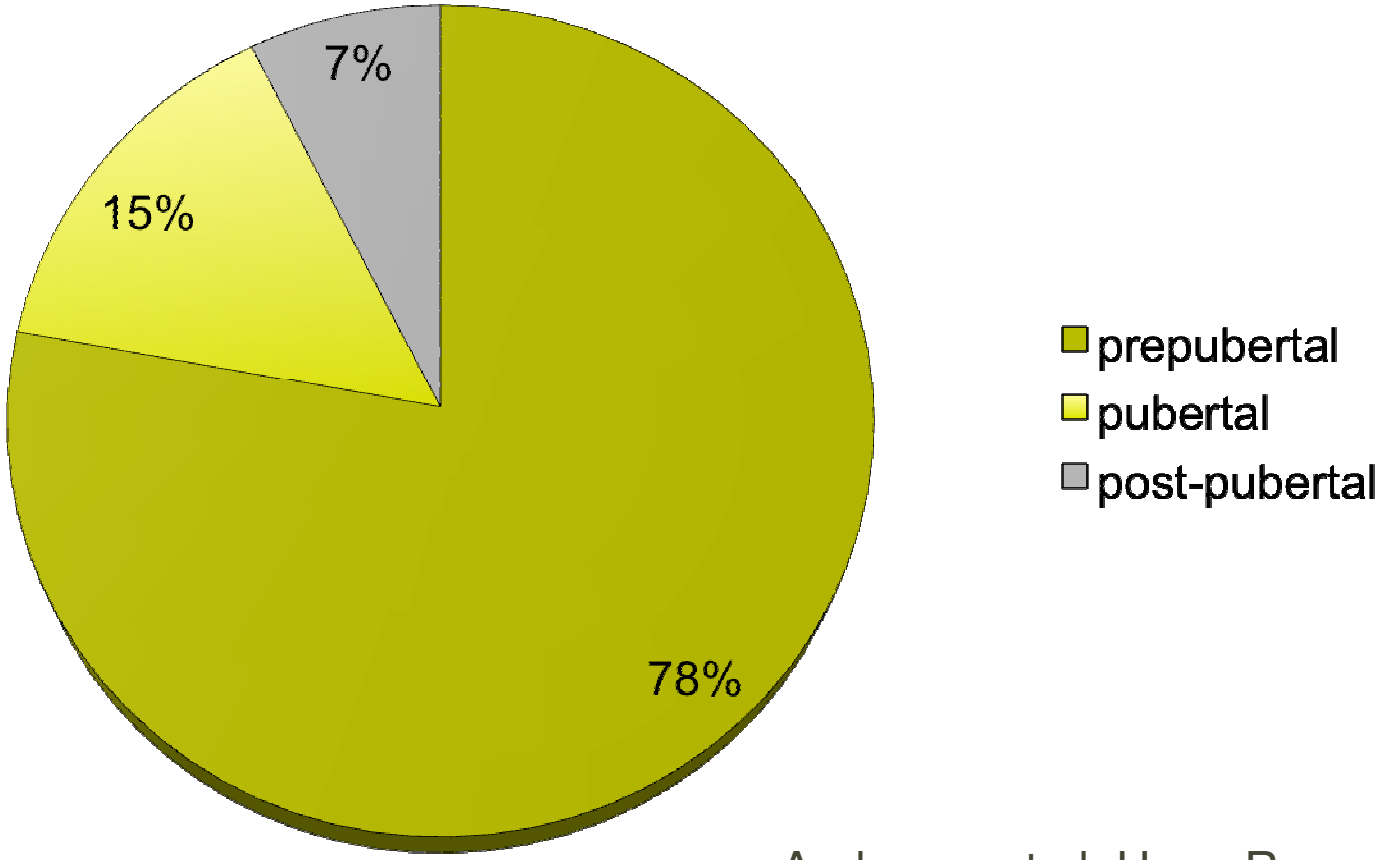
Methods
An expert panel and a writing committee were formed. The questions to be addressed by the guideline were determined, and a systematic review of the literature from 1987 to 2005 was performed, and included a search of online databases and consultation with content experts.

Results
The literature review found many cohort studies, case series, and case reports, but relatively few randomized or definitive trials examining the success and impact of fertility preservation methods in people with cancer. Fertility preservation methods are used infrequently in people with cancer.

Recommendations
As part of education and informed consent before cancer therapy, oncologists should address the possibility of infertility with patients treated during their reproductive years and be prepared to discuss possible fertility preservation options or refer appropriate and interested patients to reproductive specialists. Clinician judgment should be employed in the timing of raising this issue, but discussion at the earliest possible opportunity is encouraged. Sperm and embryo cryopreservation are considered standard practice and are widely available; other available fertility preservation methods should be considered investigational and be performed in centers with the necessary expertise.

Conclusion
Fertility preservation is often possible in people undergoing treatment for cancer. To preserve the full range of options, fertility preservation approaches should be considered as early as possible during treatment planning.

Distribution Children's Cancer Research Group UK



Anderson et al. Hum. Reprod. 2008

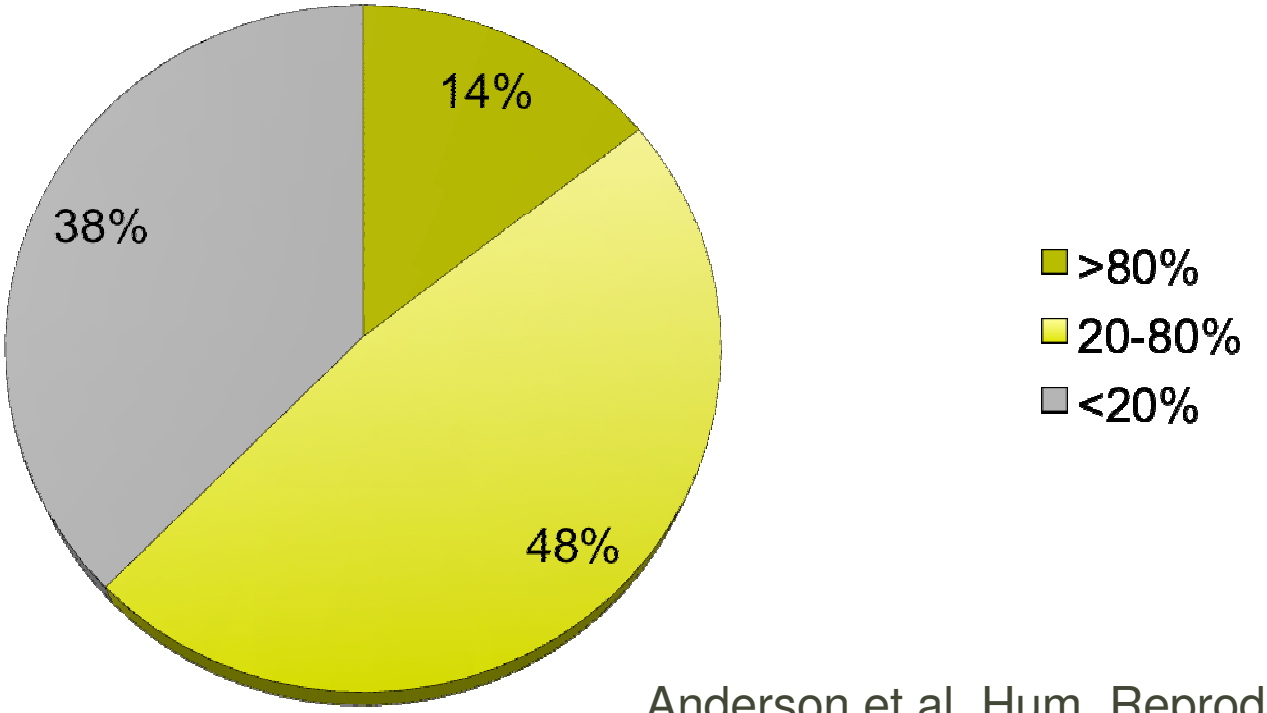
>80% risk for sterility after cytostatic treatment

- ✓ whole body irradiation
- ✓ conditioning for bone-marrow transplantation
- ✓ Hodgkin treated with alkylating agents
- ✓ metastatic Ewing's sarcoma
- ✓ metastatic soft-tissue sarcoma
- ✓ testicular radiotherapy

Wallace et al. Lancet Oncol. 2005

Risk for sterility after cytostatic treatment

pre-pubertal boys CCRG in whom fertility was discussed, i.e. 61%



Anderson et al. Hum. Reprod. 2008

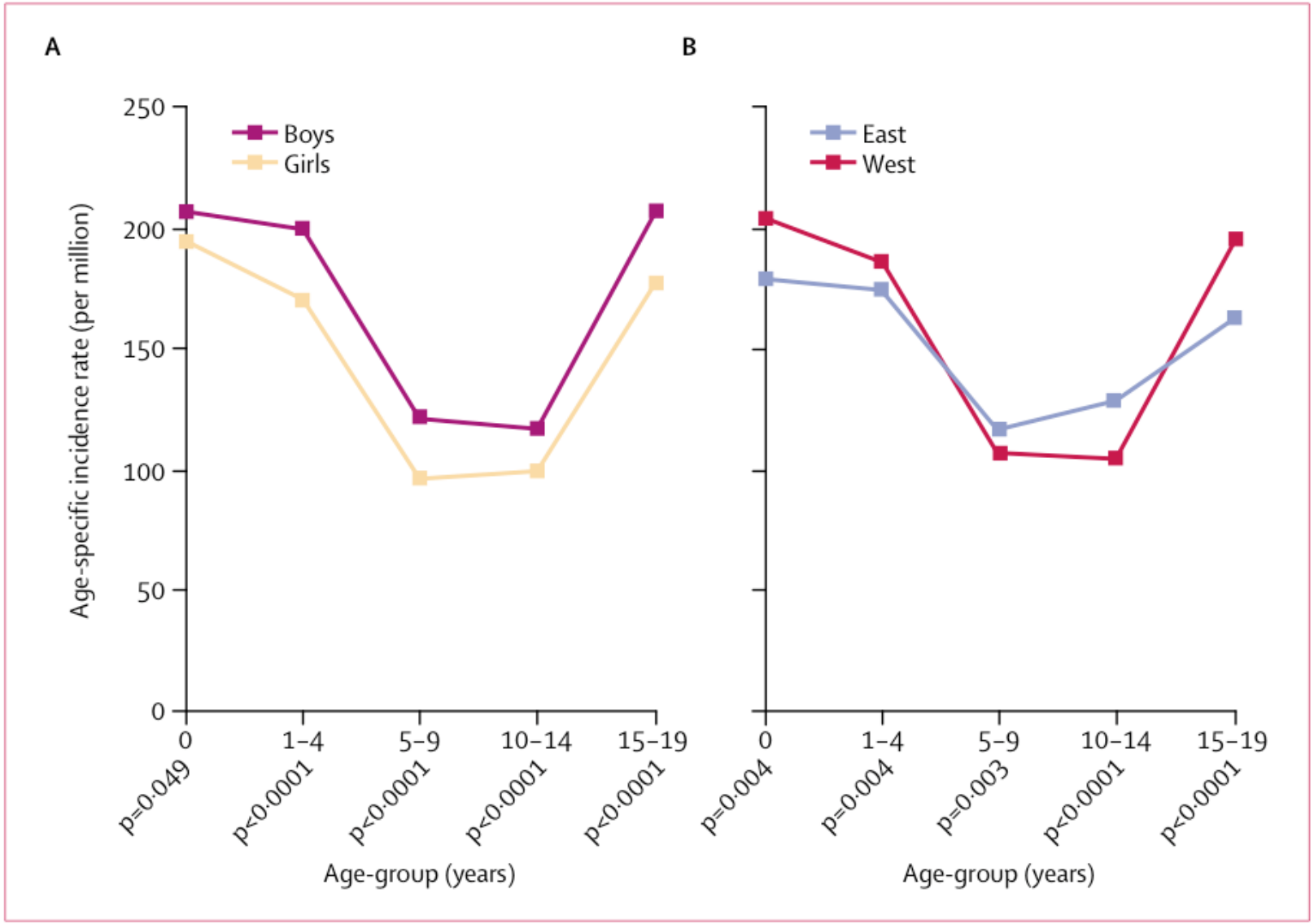


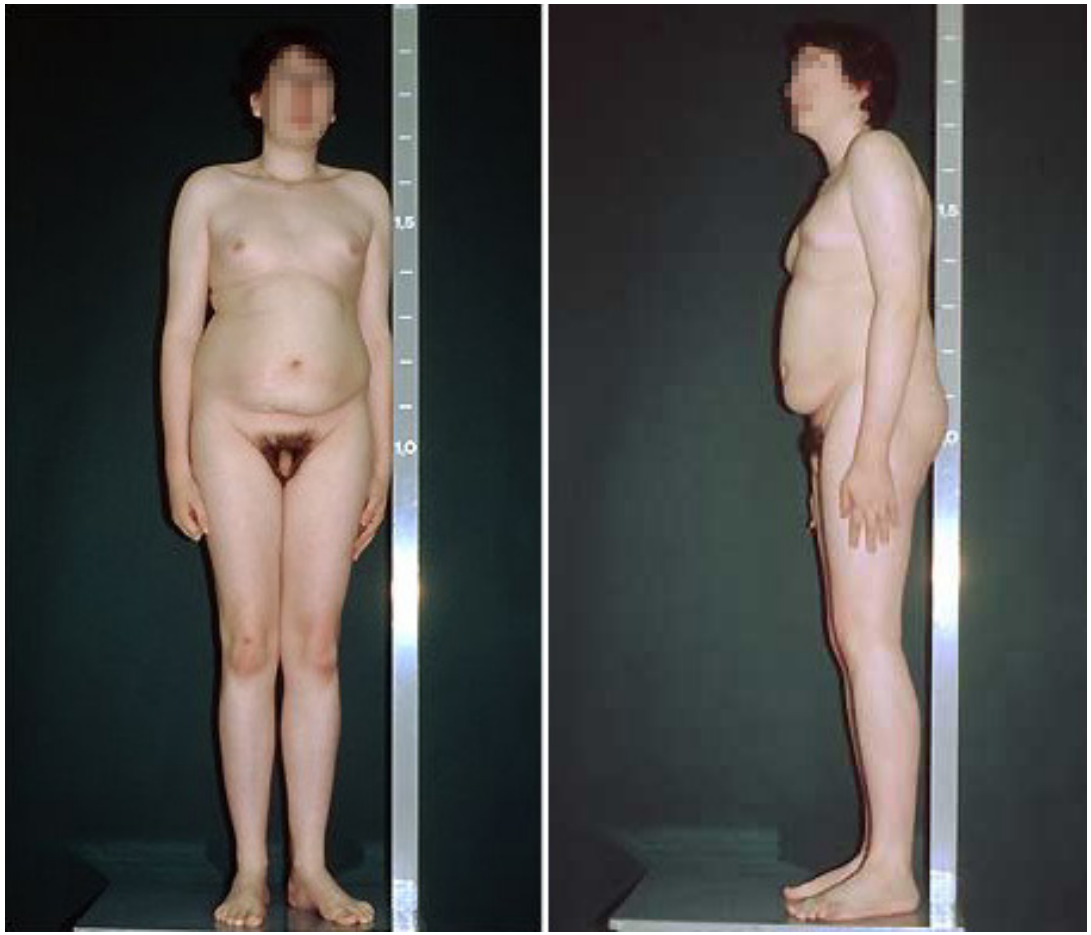
Figure 2: **Age-specific incidence rates of cancer in Europe for the 1990s by (A) sex and (B) geographical area**

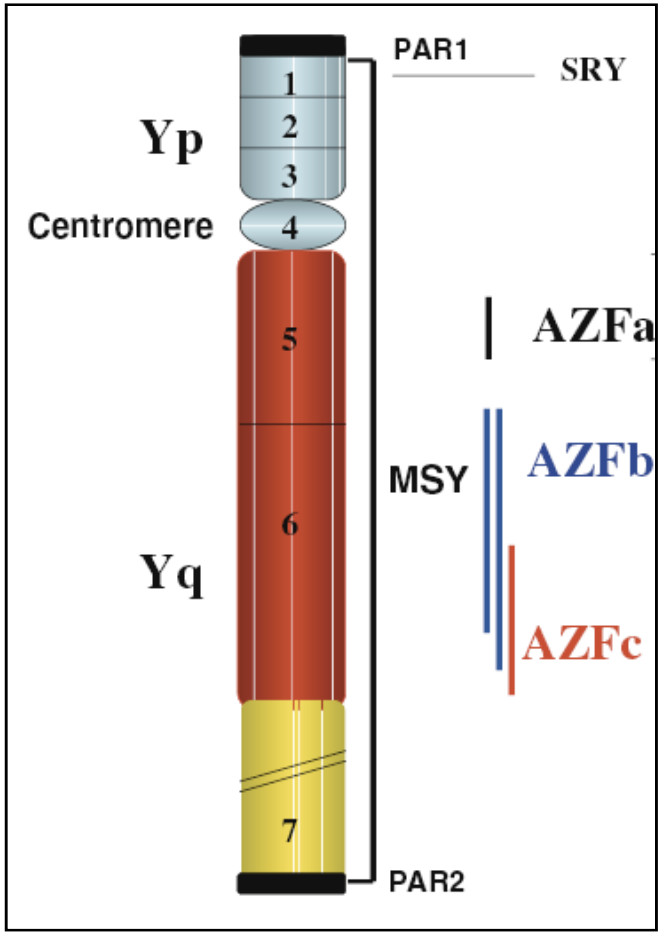
Steliarova-Foucher et al. 2004

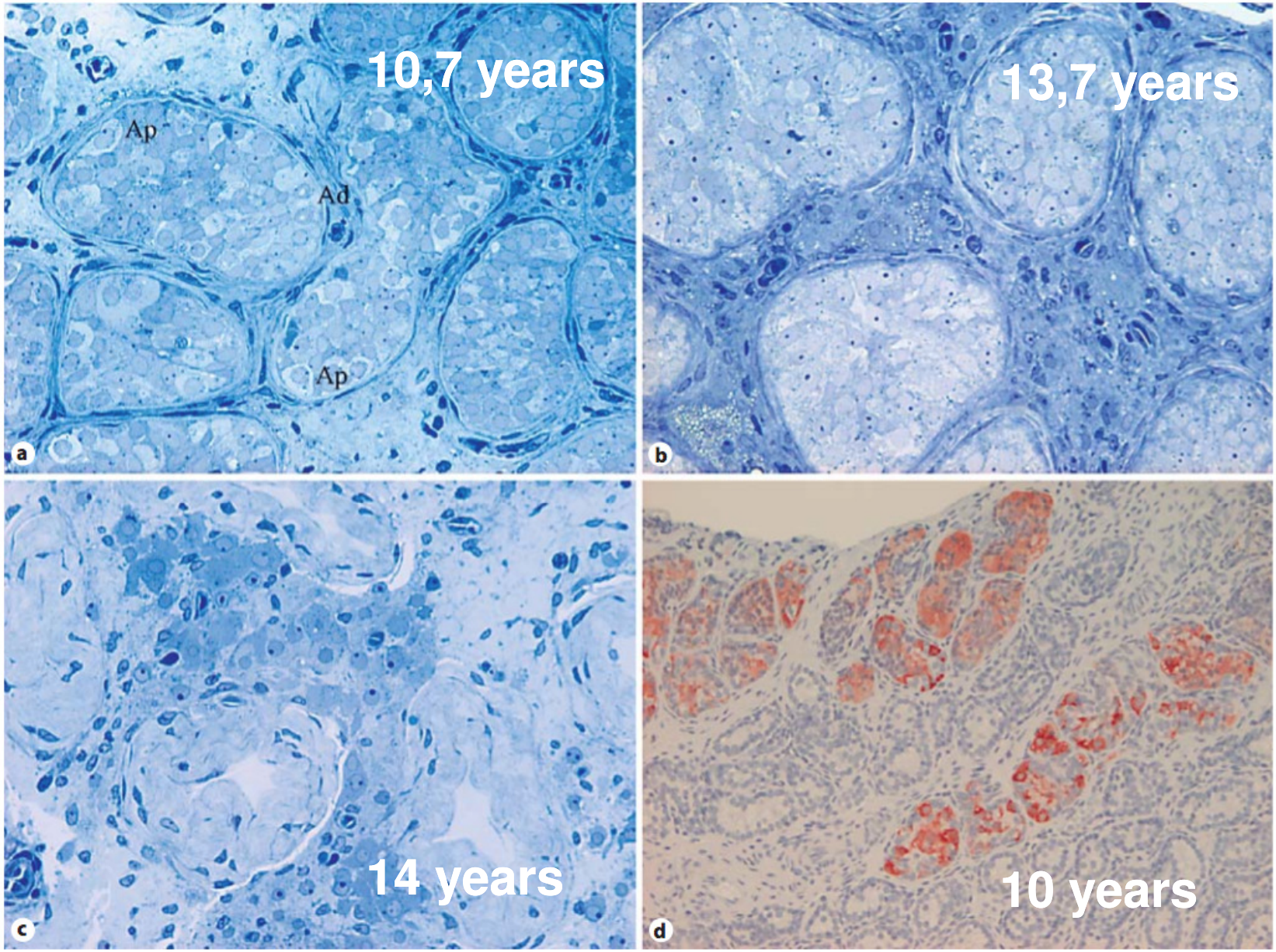
Cell and Tissue Banking (2006) 7:105–112

Sperm cryopreservation in male infertility due to genetic disorders

Csilla Krausz* and Gianni Forti
Andrology Unit, Department of Clinical Physiopathology







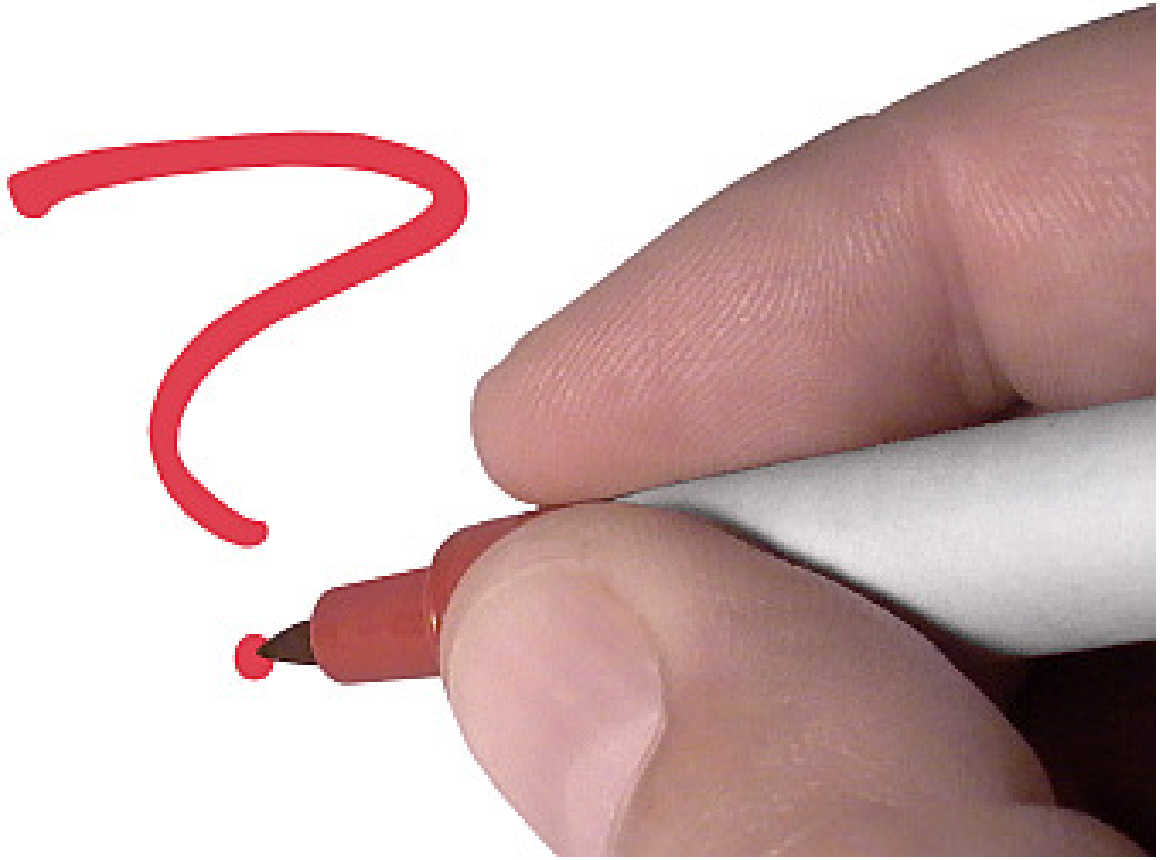
Wikstrom and Dunkel Horm. Res. 2008

Fertility Preservation in Prepubertal Males

What ?

How ?

When?



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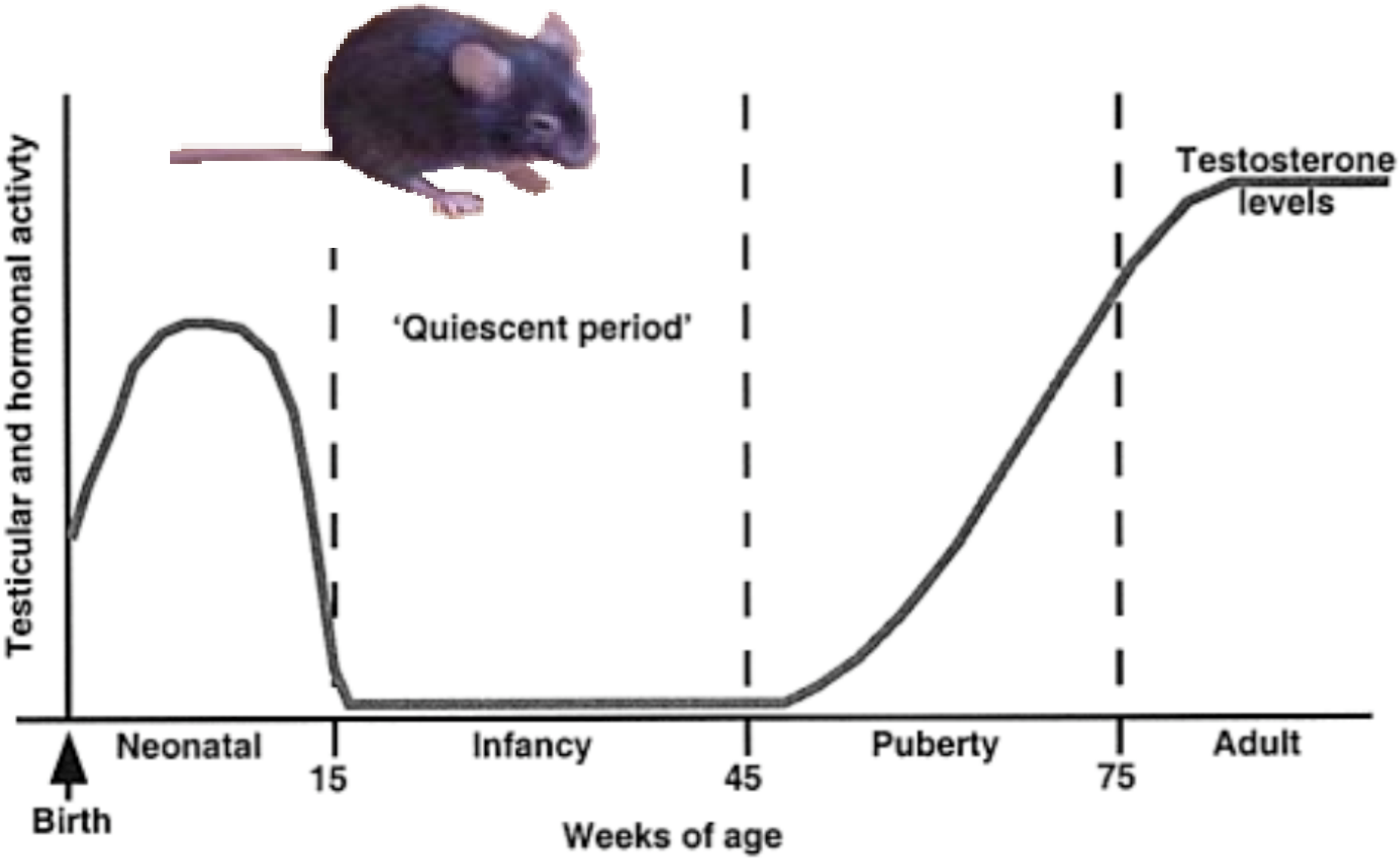
Induction of ‘testicular quiescence’



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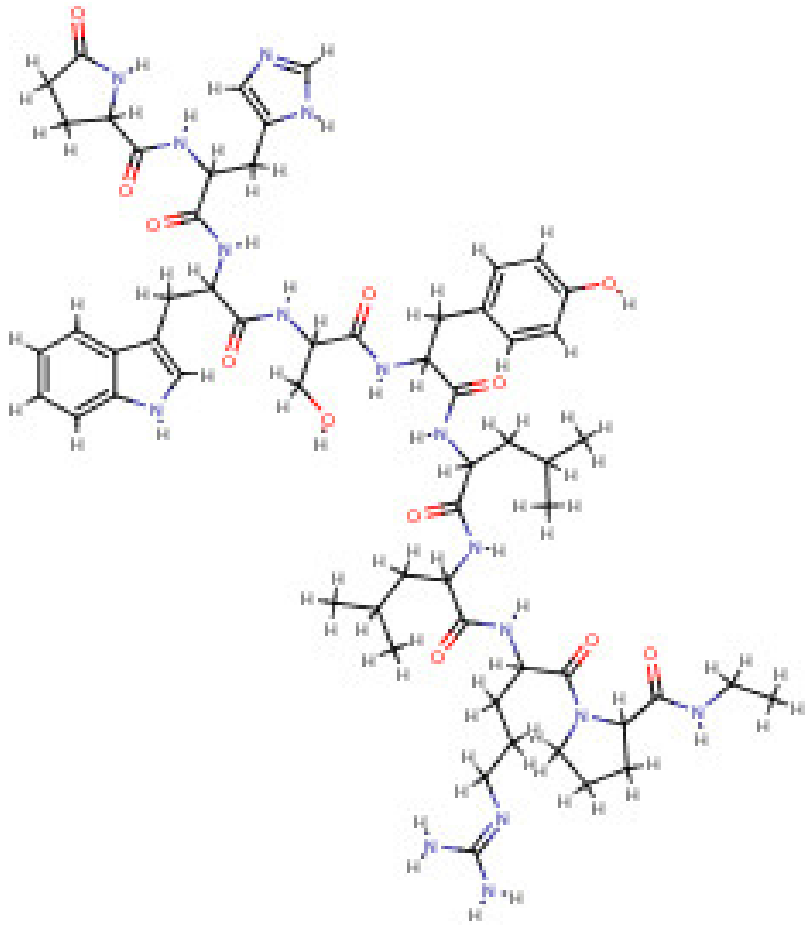
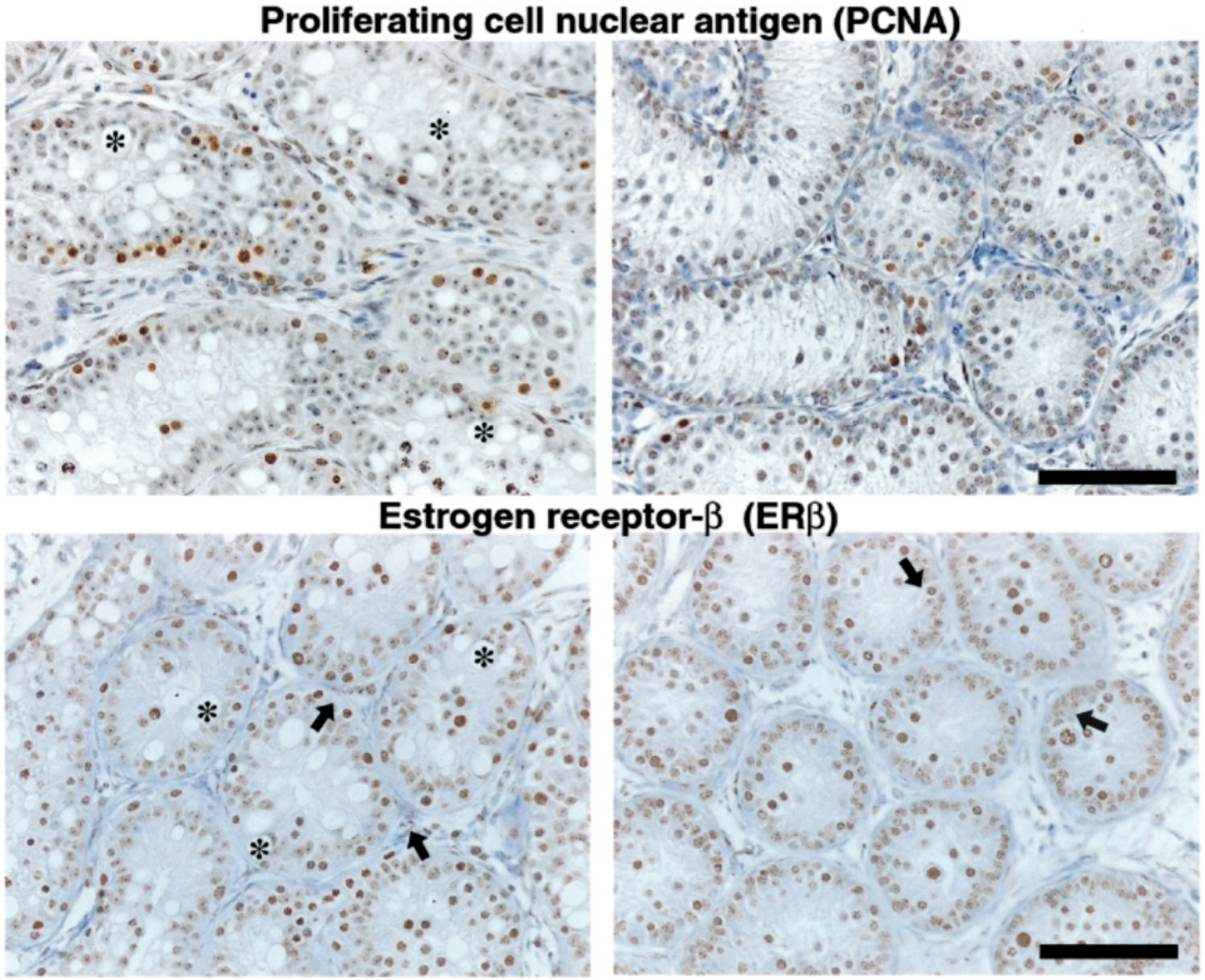


Testis is ‘quietly active’ rather than ‘truly quiescent’



Kelnar et al. Hum. Reprod. 2002

Control marmoset

GnRHa-treated marmoset

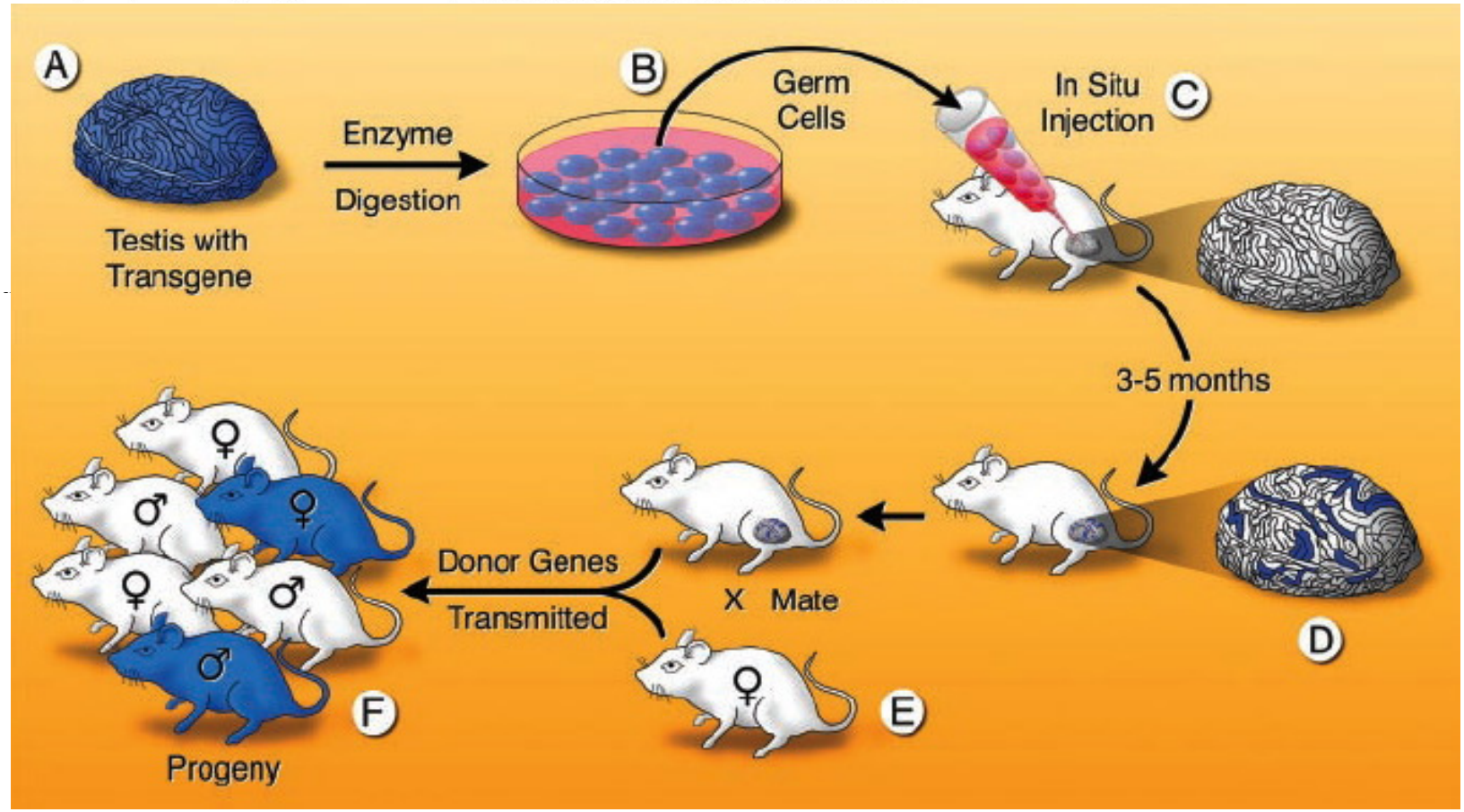
Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 11298–11302, November 1994
Developmental Biology

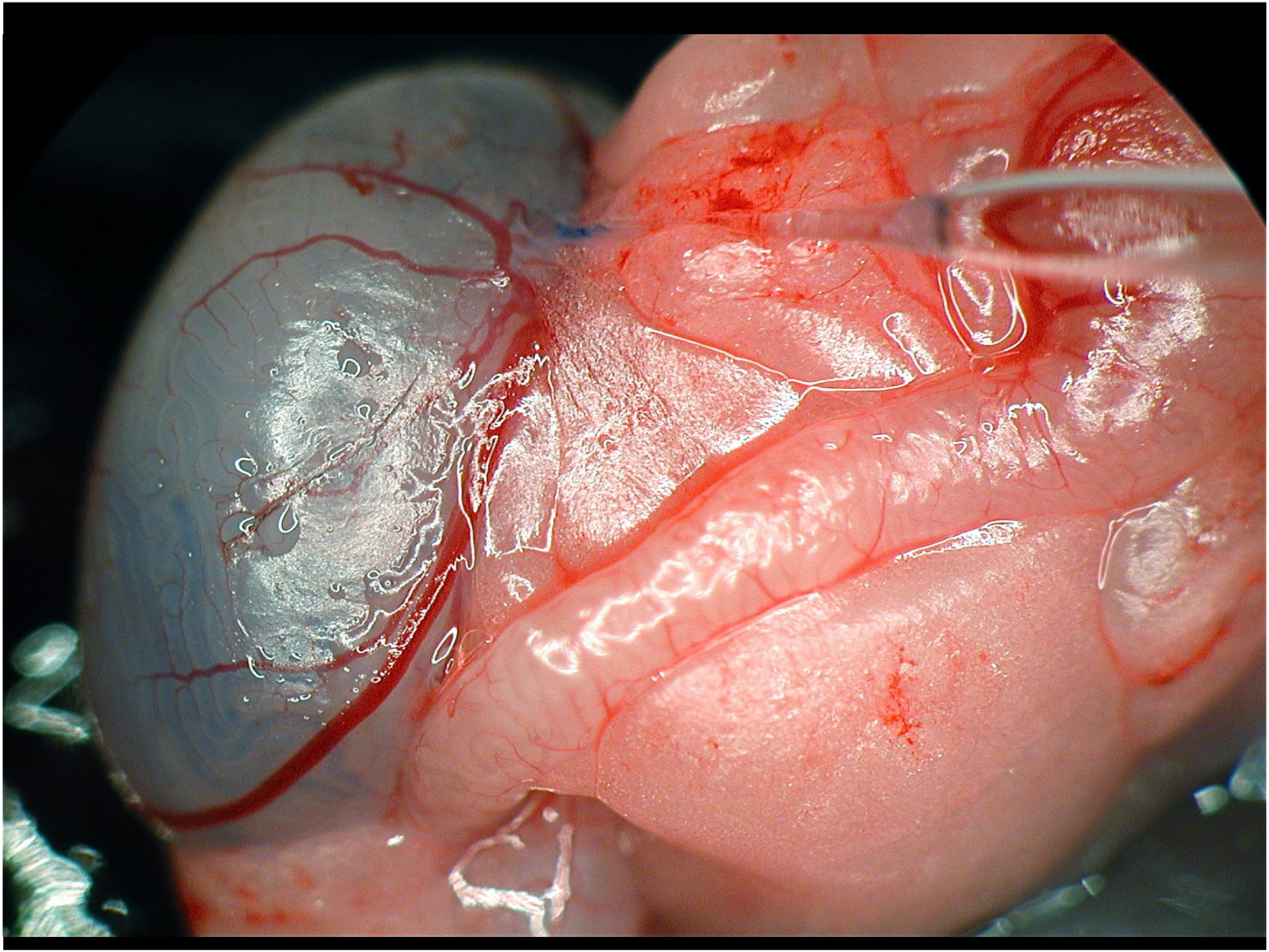
Spermatogenesis following male germ-cell transplantation

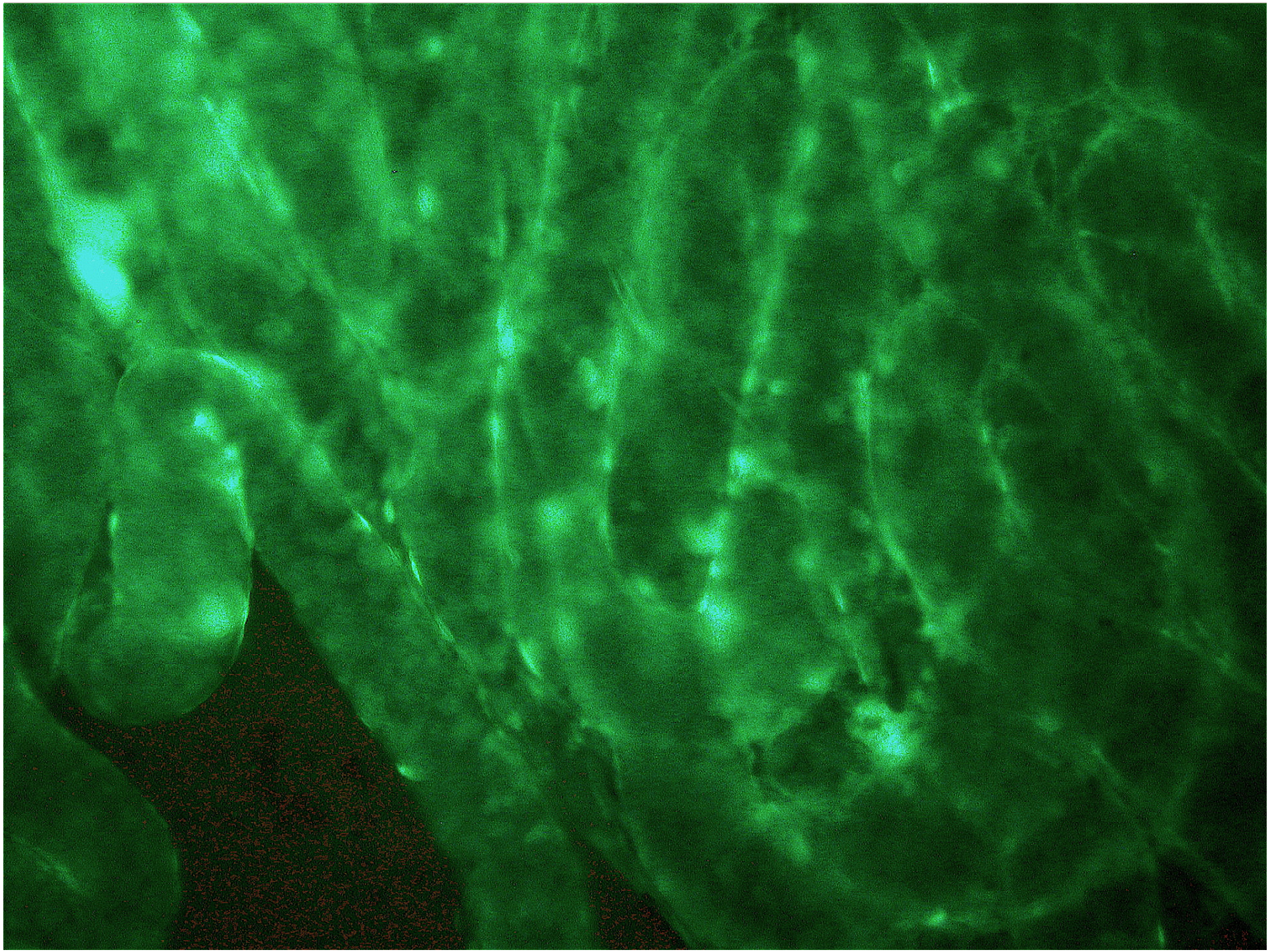
(spermatogonia/stem cells/testes/transgenic mice)

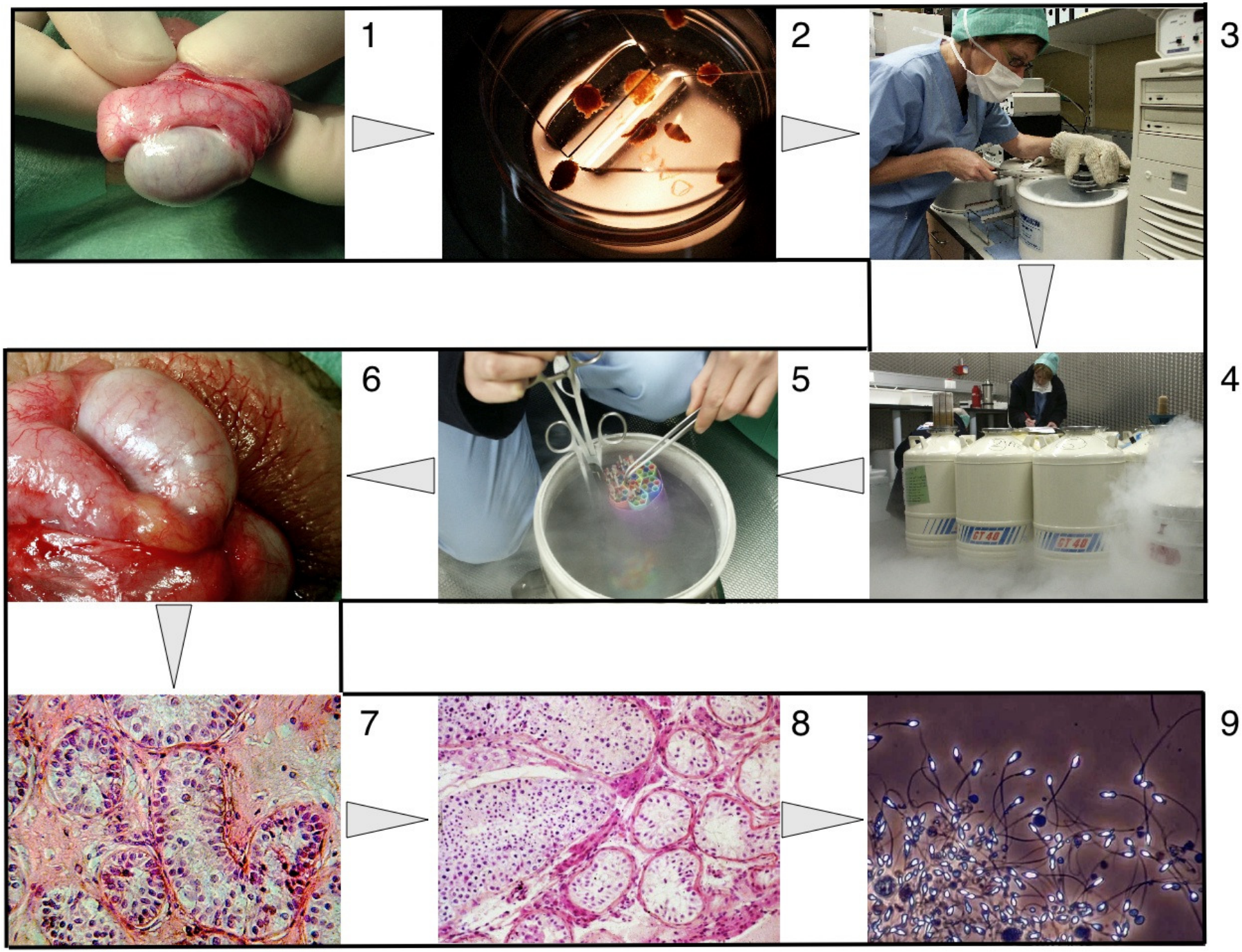
RALPH L. BRINSTER* AND JAMES W. ZIMMERMANN†

Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104








1
2
3
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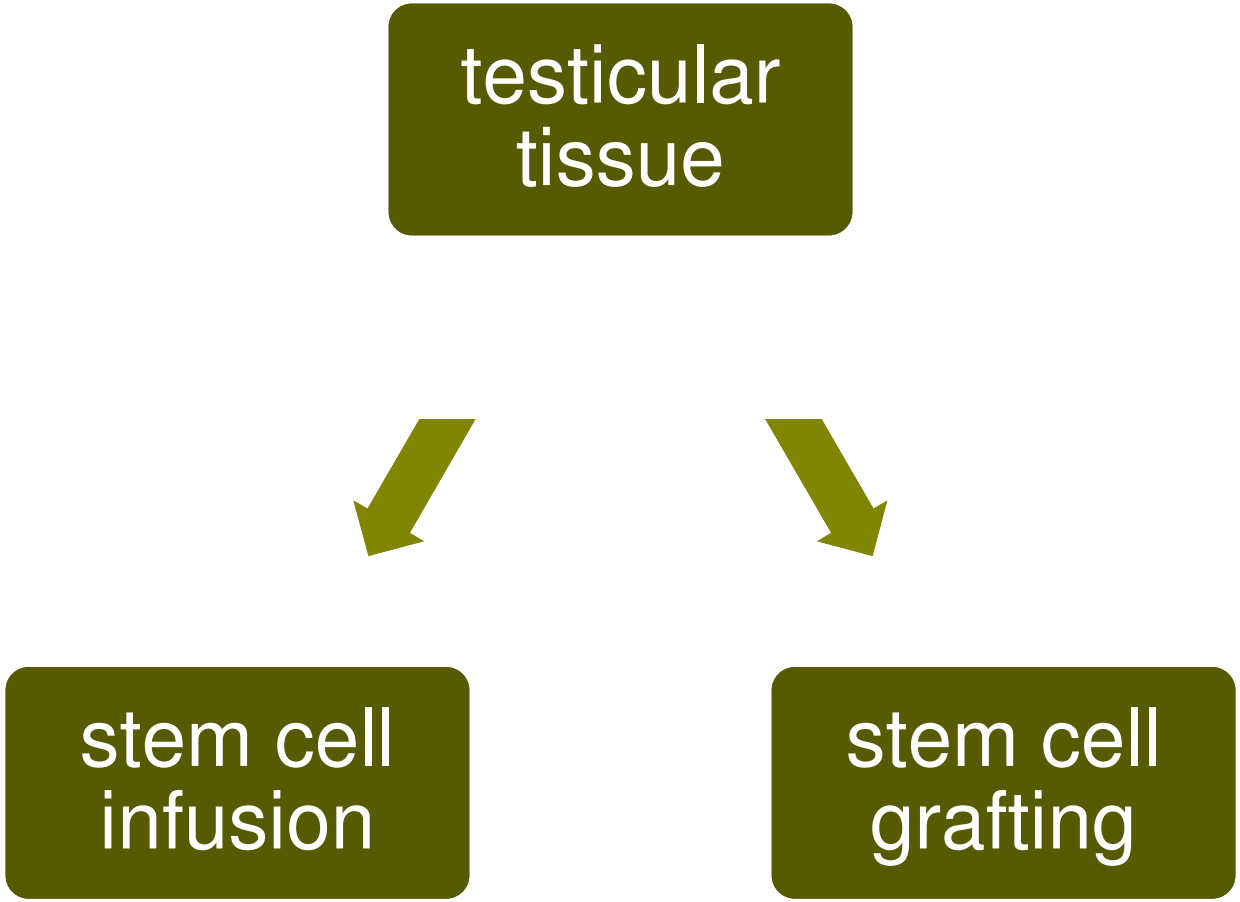
9

Parental acceptability prepubertal boys

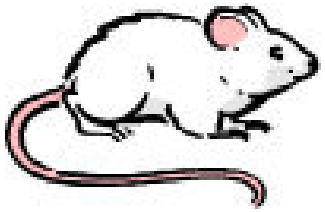
- ✓ biopsy: 100/162 62%
- ✓ hemicastration: 55/162 34%

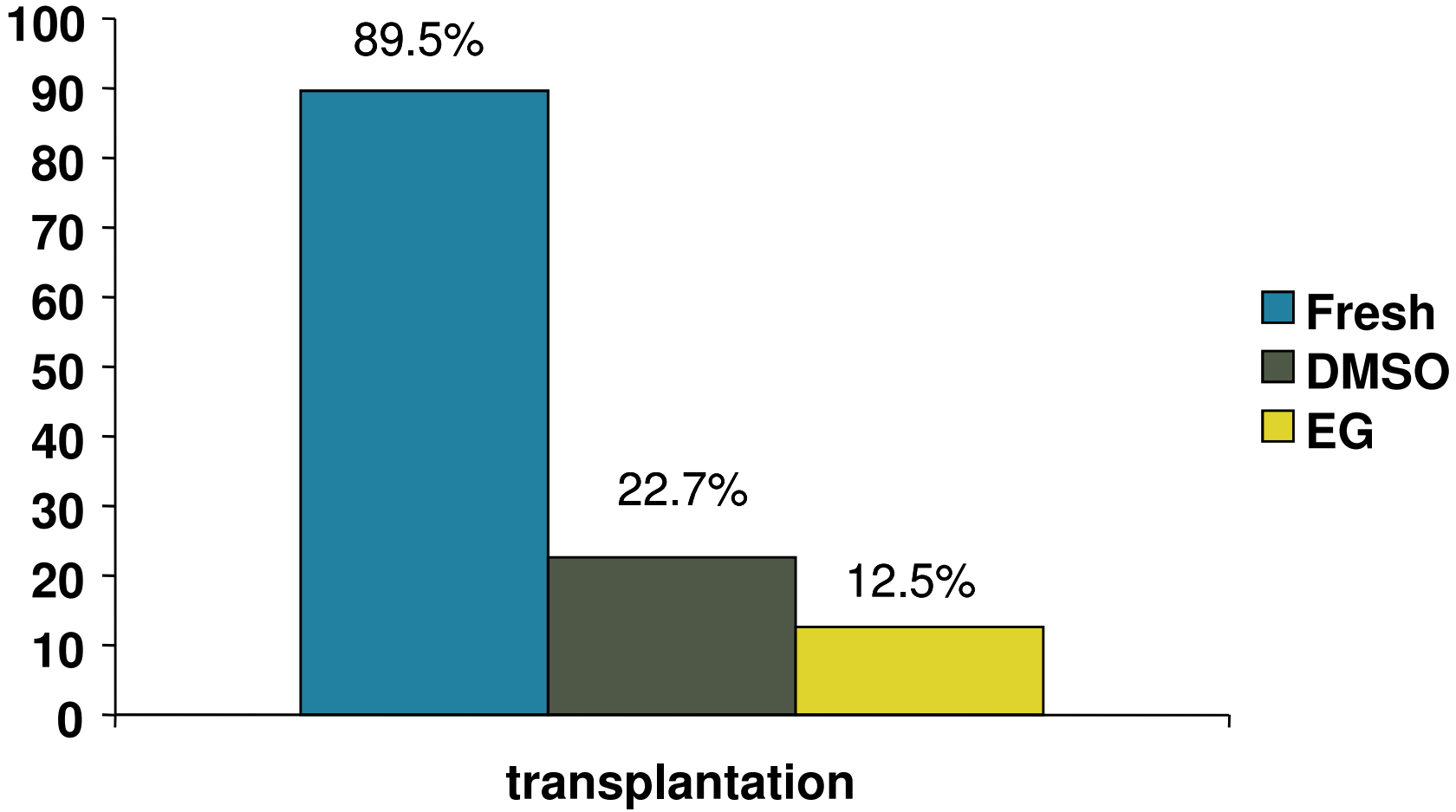
Van den Bergh et al. Hum. Reprod. 2007

Two possible clinical strategies



Transplantation after cryopreservation





Frederickx et al. 2004 Hum Reprod

Cryosurvival and spermatogenesis after allografting prepubertal mouse tissue: comparison of two cryopreservation protocols

TABLE 1

Evaluation of fresh and cryopreserved prepubertal mouse allografts.

	No. of grafts	Sclerosis/ atrophy	Most advanced cell stage in graft				No. of tubules analyzed	No. of tubules containing spz	No. of damaged tubules
			spg	spc	spd	spz			
Fresh	28	0 (0%)	—	—	—	28 (100%)	1538	354 (23%)[a,b]	603 (39%)[c]
EG	14	3 (21%)	—	—	—	11 (79%)	547	176 (32%)[a]	231 (42%)[d]
DMSO	14	1 (7%)	2 (14%)	—	—	11 (79%)	949	308 (32%)[b]	302 (32%)[c,d]

Note: spg = spermatogonia; spc = spermatocyte; spd = spermatid; spz = spermatozoa.

[a,b] $P<.0001$ by chi-squared test.

[c,d] $P<.001$ by chi-squared test.

Goossens. Cryosurvival of murine testicular tissue. Fertil Steril 2008.

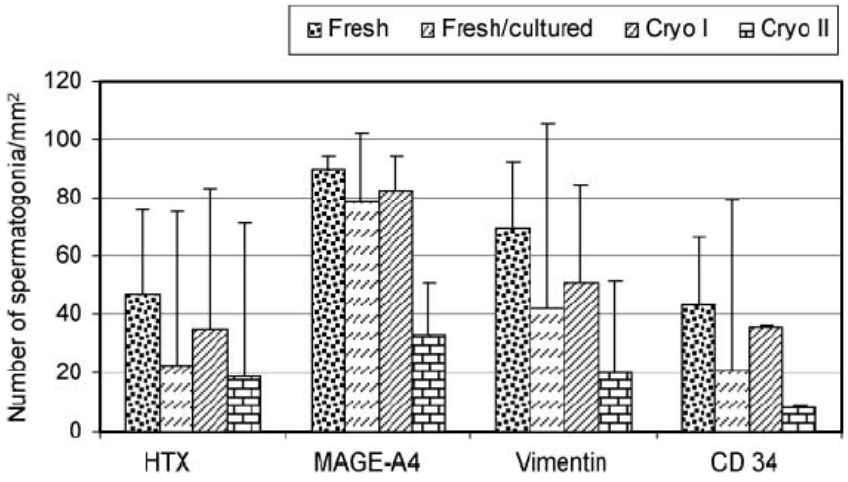
biology of the testis

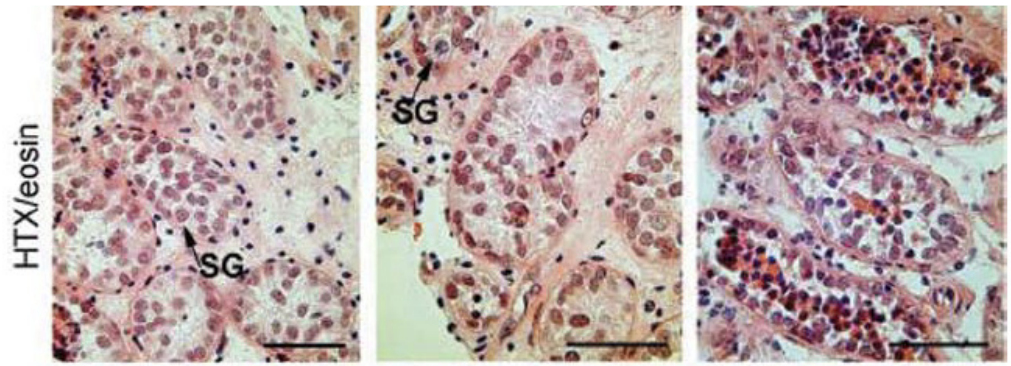
Goossens et al. 2008 Fertil Steril

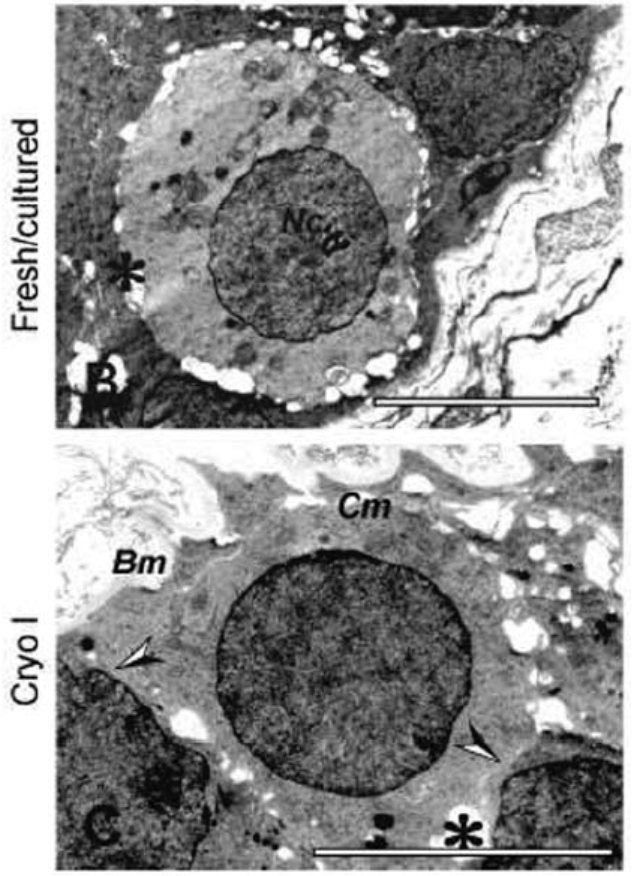
Methods of cryopreservation of testicular tissue with viable spermatogonia in pre-pubertal boys undergoing gonadotoxic cancer treatment

Victoria Keros[1,5], Kjell Hultenby[2], Birgit Borgström[3], Margareta Fridström[1], Kirsi Jahnukainen[4] and Outi Hovatta[1]

[1]Karolinska Institute, Division of Obstetrics and Gynaecology, Department of Clinical Science, Technology and Intervention, [2]Clinical Research Centre, [3]Department of Paediatrics, Karolinska University Hospital, Huddinge, SE 141 86 Stockholm, Sweden and [4]Paediatric Endocrinology Unit, Astrid Lindgren Children's Hospital, Karolinska Institute, Stockholm, Sweden







Two possible clinical strategies

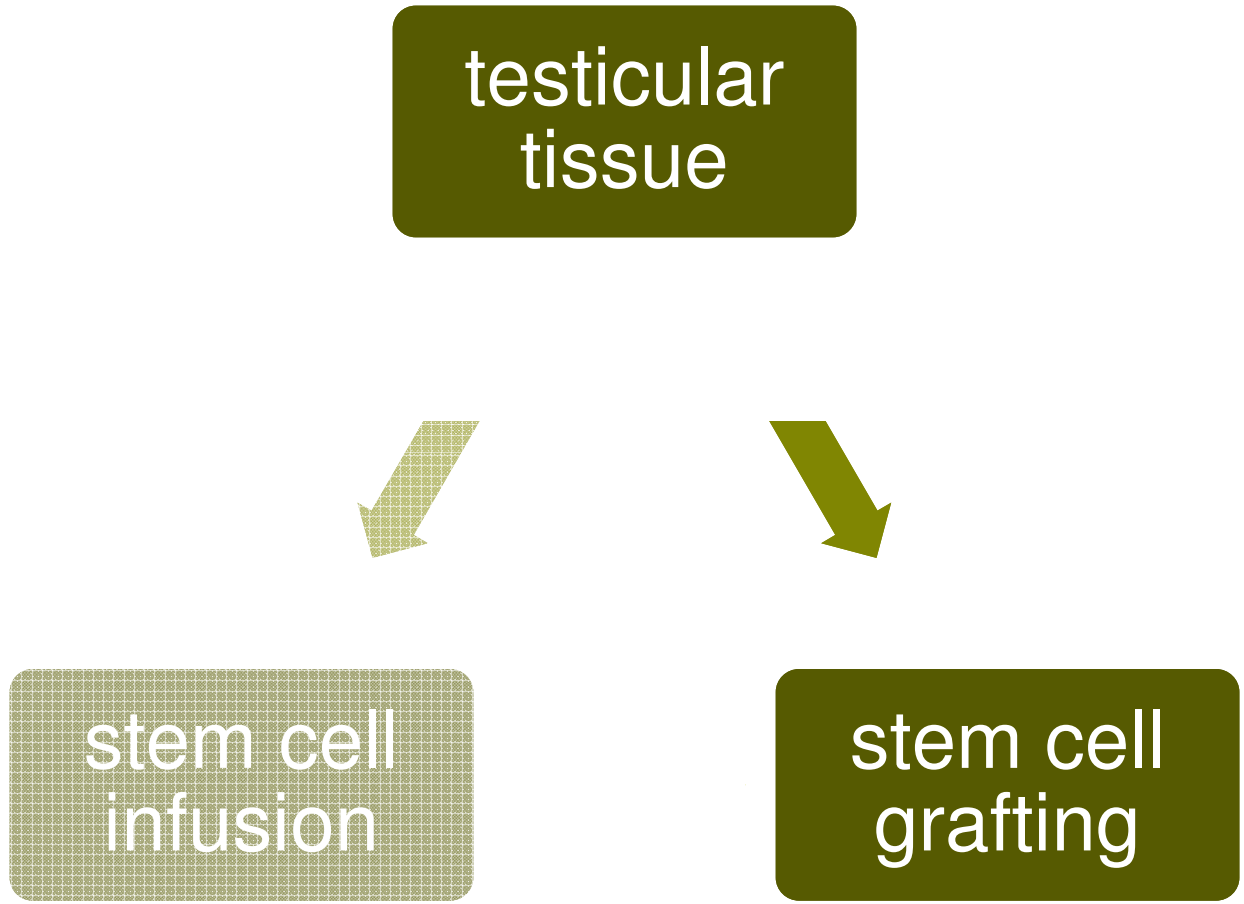
testicular tissue

?

stem cell infusion

stem cell grafting

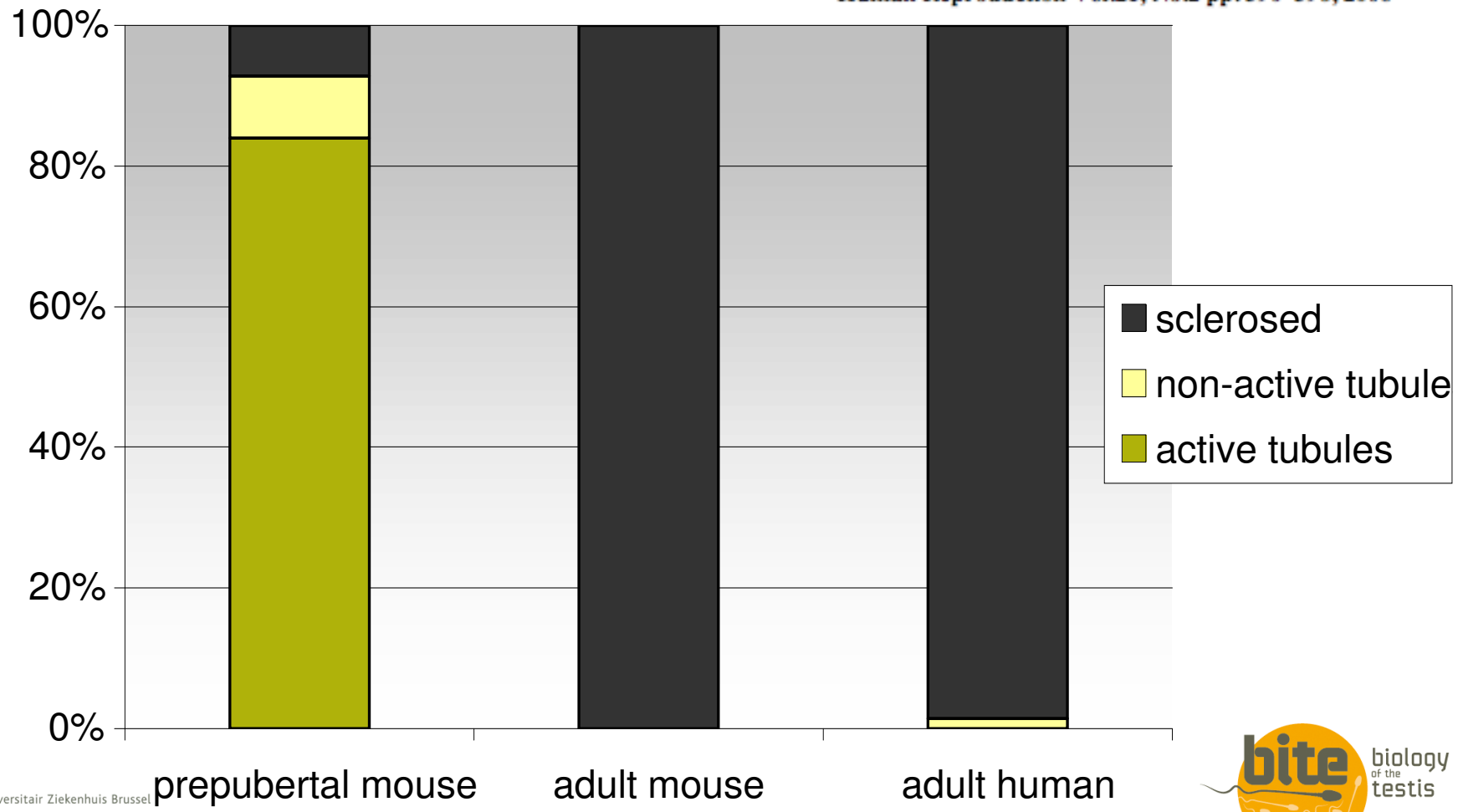
Two possible clinical strategies

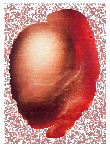


Spermatogonial survival after grafting human testicular tissue to immunodeficient mice

Mieke Geens, Gert De Block, Ellen Goossens, Veerle Frederickx, André Van Steirteghem and Herman Tournaye[1]

Human Reproduction Vol.21, No.2 pp. 390–396, 2006





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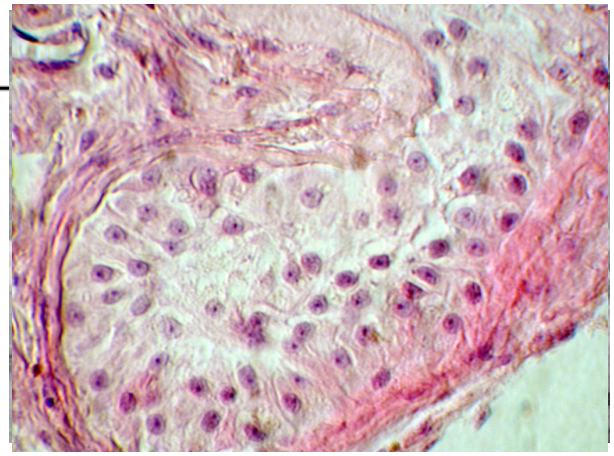
bite biology of the testis

Spermatogonial survival after grafting human testicular tissue to immunodeficient mice

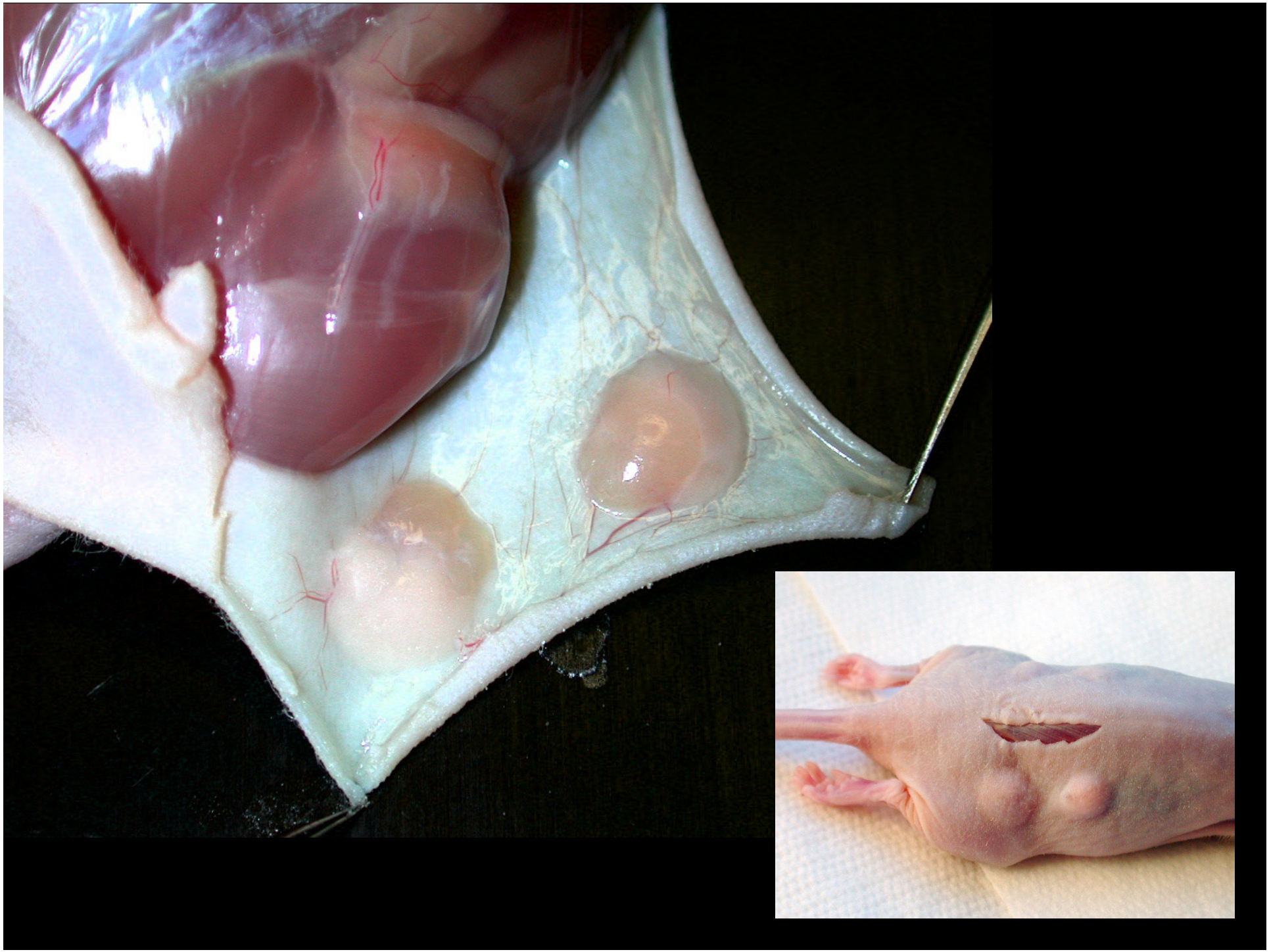


Mieke Geens, Gert De Block, Ellen Goossens, Veerle Frederickx, André Van Steirteghem and Herman Tournaye[1]

Centre for Reproductive Medicine and Research Centre for Reproduction and Genetics, Universiteit Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium

[1]To whom correspondence should be addressed: tournaye@az.vub.ac.be

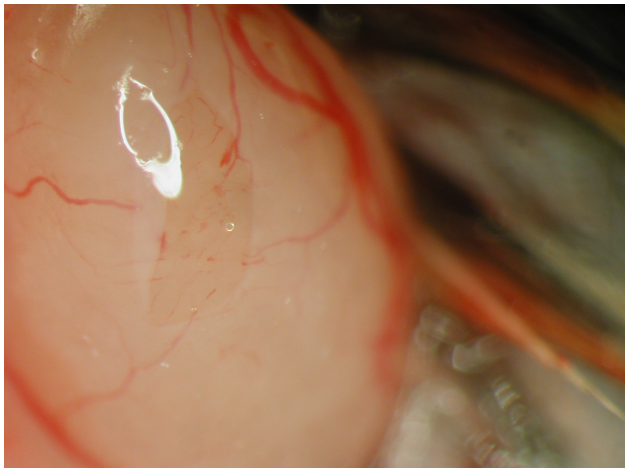
BACKGROUND: The xenografting of pre-pubertal human testicular tissue to an immunodeficient mouse is a theoretical strategy for restoring fertility in childhood cancer patients, while circumventing the risk of malignant recurrence. This study aimed at comparing the grafting of pre-pubertal and adult murine testicular tissue, as well as that of human adult testicular tissue, to two immunodeficient recipients, i.e. Swiss Nude mice and SCID-NOD mice. MATERIALS AND METHODS: In this study, we evaluated the survival of pre-pubertal and adult murine testicular tissues, and that of adult human testicular tissue after subcutaneous grafting to immunodeficient mice. RESULTS: After allografting pre-pubertal testicular tissue pieces, meiotic cells were observed in 69.1% of the grafts, while complete spermatogenesis was observed in 30.9%. All grafts of adult murine testicular tissue and 59.5% of the adult human testicular grafts showed sclerosis. However, in 21.6% of the adult human testicular grafts, spermatogonia were still observed, with increasing sclerosis in time. No significant differences were observed between the two mouse models under evaluation. CONCLUSION: After xenografting human adult testicular tissue to a recipient mouse, spermatogonia were maintained over a period of >195 days. However, in order to prove xenografting as a method for external germ line storage, the transplants should have a more immature developmental stage. Moreover, not only the developmental status of the tissue at the time-point of grafting, but also the structural organisation of the seminiferous epithelium, might influence the development of the testicular tissue.

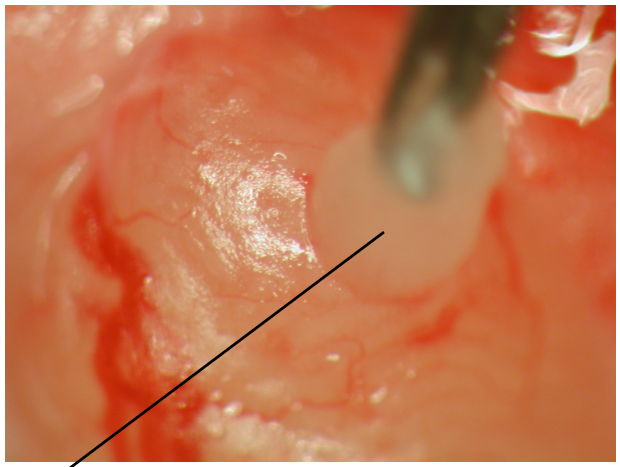


Regeneration of spermatogenesis by grafting testicular tissue or injection of testicular cells into the testes of sterile mice: a comparative study

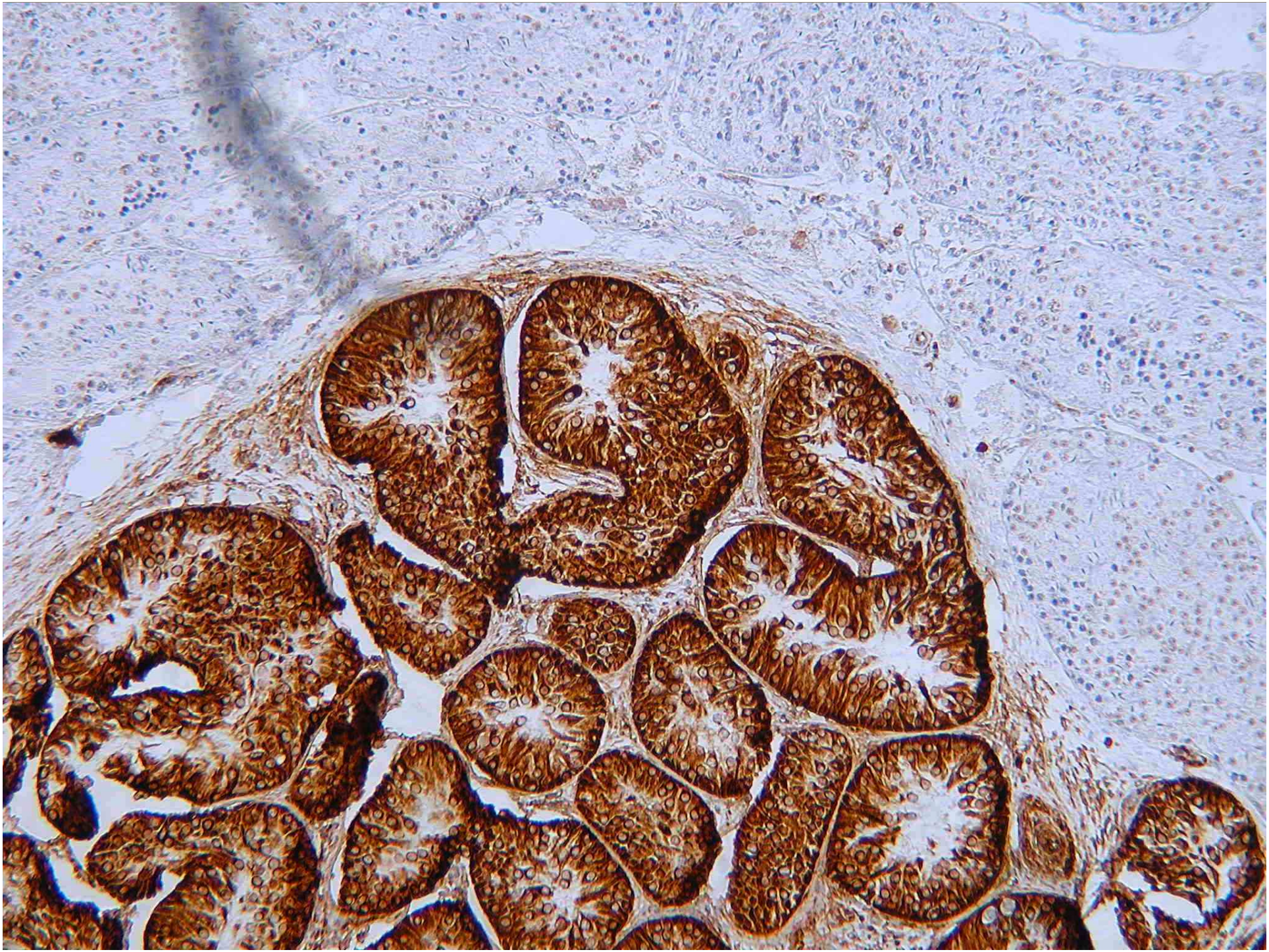
Dorien Van Saen, M.Sc., Ellen Goossens, Ph.D., Gert De Block, and Herman Tournaye, M.D., Ph.D.

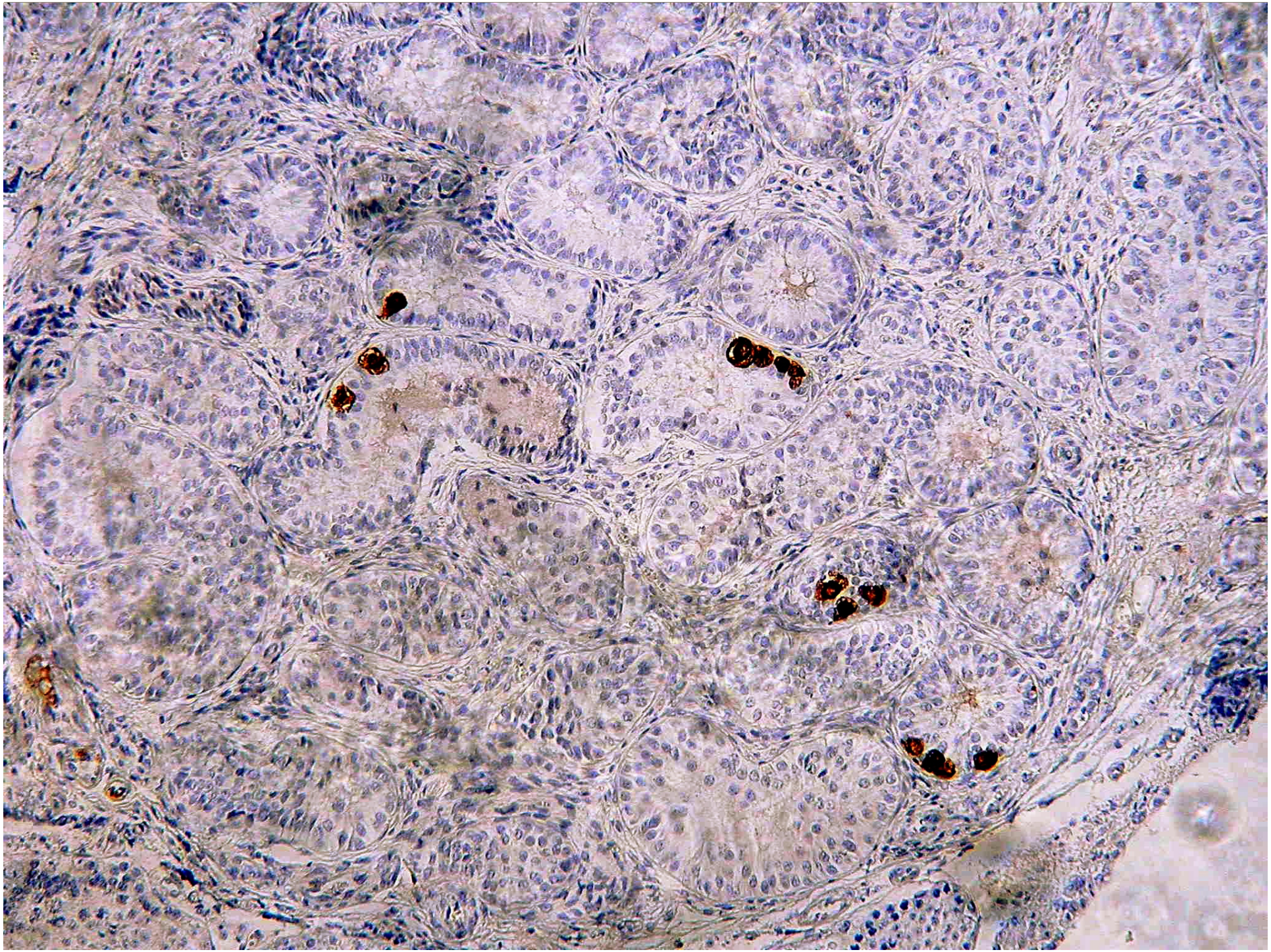
Center for Reproductive Medicine and Research Laboratories for Reproductive Medicine, University Hospital and Medical School, Dutch-Speaking Brussels Free University (Vrije Universiteit Brussel), Brussels, Belgium





Prepubertal tissue





[CANCER RESEARCH 61, 706–710, January 15, 2001]

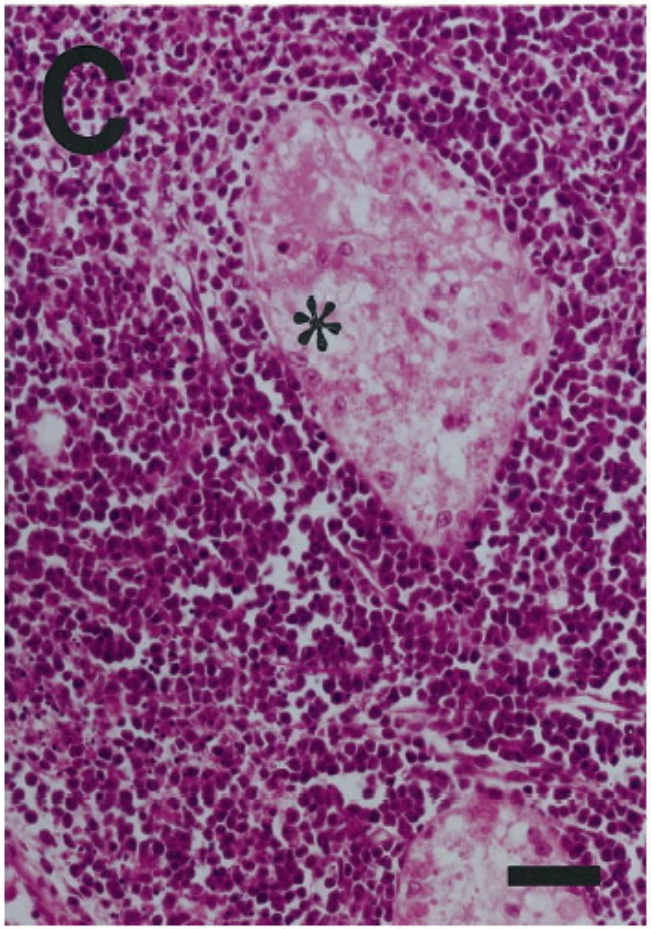
Intratesticular Transplantation of Testicular Cells from Leukemic Rats Causes Transmission of Leukemia[1]

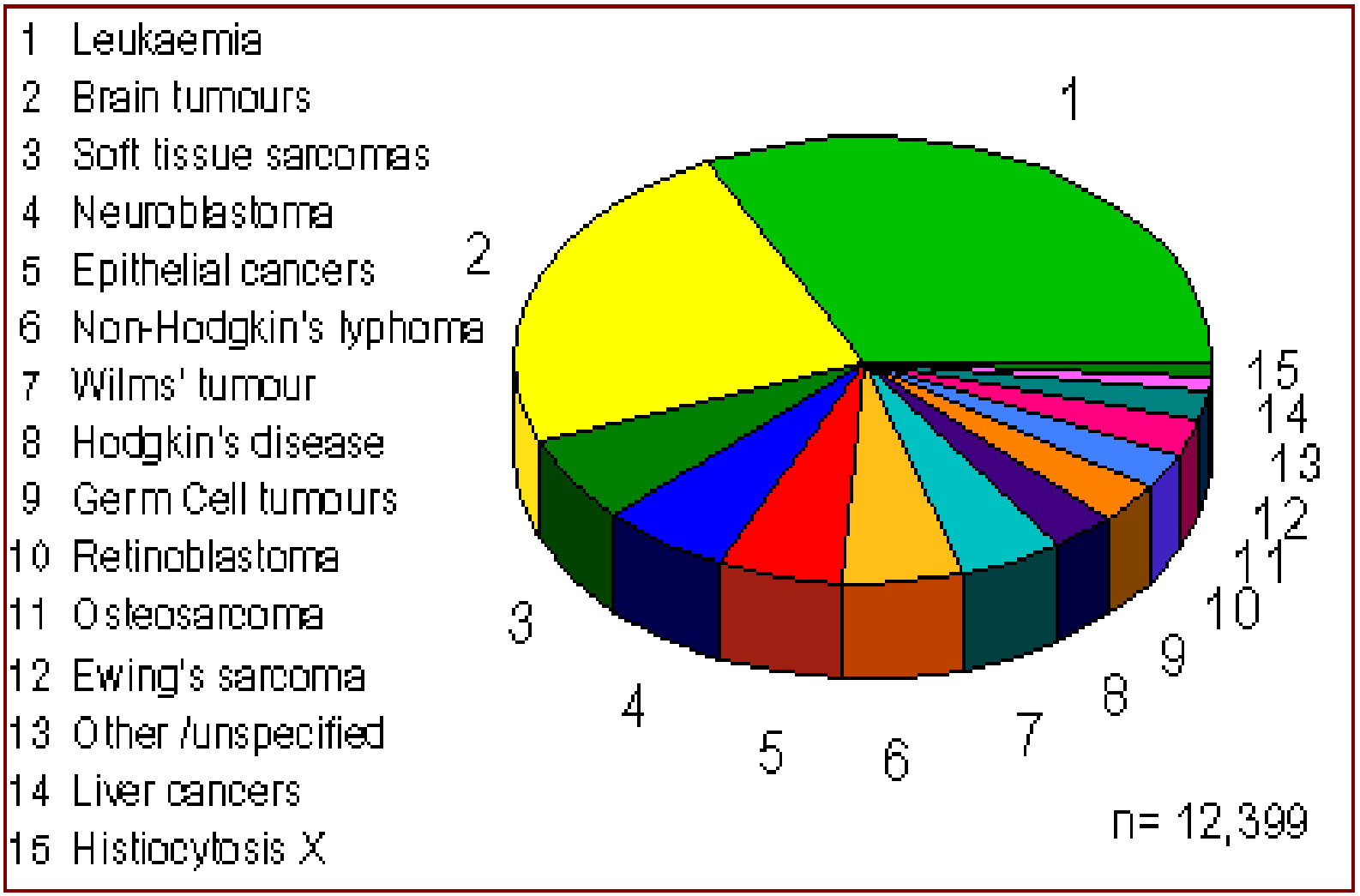
Kirsi Jahnukainen,[2] Mi Hou, Cecilia Petersen, Brian Setchell, and Olle Söder

Pediatric Endocrinology Unit, Karolinska Institute, Karolinska Hospital, 171 76 Stockholm, Sweden [K. J., M. H., C. P., B. S., O. S.], and Department of Pediatrics, University of Turku, 20520 Turku, Finland [K. J.]

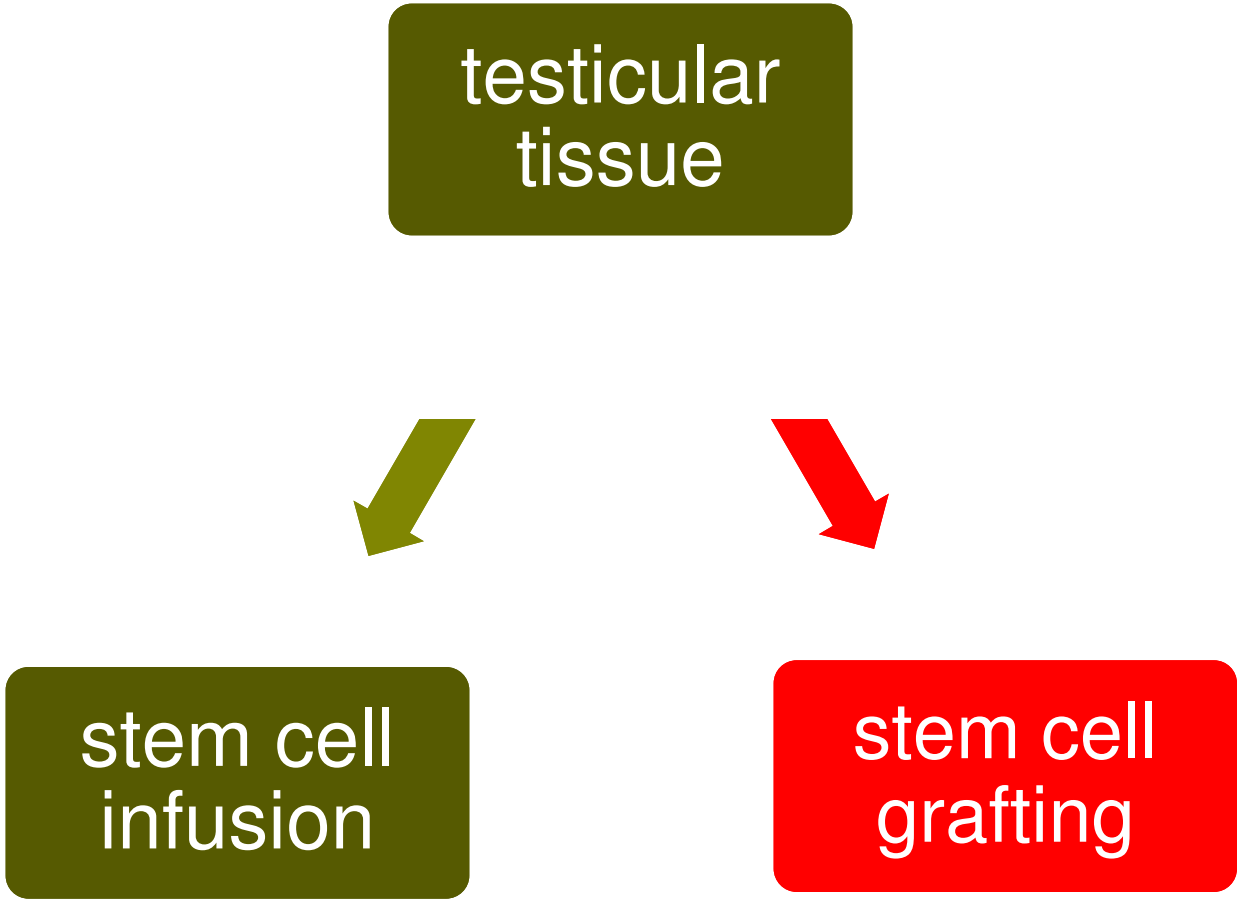
ABSTRACT

A rat T-cell leukemia model was used to study the safety of germ cell transplantation as a mean of preventing infertility in males undergoing gonadotoxic cancer treatment. Donor germ cells were harvested from the testes of terminally ill leukemic rats and were either used directly or cryopreserved and thawed before transplantation by rete testis microinjection. All rats transplanted with testicular cells from leukemic donors developed signs of terminal rat T-cell leukemia, whereas control animals remained healthy. Cryopreservation of the donor germ cells caused a 3- to 6-day delay in the terminal phase of leukemia. When a known number of leukemic cells were mixed with germ cells and microinjected into the testis, the rate of appearance of terminal leukemia was directly related to the number of transferred leukemic lymphoblasts. As few as 20 leukemic cells were able to cause a cancer relapse resulting in terminal leukemia 21 days after transplantation in three of five transplanted animals. Our results demonstrate that germ cell transplantation with the presently used techniques is not safe enough for clinical use. Improved methods for purging testicular specimens of cancer cells or totally new approaches with transient xenogenetic host models to detect contamination of malignant cells must be developed before this technique can be offered to patients without fear of disease relapse.




1 Leukaemia
2 Brain tumours
3 Soft tissue sarcomas
4 Neuroblastoma
5 Epithelial cancers
6 Non-Hodgkin's lyphoma
7 Wilms' tumour
8 Hodgkin's disease
9 Germ Cell tumours
10 Retinoblastoma
11 Osteosarcoma
12 Ewing's sarcoma
13 Other /unspecified
14 Liver cancers
15 Histiocytosis X
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
n= 12,399

One possible clinical strategy

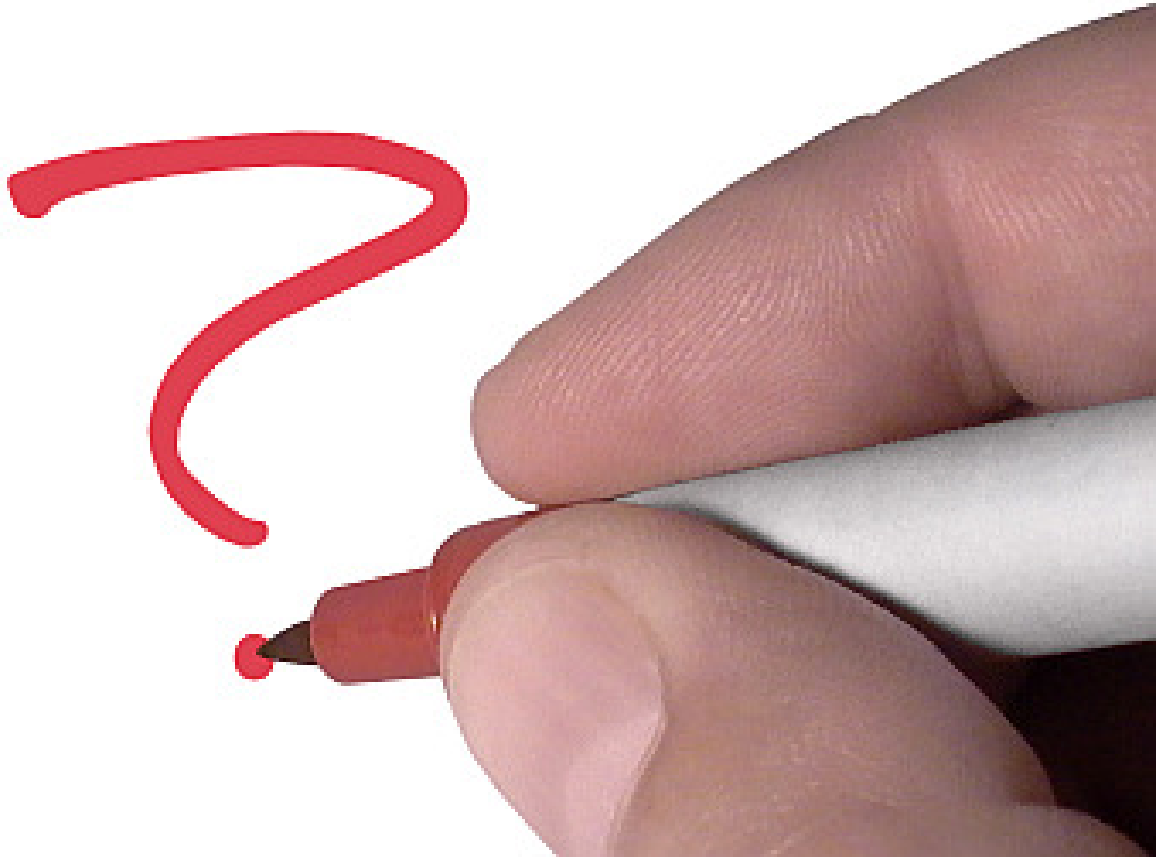


Fertility Preservation in Prepubertal Males

Why ?

How ?

When?



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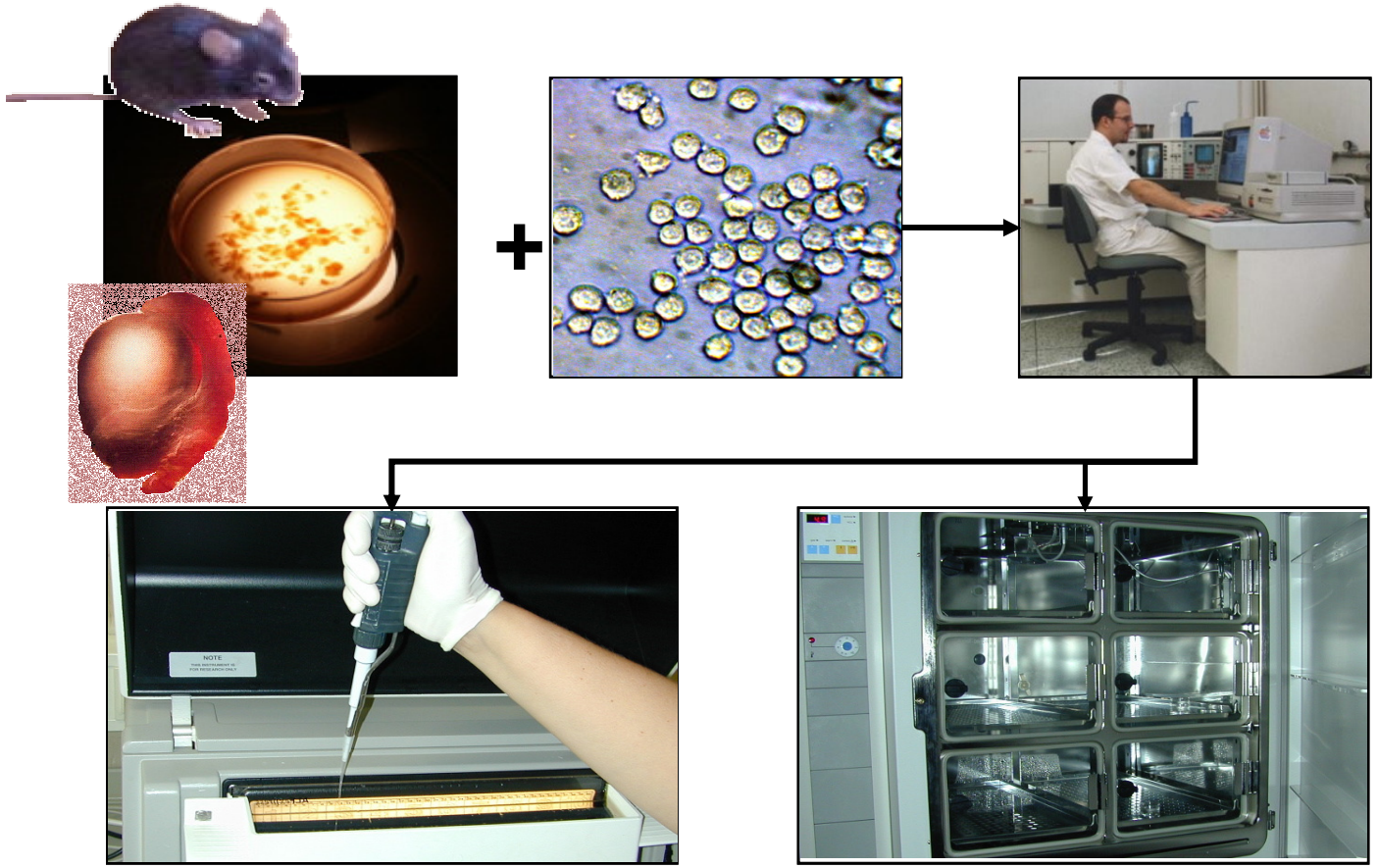
Centrum voor Reproductieve Geneeskunde

Human Reproduction Vol.22, No.3 pp. 733–742, 2007

The efficiency of magnetic-activated cell sorting and fluorescence-activated cell sorting in the decontamination of testicular cell suspensions in cancer patients

M.Geens[1,3], H.Van de Velde[2], G.De Block[1], E.Goossens[1], A.Van Steirteghem[2] and H.Tournaye[2]

[1]Research Centre for Reproduction and Genetics, [2]Centre for Reproductive Medicine, University Hospital and Medical School, Vrije Universiteit Brussel, Brussels, Belgium



bite biology of the testis

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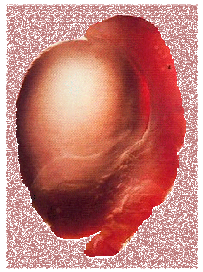

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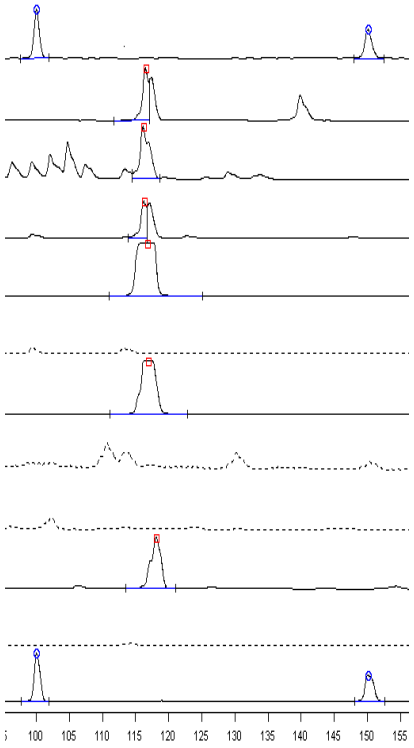

Table IV. Results of the human testicular suspensions, contaminated with 0.05% SB cells

Patient	HLA class I positivity (%) (Corrected for isotype control)		Tumour growth in culture (%)		PCR
	Before sort	After sort	Before sort	After sort	B cell
1b	6.42	0.32	100	50	Positive
2b	9.85	1.54	100	25	Positive
3b	5.64	0.02	75	50	Positive
4b	4.56	0.11	75	25	Positive
5b	8.41	0.00	100	0	Negative
6b	5.31	0.40	100	50	Positive
Average (SD)	6.70 (± 2.03)	0.40 (± 0.58)	91.7 (± 12.9)	33.3 (± 20.4)	

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Human Reproduction Vol.21, No.8 pp. 2057–2060, 2006

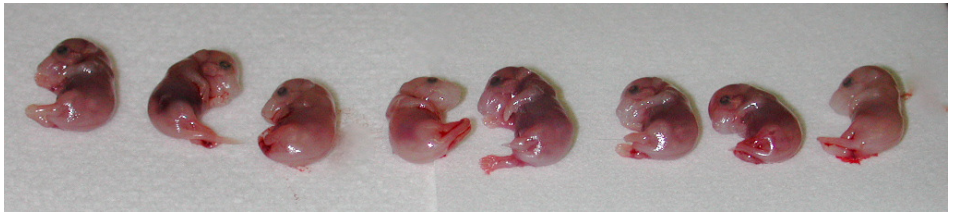
Evaluation of *in vivo* conception after testicular stem cell transplantation in a mouse model shows altered post-implantation development

Ellen Goossens[1], Veerle Frederickx, Gert de Block, André van Steirteghem and Herman Tournaye

Table III. Litter sizes of subsequent generations after testicular stem cell transplantation

Litter size	First generation (average)[a]	Second generation (average)	Third generation (average)
Transplanted mice	1–6 (3.5)[b]	5–11 (8.0)	6–8 (7.2)
Control mice	7–9 (8.3)[b]	5–11 (7.6)	4–8 (5.3)

[a]The first generation of offspring is the offspring derived from the transplanted male with a fertile female.
[b]$P = 0.03$ by Mann–Whitney.



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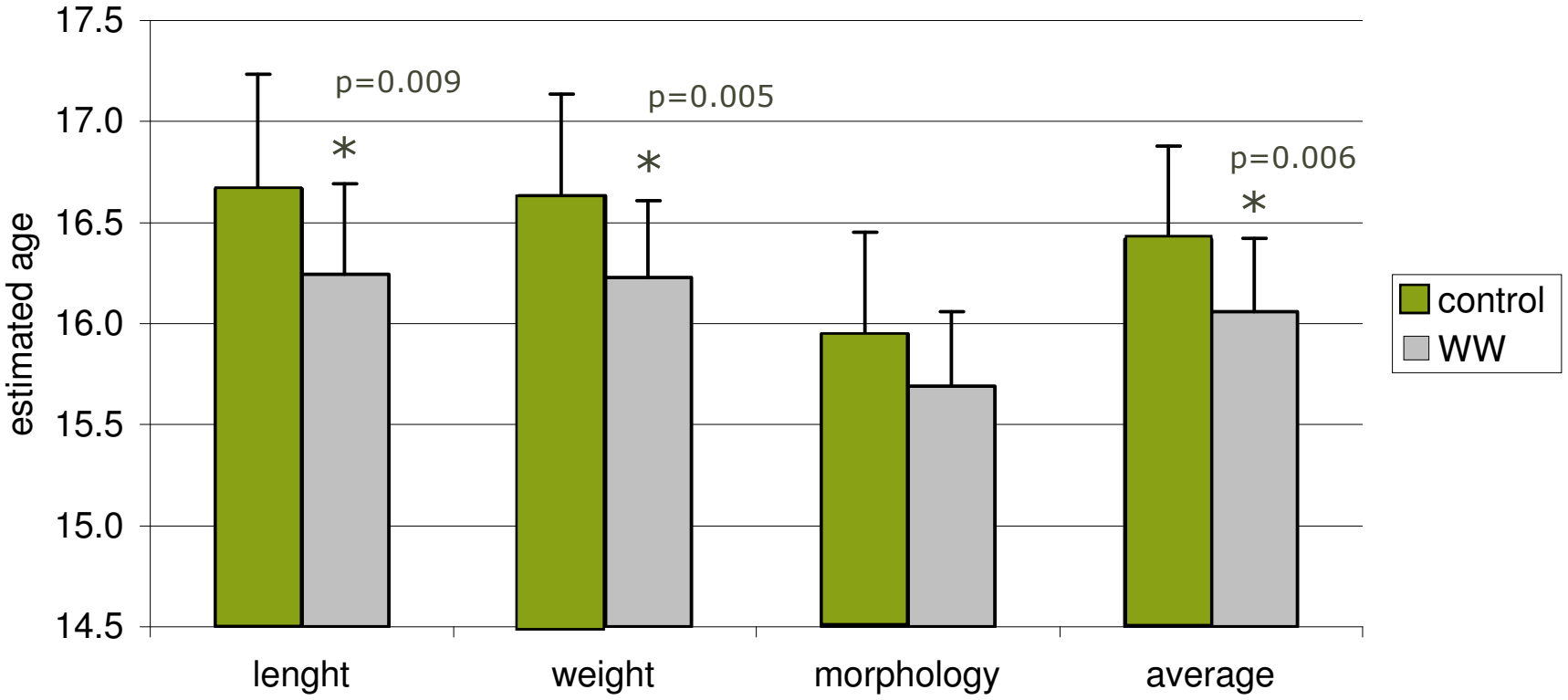

Figure 1. Developmental characteristics on gestational day 17 of fetuses obtained after testicular stem cell transplantation (TSCT). $^{a}P = 0.005$; $^{b}P = 0.0086$; $^{c}P = 0.0055$.

Spermatogonial stem cell transplantation between syngeneic mice

no differences in DNA methylation pattern of

Igf2 (Insuline-like Growth Factor-2 (*Igf2*)
= maternally methylated gene)

*Peg1 (*Paternally Expressed Gene-1)

alpha-Actin (not imprinted gene)

in spermatozoa obtained after SSCT

in liver, kidney and placental tissues of two subsequent generations of offspring obtained after SSCT.

biology of the testis

Goossens et al. Hum Reprod 2009

A Strategy for Fertility Services for Survivors of Childhood Cancer

Author Multidisciplinary Working Group—British Fertility Society

Over the last 20 years there has been a very significant improvement in the outcome of treatments for children with cancer. Unfortunately, one of the side effects is either severe compromise or total destruction of fertility potential. For those of us practising in the reproductive endocrinology environment, this has become a very real problem. To that end, the British Fertility Society convened a multidisciplinary working group, which produced the above document under Ian Cooke's guidance. This is a tour de force. However, it is not really aimed at the general gynaecologist but at the organisations and units responsible for delivering such treatments and also as an aid to the Government in designing its strategies, nationally and regionally, for the provision of such services.

Fertilisation and Embryology Act must change to keep pace with the changing requirements of service provision. It also highlights the necessity of cancer networks involved in the management and coordination of care for these children to include fertility experts in their network.

The document has sections on counselling and sensibly suggests a combination approach, using those experienced in counselling children with childhood cancers and those experienced in counselling in the ART unit environment. Having said this, I am not convinced that the document deals adequately with how one copes with the teenager who requests storage of gametes but whose long-term survival chances are negligible. This is a

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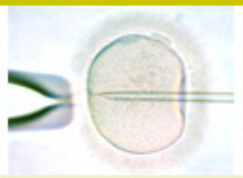


Welcome to the Centre for Reprod

September 2008
25 years of IVF at UZ Brussel
At the occasion of the 25th anniversary of its fertility clinic UZ Brussel presents a captivating scientific programme on Friday, September 12 and Saturday, September 13.

February 2008
CRG intesifies sperm donor campaign
The number of women that can be inseminated with sperm from a single donor has been limited to 6

December 2007
There is more in you!
In 2007 the CRG initiated an awareness campaign to sensibilize young women about egg cell donation.

October 2007
Open-Door Day for Companies at the CRG
On Sunday 7 October, the University Hospital of Brussels (UZ Brussels) opened its doors to the interested general public.

October 2007
Prof. Dr. Paul Devroey receives IVI award 2007
Prof. Devroey has received the international award of the IVI Foundation for the number of scientific articles which



WHO WE ARE?
The Centrum voor Reproductieve Geneeskunde (CRG) belongs to UZ Brussel, the universitary hospital of Brussels.
- Introduction CRG
- Long list of telephone numbers for all departments
- Concise list
- Faces and names of all staff members.

WHAT WE DO?
The CRG is specialized in human fertility problems and in sethe different techniques, investigations and treatments of reproductive medicine.
- Theory
- Investigations and treatments - for man and woman
- Medically assisted pregnancy
- IVF|ICSI in detail
- IVF|ICSI for overseas patients
- Artificial insemination
- Prenatal examinations
- Storage of cells and tissue
- Donation programs

WHERE TO FIND US?
General maps and route descriptions.
- How to get to the CRG
- On the UZ Brussel campus
- A look inside
- Getting around: the specific trajectory that you cover onsite for specific treatments

practical issues:
- Investigations and treatments: how and where?
- Prices of treatments for (foreign) patients
- Anaesthetics procedure
- Treatment at the CRG: practical information
- All relevant information for foreign patients collected from the website
- Forms, contracts & treatment schedules in PDF format
- The brochure 'A guide to your treatment' in PDF format

the CRG:
- Scientific network
- Publications
- Scientific meetings
- History of the CRG
- Press clippings
- Training
- > 1-to-1 seminars

non-profit CRG

QUESTIONNAIRE
When coming to the CRG for a treatment for the first time, you will be asked to fill in a questionnaire regarding your medical history and family status. You can download the questionnaire here, print it and fill it out in advance: questionnaire woman, questionnaire man. You will need Adobe Acrobat Reader, which can be downloaded from this address: http://www.adobe.com/products/acrobat/readstep2.html.

www.brusselsivf.be

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Centrum
Reproductieve Geneeskunde | Laarbeeklaan 101 – 1090 Brussel | Tel. +32 (0)2 477 66 99 – Fax +32 (0)2 477 66 49 | www.brusselsivf.be

PERMISSION TO BANK TESTICULAR TISSUE AND CONDUCT RESEARCH WITH IT

CON-PREPUB-E

The Universitair Ziekenhuis Brussel,
represented by Professor Dr. P. Devroey,
clinical and scientific head of department
of the Centrum voor Reproductieve Geneeskunde,
hereinafter referred to as UZ Brussel,

and Ms. ► .....
Mr. ► .....
living at ► .....
.....
.....

are agreed on the following.

Fertility after treatment for cancer

Questions remain over ways of preserving ovarian and testicular tissue

11 men have had testicular tissue harvested and cryopreserved as a single cell suspension (J A Radford et al, British Cancer Research meeting, Edinburgh, July 1999, and PF Brook et al, unpublished), and five who have now successfully completed treatment for cancer have had this material injected back into the donor testis. Results of follow up semen analysis are awaited with interest.

JA Radford *senior lecturer in medical oncology*
SM Shalet *professor of endocrinology*
Christie Hospital, Manchester M20 4BX

BA Lieberman *consultant gynaecologist*
St Mary's Hospital, Manchester M13 0JH

Last Updated: Tuesday, 1 July, 2003, 09:03 GMT 10:03 UK

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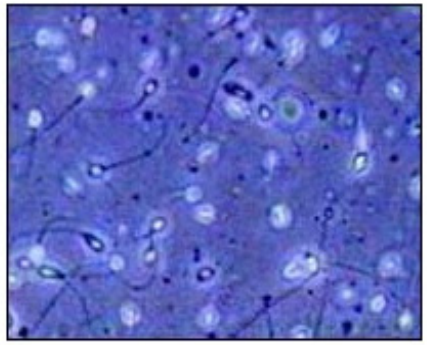
Testicle transplant makes sperm

By Martin Hutchinson
BBC News Online health staff in Madrid



Men facing cancer treatment may not have to rely on a limited supply of frozen sperm to have children, as doctors hail the success of putting testicle tissue in storage instead.

The new technique preserves the "germ cells" which make sperm, which are frozen and then transplanted back into the man when he is given the all-clear from the disease.

Remarkably, the frozen cells then "re-colonise" the testicle, and start producing enough sperm to allow fertility doctors to extract it from semen.

The Greek scientist behind the advance has already managed to grow these germ cells within the testicle of a rat, and says that storing testicle tissue instead of sperm will be a much better idea for would-be fathers.

bite biology of the testis

Herman Tournaye, MD PhD
Ellen Goossens, PhD
Mieke Geens, MSc
Dorien Van Saen, MSc
Ning Liang, MD
Pierre Hilven
Fabian Van Haelst

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Research Council VUB
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