

# Clinical aspects of sperm and testicular tissue cryopreservation



ESHRE Campus  
Athens, Greece  
25-26 September 2009

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Centre for Reproductive Medicine Brussels



Universitair Ziekenhuis Brussel



Vrije Universiteit Brussel



Centrum voor  
Reproductieve Geneeskunde



biology  
of the  
testis

# Outline of the presentation

- banking spermatozoa
- troubleshooting problem patients
- banking testicular tissue
- what will the future bring?

# Cryopreservation of ejaculated spermatozoa

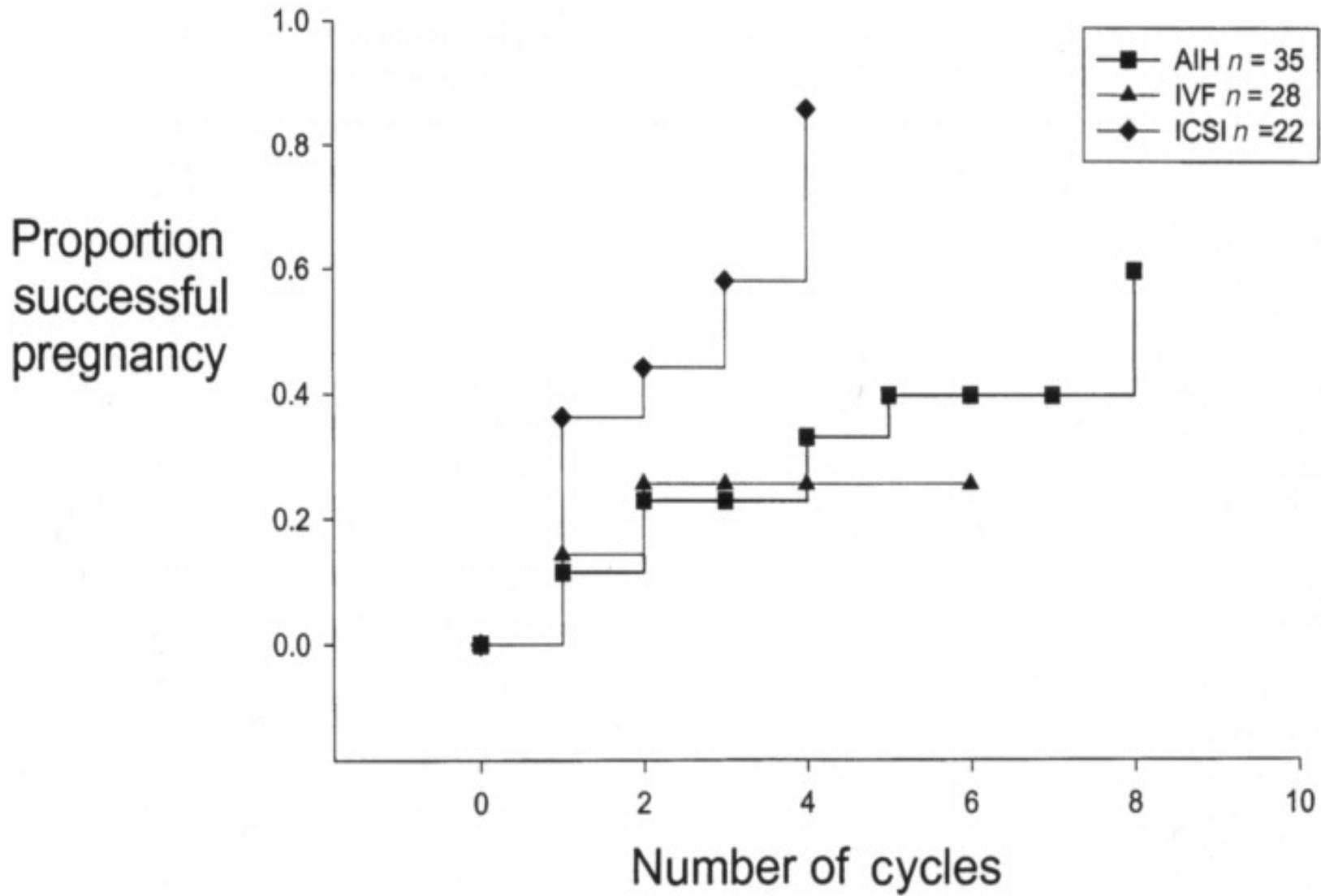
## Well-established and accepted strategy

- to quarantine donor semen
- to ensure availability of sperm for ART (cryptozoospermia, business men, ....)
- to preserve fertility potential before starting gonadotoxic treatments

# Fertility-friendly chemotherapy trap

- no chemotherapy guarantees 100% fertility
- oligozoospermia  $\neq$  fertility
- aneuploidy and mutagenesis
- you know which treatment your patient starts,  
you never know his final treatment course

# Time to pregnancy



Κε

# Banking after starting chemotherapy



Risks:            increased aneuploidy  
                      increased DNA damage  
                      mutagenesis

But:                children conceived during  
                      chemotherapy do not show an  
                      increase in congenital malformations

                      neither is there an increase in  
                      pregnancy wastage

                      role PGD-AS ?

# Nothing to bank?

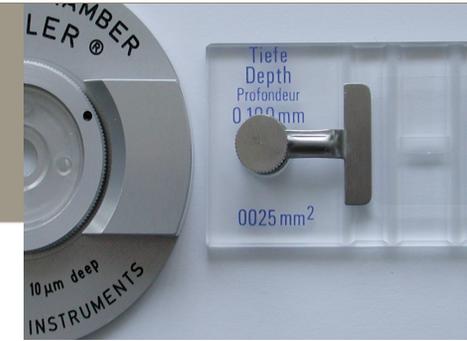


Azoospermia at time of diagnosis  
recent data for various cancers

Variable prevalence: 3 - 100 %

- |                      |               |      |
|----------------------|---------------|------|
| •Lass et al. 1998    | 40 out of 231 | 17%  |
| •Kelleher et al.2001 | 31 out of 930 | 3.3% |

# What if azoospermia at time of diagnosis?



If extended preparation fails to show sperm

- vas aspiration or MESA at orchiectomy

*Baniel and Sella F&S 2001*

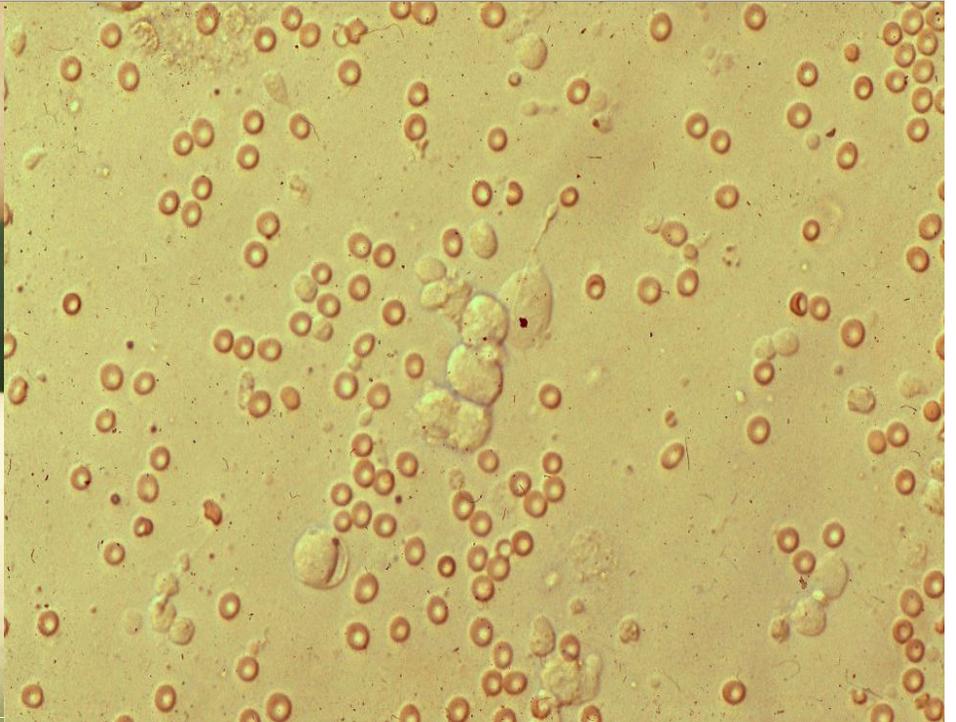
- Testicular sperm extraction (TESE)

*Rosenlund et al. HR 1998*

*Res et al. HR 2000*

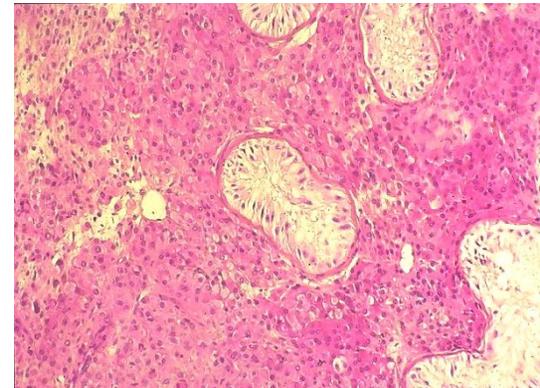
*Kohn et al. HR 2001*

*Schrader et al., Urology 2003*



# Should we cryopreserve testicular tissue in NOA patients? Cumulative retrieval rates after TESE

Vernaeve et al. (2006) Hum. Reprod.



# Should diagnostic testicular sperm retrieval followed by cryopreservation for later ICSI be the procedure of choice for all patients with non-obstructive azoospermia?

G.Verheyen<sup>1</sup>, V.Vernaeve, L.Van Landuyt, H.Tournaye, P.Devroey and A.Van Steirteghem

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**BACKGROUND:** This was a retrospective study to determine if diagnostic testicular biopsy followed by cryopreservation should be the procedure of choice for all patients with testicular failure. **METHODS:** The first part of the study analysed 97 ICSI cycles scheduled with frozen–thawed testicular sperm for 69 non-obstructive azoospermia (NOA) patients. The second part focused on a subgroup of 32 patients who underwent 42 ICSI cycles with frozen and 44 cycles with fresh testicular sperm. Sperm characteristics, fertilization, embryo quality, pregnancy and implantation rates were evaluated. **RESULTS:** Part I: The average time needed to find sperm was 113 min per cycle and 17 min per individual sperm. Fertilization rate, embryo transfer rate, ongoing pregnancy and implantation rates were 58.4%, 83%, 20.8% and 11.3%, respectively. Part II: The search time per sperm was higher ( $P = 0.016$ ) in frozen (18 min) than in fresh suspensions (13 min). A higher embryo transfer rate was observed in fresh cycles than in frozen cycles (93.2% vs 76.2%,  $P = 0.028$ ). Fertilization, ongoing pregnancy and implantation rates were comparable for the two groups. **CONCLUSIONS:** Even in a programme with low-restrictive criteria for patient allocation and for sperm cryopreservation, diagnostic testicular biopsy followed by cryopreservation can be the procedure of choice for patients with testicular failure.

# Should we cryopreserve testicular tissue in NOA patients?

**Table V.** Comparison of sperm characteristics in the ICSI cycles with fresh (44 cycles) and frozen (42 cycles) testicular sperm of 32 non-obstructive azoospermia (NOA) patients

	Fresh TESE	Frozen TESE	Mann–Whitney
Cycles	44	42	
Search time/cycle (min)	81	110	$P = 0.053$
Search time/sperm (min)	13	18	$P = 0.016$
% oocytes injected with motile sperm	82.3	83.7	NS
Cycles injected with only motile sperm (%)	33/44 (75)	31/42 (74)	NS <sup>a</sup>
Cycles injected with only immotile sperm (%)	3/44 (7)	4/42 (10)	NS <sup>a</sup>
COC/cycle	10.5 ± 6.2	9.3 ± 5.2	NS
Metaphase II/cycle	9.1 ± 5.8	7.6 ± 4.2	NS
% 2PN	58.0 ± 24.2	59.3 ± 25.5	NS
% 1PN	7.0 ± 11.0	7.8 ± 19.2	NS
% ≥ 3PN	3.6 ± 8.3	1.9 ± 4.9	NS

<sup>a</sup>Chi-square test.

Verheyen et al. 2004 Hum Reprod

# Should we cryopreserve testicular tissue in NOA patients?

**Table VI.** Results of embryo transfer, pregnancy and implantation rates after ICSI with fresh (44 cycles) and frozen (42 cycles) testicular sperm of 32 non-obstructive azoospermia (NOA) patients

	Fresh TESE	Frozen TESE	Chi-square
Cycles	44	42	
Transfers (%)	41 (93.2)	32 (76.2)	$P = 0.028$
Embryos/ET	2.6	2.5	NS
Pos hCG/cycle (%)	9/44 (20.4)	8/42 (19.0)	NS
Pos hCG/ET (%)	9/41 (21.9)	8/32 (25.0)	NS
Clinical PR/cycle (%)	7/44 (15.9)	6/42 (14.3)	NS
Clinical PR/ET (%)	7/41 (17.1)	6/32 (18.7)	NS
Implantation rate (%)	8/105 (7.6)	6/81 (7.4)	NS

ET, embryo transfer; Pos, positive; PR, pregnancy rate.

Verheyen et al. 2004 Hum Reprod

# Severe Hypospermatogenesis in Cases of Nonobstructive Azoospermia: Should We Use Fresh or Frozen Testicular Spermatozoa?

RON HAUSER,\* LEAH YOGEV,\* AMI AMIT,† HAIM YAVETZ,\* AMNON BOTCHAN,\* FUAD AZEM,\* JOSEPH B. LESSING,\* AND DALIT BEN-YOSEF†

*From the \*Institute for the Study of Fertility and the †IVF Unit, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Israel.*

**ABSTRACT:** The aim of this comparative clinical study was to examine whether the fertilizing potential of frozen-thawed testicular sperm in the most severe cases of hypospermatogenesis is reduced compared with fresh testicular sperm. The results could determine the necessity of using fresh testicular sperm cells, which mandates involving the spouse by performing simultaneous in vitro fertilization intracytoplasmic sperm injection (IVF-ICSI) treatment in this subgroup of nonobstructive azoospermia (NOA) patients. We studied 13 couples in which the husband was diagnosed as having NOA and few motile testicular sperm cells or only immotile testicular sperm cells were isolated by testicular sperm extraction (TESE). Each couple underwent both an ICSI cycle, in which fresh testicular sperm that were retrieved shortly beforehand were injected, and a consecutive cycle, which used frozen-thawed sperm that were retrieved in the original TESE procedure but were cryopreserved and stored until use. We found that motility was lost during the freezing and thawing

process in some cases, which resulted in significantly more cycles with only immotile sperm cells for injection in the frozen-thawed sperm group (38.5%) than in the fresh sperm group (7.7%;  $P < .05$ ). Availability of only immotile sperm cells significantly reduced fertilization rates in both fresh and frozen-thawed groups, but the respective overall fertilization rate (44.9% vs 41.1%) and quality of embryos and pregnancy rate (18.2% vs 15.4%) were not significantly different between groups. Implantation rates were more favorable in the fresh sperm group (10.5% vs 5.9%), but not significantly so. We conclude that, although cryopreservation does impair motility, which results in significantly more cycles with only immotile sperm cells for ICSI in the most severe forms of hypospermatogenesis, fertilization and pregnancy rates are not significantly compromised.

Key words: Testicular sperm extraction, fertilization, implantation, IVF-ICSI.

**J Androl 2005;26:772–778**

# Should we cryopreserve testicular tissue in NOA patients?

Yes, we should

- similar outcome after ICSI
- but...works for 4 out of 5 patients
- counsel your patient towards back-up TESE

Verheyen et al. 2004 Hum Reprod  
Hauser et al. 2005 Androl.

## “ONCO-TESE”: TESTICULAR SPERM EXTRACTION IN AZOOSPERMIC CANCER PATIENTS BEFORE CHEMOTHERAPY—NEW GUIDELINES?

M. SCHRADER, M. MÜLLER, N. SOFIKITIS, B. STRAUB, H. KRAUSE, M. SCHOSTAK, AND K. MILLER

**TABLE I.** *Patients with testicular germ cell tumors and azoospermia before chemotherapy*

Clinical Stage	Patients with Azoospermia (n)	Patients with Successful Sperm Retrieval (n)	Patients with Maturation Arrest (JS 3–5) (n)	Patients with SCOS (JS 1–2) (n)
I	2	2/2	0/2	0/2
IIA–IIB	8	3/8	3/8	2/8
>IIC	4	1/4	0/4	3/4

KEY: JS = Johnsen score; SCOS = Sertoli cell-only syndrome.

Histologic examination results according to clinical tumor stage; classification into clinical tumor stages followed World Health Organization guidelines.

## “ONCO-TESE”: TESTICULAR SPERM EXTRACTION IN AZOOSPERMIC CANCER PATIENTS BEFORE CHEMOTHERAPY—NEW GUIDELINES?

M. SCHRADER, M. MÜLLER, N. SOFIKITIS, B. STRAUB, H. KRAUSE, M. SCHOSTAK, AND K. MILLER

**TABLE III.** *Patients with Hodgkin’s/non-Hodgkin’s disease and azoospermia before chemotherapy*

Disease	Patients with Azoospermia (n)	Patients with Successful Sperm Retrieval (n)	Patients with Maturation Arrest (JS 3–5)	Patients with SCOS (JS 1–2)
Hodgkin’s disease	7	3/7	2/7	2/7
Non-Hodgkin’s disease	10	5/10	3/10	2/10

*KEY: JS = Johnsen score; SCOS = Sertoli cell-only syndrome. Histologic examination results according to the disease type.*

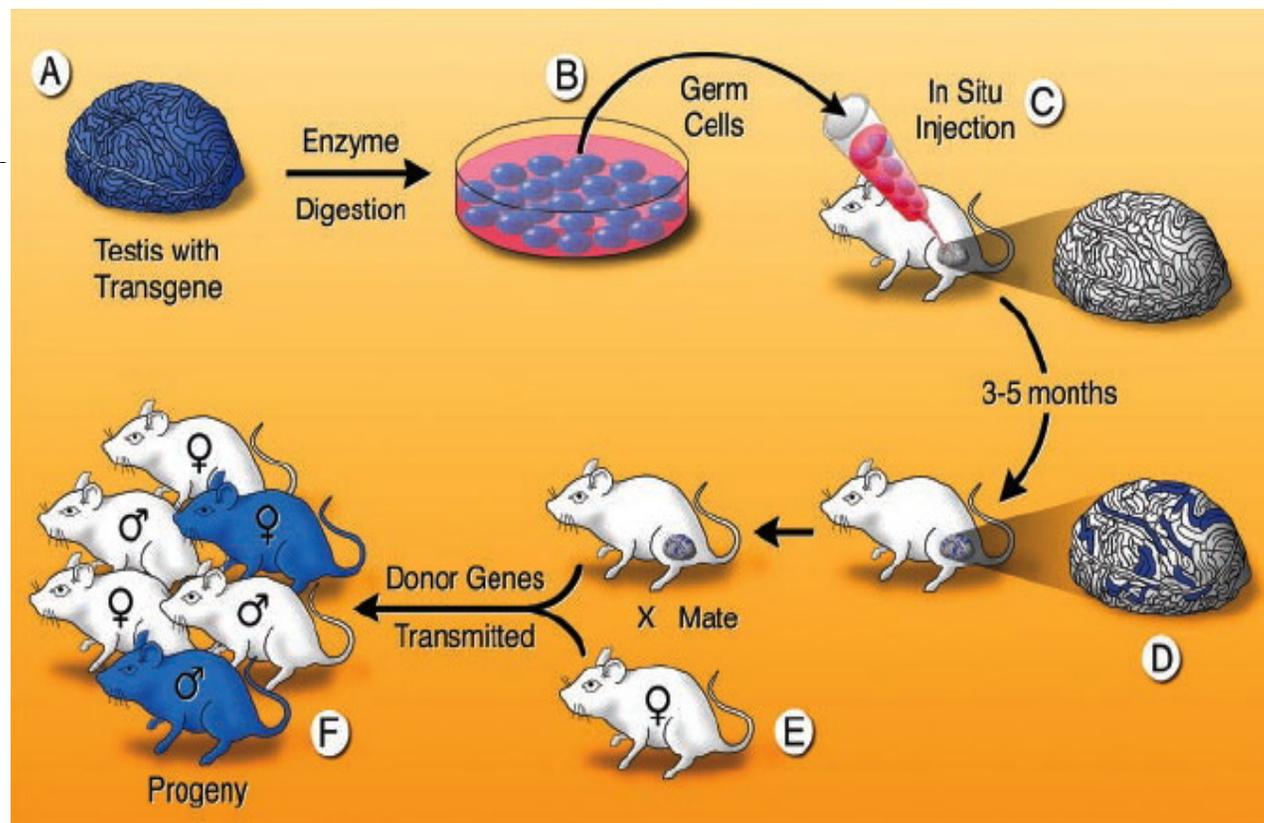
# Spermatogenesis following male germ-cell transplantation

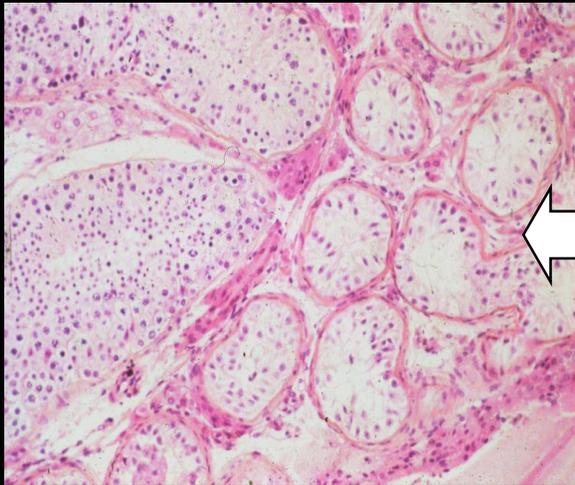
(spermatogonia/stem cells/testes/transgenic mice)

RALPH L. BRINSTER\* AND JAMES W. ZIMMERMANN†

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*Contributed by Ralph L. Brinster, August 11, 1994*





# Cryopreservation of testicular tissue in adults

BMJ VOLUME 319 9 OCTOBER 1999 www.bmj.com

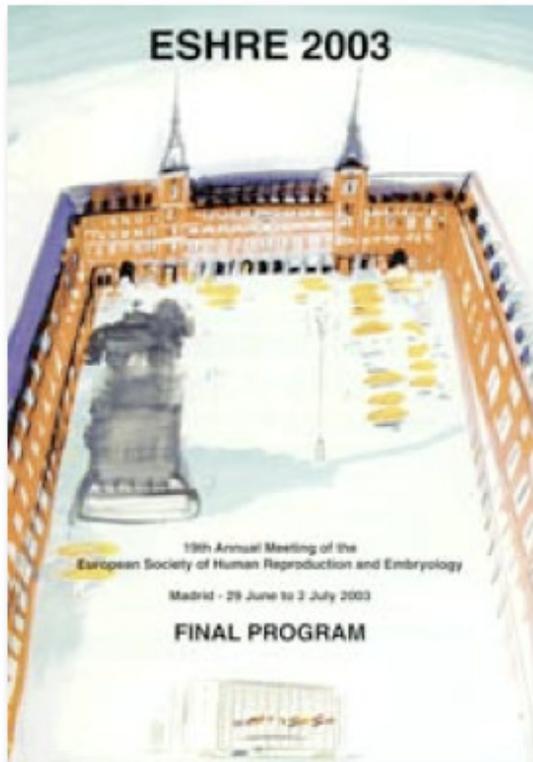
## Fertility after treatment for cancer

*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



 **BBC NEWS** UK EDITION

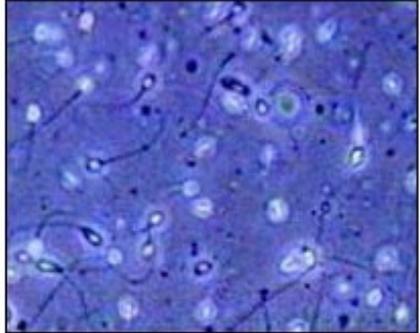
Last Updated: Tuesday, 1 July, 2003, 09:03 GMT 10:03 UK

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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.

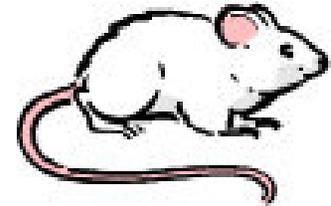
Remarkably, the frozen cells then "re-colonise" the testicle, and start producing enough sperm to allow fertility doctors to extract it from semen.

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.

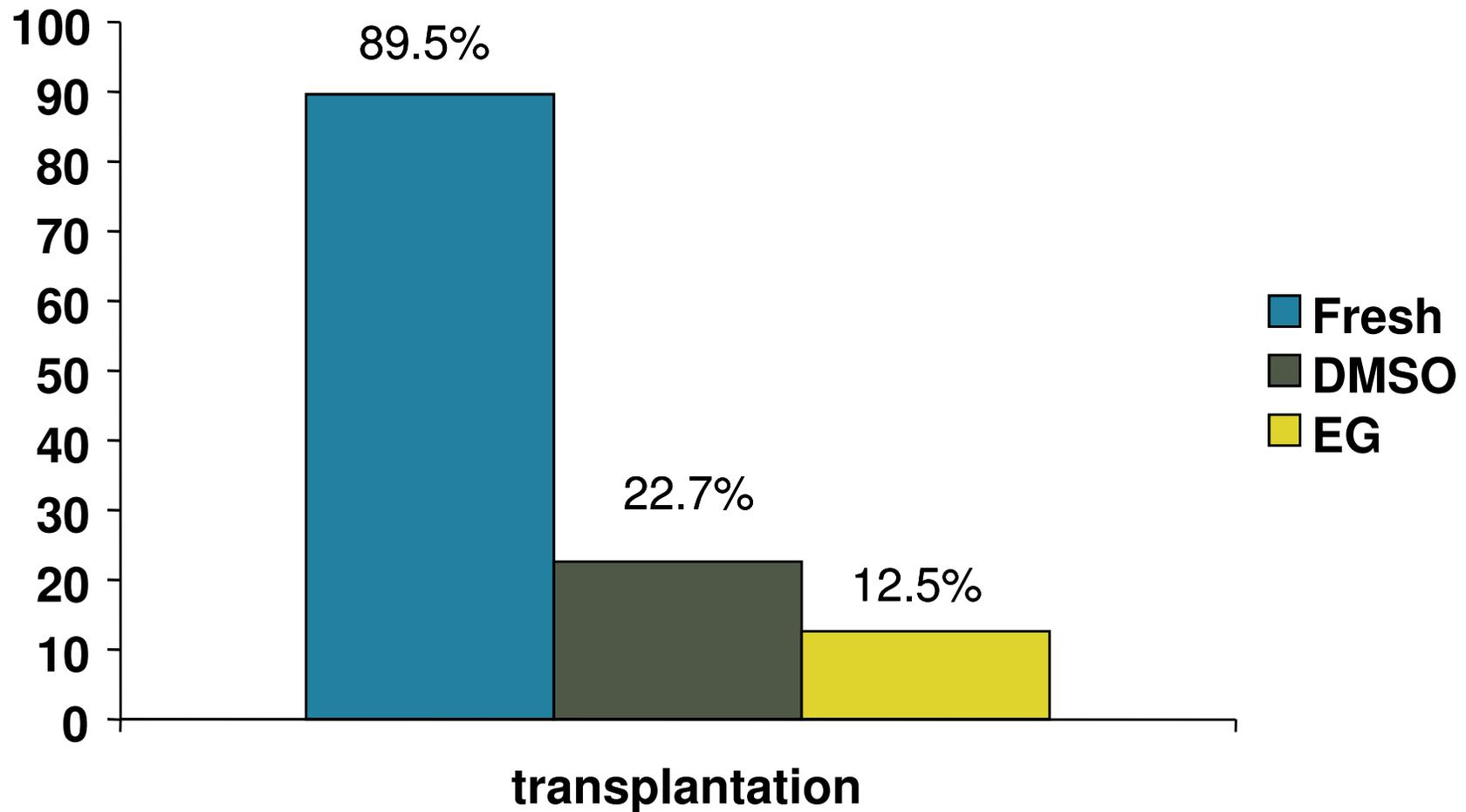
[BBC SPORT](#)  
[BBC WEATHER](#)

# Where do we stand anno 2009 ?

- Technically feasible ?
  - transplantation protocol
  - storage protocol
- Reproductive efficiency ?
- Reproductive safety ?



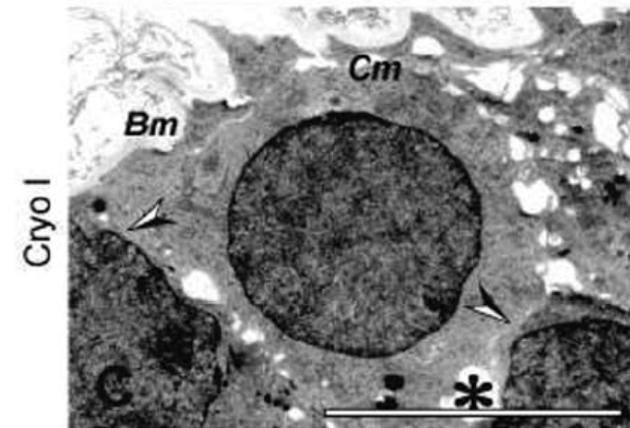
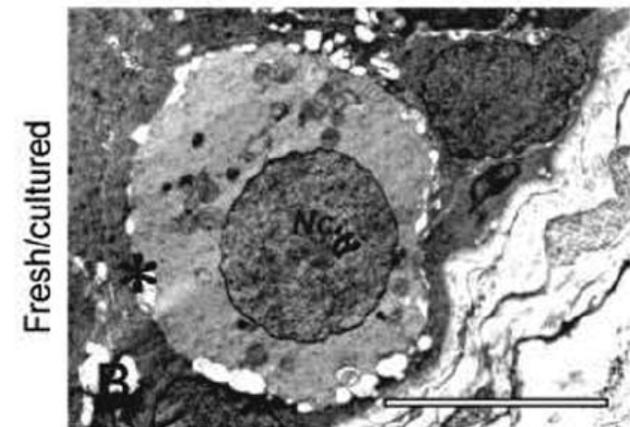
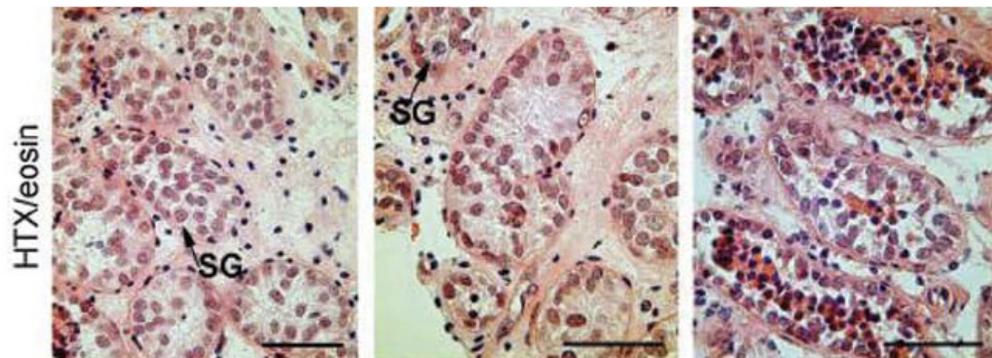
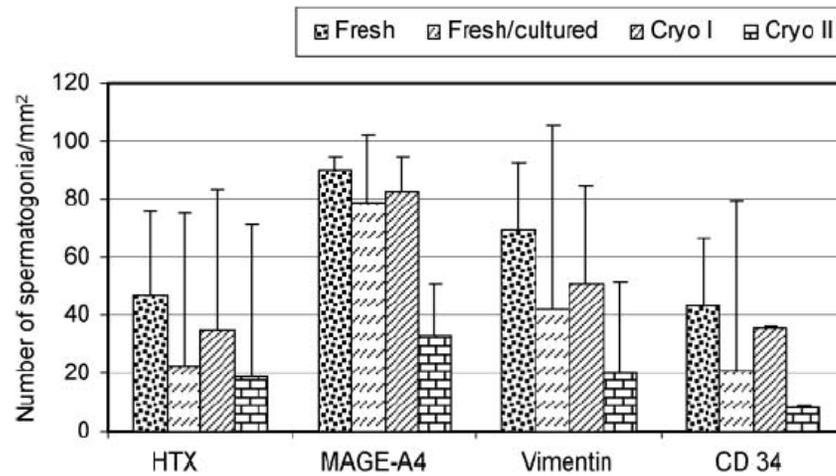
## Transplantation after cryopreservation



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

Victoria Keros<sup>1,5</sup>, Kjell Hultenby<sup>2</sup>, Birgit Borgström<sup>3</sup>, Margareta Fridström<sup>1</sup>, Kirsi Jahnukainen<sup>4</sup> and Outi Hovatta<sup>1</sup>

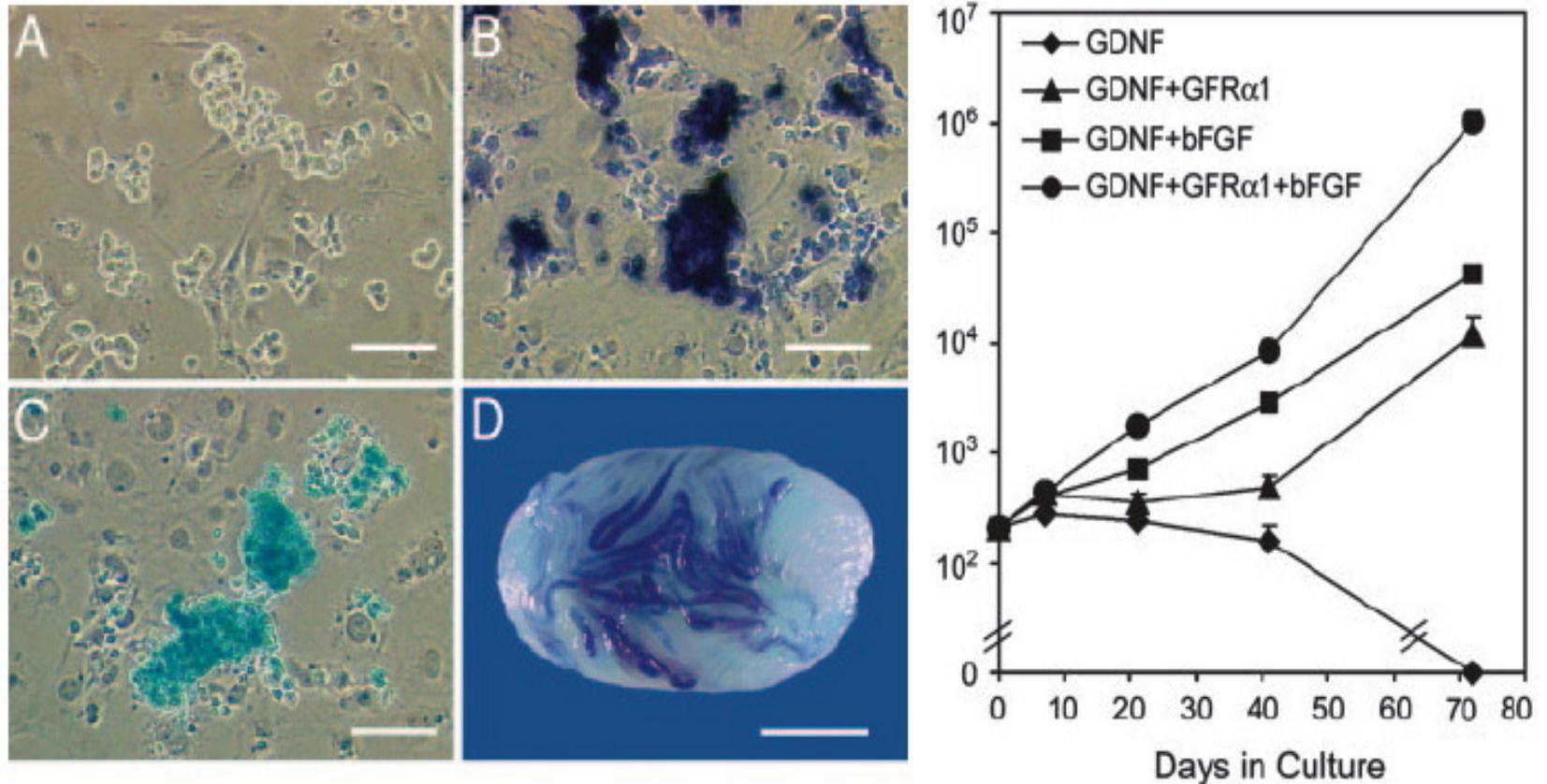
<sup>1</sup>Karolinska Institute, Division of Obstetrics and Gynaecology, Department of Clinical Science, Technology and Intervention, <sup>2</sup>Clinical Research Centre, <sup>3</sup>Department of Paediatrics, Karolinska University Hospital, Huddinge, SE 141 86 Stockholm, Sweden and <sup>4</sup>Paediatric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



# Growth factors essential for self-renewal and expansion of mouse spermatogonial stem cells

Hiroshi Kubota, Mary R. Avarbock, and Ralph L. Brinster\*

Department of Animal Biology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19104



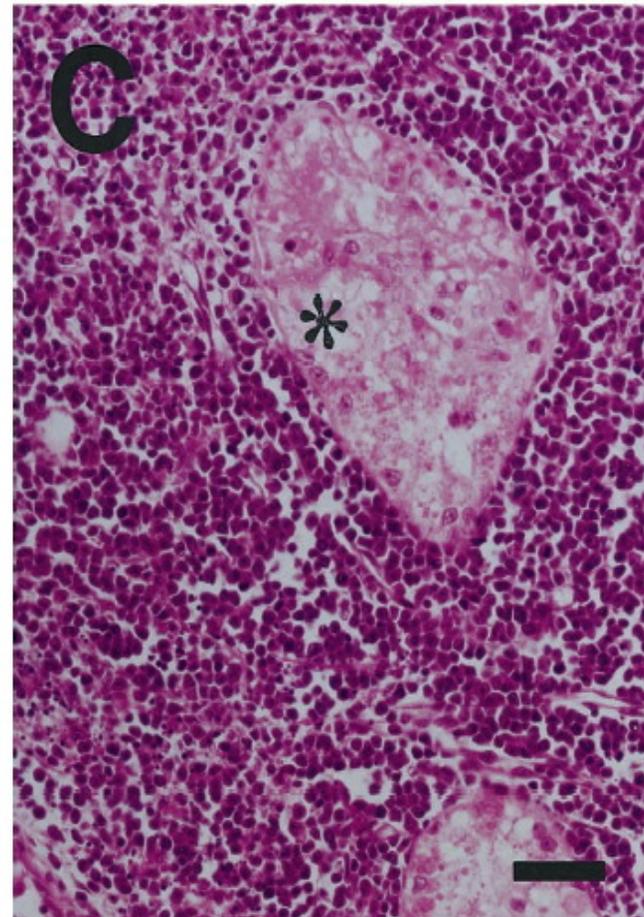
# Intratesticular Transplantation of Testicular Cells from Leukemic Rats Causes Transmission of Leukemia<sup>1</sup>

Kirsi Jahnukainen,<sup>2</sup> 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

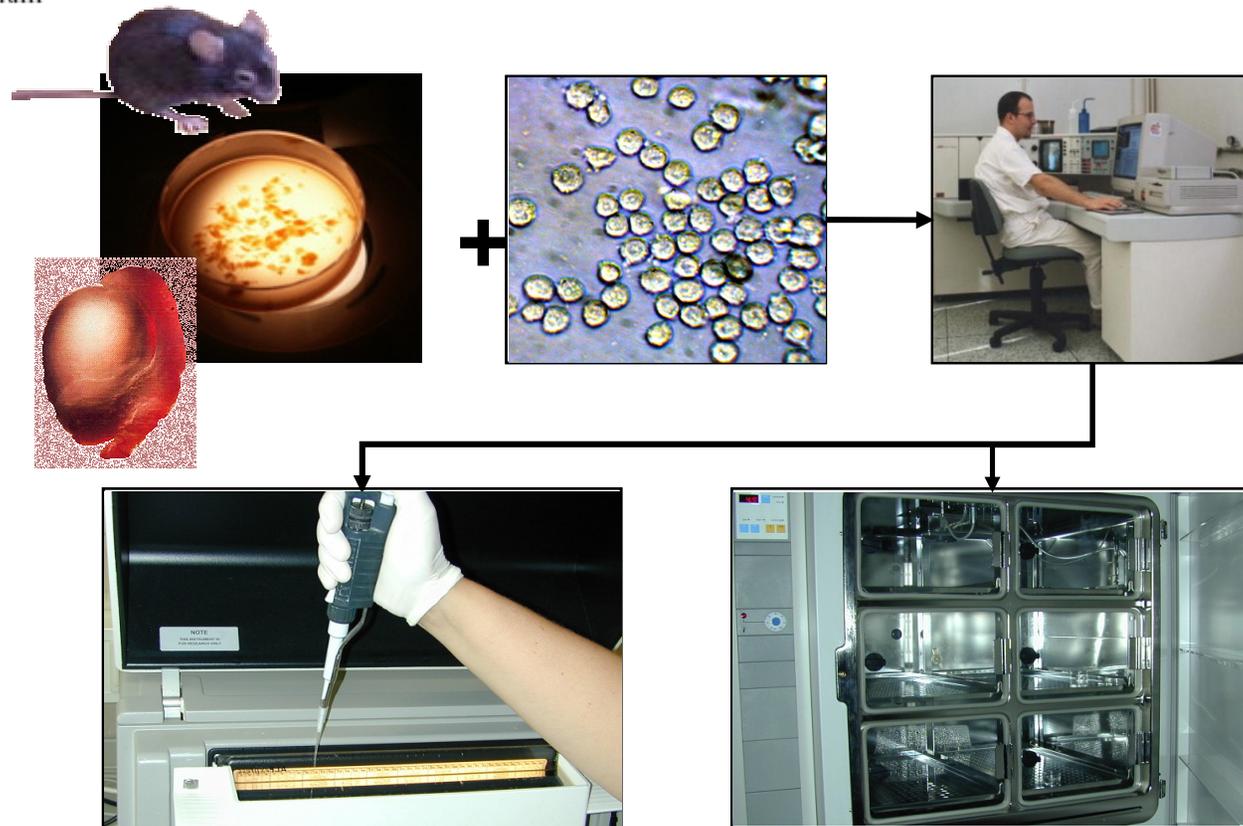
A rat T-cell leukemia model was used to study the safety of germ cell transplantation as a mean of preventing infertility in males undergoing gonadotoxic cancer treatment. Donor germ cells were harvested from the testes of terminally ill leukemic rats and were either used directly or cryopreserved and 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.



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

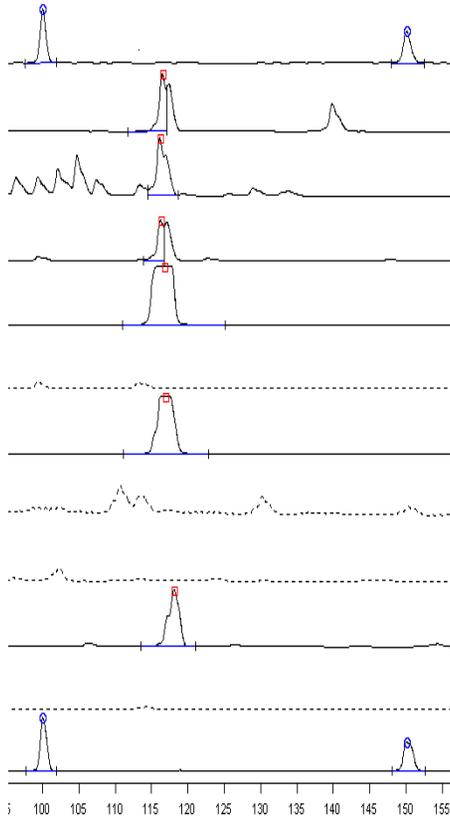
M.Geens<sup>1,3</sup>, H.Van de Velde<sup>2</sup>, G.De Block<sup>1</sup>, E.Goossens<sup>1</sup>, A.Van Steirteghem<sup>2</sup> and H.Tournaye<sup>2</sup>

<sup>1</sup>Research Centre for Reproduction and Genetics, <sup>2</sup>Centre for Reproductive Medicine, University Hospital and Medical School, Vrije Universiteit Brussel, Brussels, Belgium





# Malignant decontamination by FACS (HLA1-)



0,05% SB	HLA-type 1 positivity (%)		Tumorgrowth in culture (%)		PCR	
	patient	pre sort	post sort	pre sort		post sort
	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	<b>0.00</b>	100	<b>0</b>	<b>negative</b>
	6b	5.31	0.40	100	50	positive
<b>average</b>		<b>6.698</b>	<b>0.398</b>	<b>91.7</b>	<b>33.3</b>	

## Ethics of testicular stem cell medicine

**G.Bahadur**

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**The ethical issues raised by advances in reproductive technology allowing the transplantation of testicular stem cells to enable infertile men and cancer patients, including the pre-pubertal, to have children, and to provide new contraceptive prospects for fertile men are discussed. Consideration of respect for the patient's autonomy, the need for informed consent and the health of any offspring resulting from such a procedure are included. Topics covered include: the problems raised by cases needing consent for the transplantation of testicular stem cells from pre-pubertal and adolescent patients; the legal status of stem cells; the arguments for treating such tissue as property which might serve as a means of guaranteeing respect for patients' rights in disputed cases; aspects of patents and the ethics of allowing commercial traffic of such material; questions relating to health and safety, as well as xenotransplantation technology in humans; and posthumous procurement use of germ cells from minors.**



## 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

# Clinical application Brussels



inquiries: n=24

accepted n=16

- sickle cell anemia (8)
- thalassemia major (1)
- leukemia (3)
- idiopathic aplastic anemia (1)
- granulomatosis (1)

refused n=1

- leukemia (pretreated)

parents declined after counselling n=7

- n=4 leukemia
- n=2 sickle cell anemia
- n=1 idiopathic aplastic anemia



Centrum voor  
Reproductieve  
Geneeskunde



Who we are



What we do



Where to find us



--> Search

News

**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 intensifies 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 sensitize 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

## Welcome to the Centre for Reproductive Medicine



### WHO WE ARE?

The Centrum voor Reproductieve Geneeskunde (CRG) belongs to UZ Brussel, the university 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



### 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>.

Practical Information

Influence

Administrative information

Treatments | Operations Practical

Practical Information regarding Treatment

Procedures for Anaesthetics

Overseas Patients

Financial Information

Documents

Printed Guide

Treatment Schedules

Glossary

Information

Contracts

Instructions

Information for professionals

More information about 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

About the scientific role of the CRG:

Scientific network  
Publications

Scientific meetings  
History of the CRG

Press clippings

Training

> 1-to-1 seminars



non-profit CRG

www.brusselsivf.be



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# PERMISSION TO BANK TESTICULAR TISSUE AND CONDUCT RESEARCH WITH IT

CON-PREUB-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,

are agreed on the following.

and Ms. > .....

Mr. > .....

living at > .....

.....

.....



Universitair Ziekenhuis Brussel



Vrije Universiteit Brussel



Centrum voor  
Reproductieve Geneeskunde

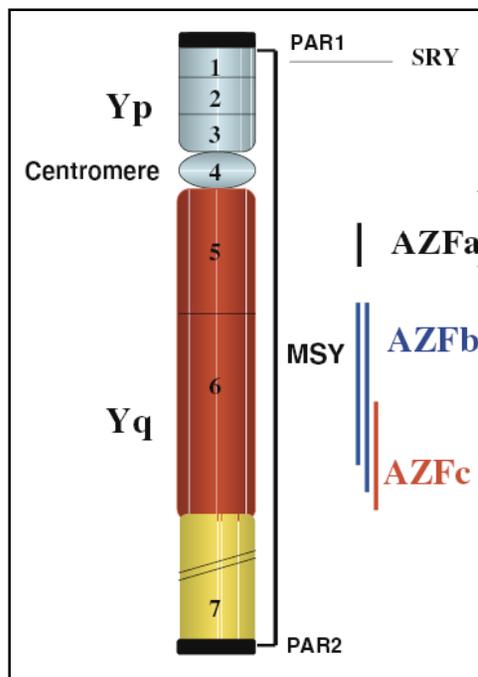
# Testicular tissue banking in adolescents

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

## Sperm cryopreservation in male infertility due to genetic disorders

Csilla Krausz\* and Gianni Forti

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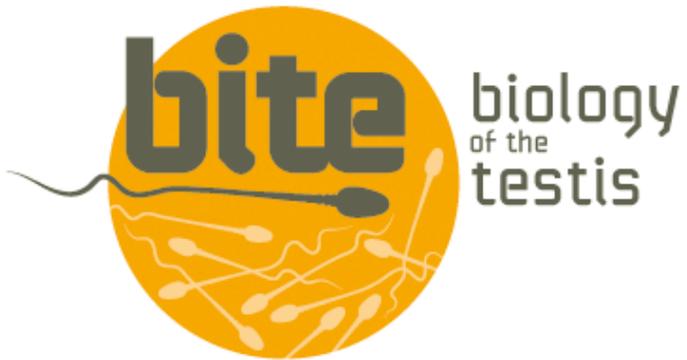


# When should we cryopreserve spermatozoa?

- in ALL men undergoing a potential gonadotoxic treatment
- consider cryopreservation in men undergoing ART prone to anejaculation
- consider cryopreservation in men undergoing vasectomy

# When should we cryopreserve testicular tissue ?

- we should cryopreserve testicular tissue in ICSI candidates with non-obstructive azoospermia
- we may consider cryopreserving testicular tissue in boys and men undergoing sterilising treatments
- we should maybe cryopreserve testicular tissue in pubertal azoospermic Klinefelter's boys and young adults with Yq deletions or Klinefelter's syndrome



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Patricia de Vos, BSc  
Pierre Hilven  
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#### Funding

Research Foundation FWO  
Research Council VUB  
Research Fund Willy Gepts



Universitair Ziekenhuis Brussel



Vrije Universiteit Brussel



Centrum voor  
Reproductieve Geneeskunde