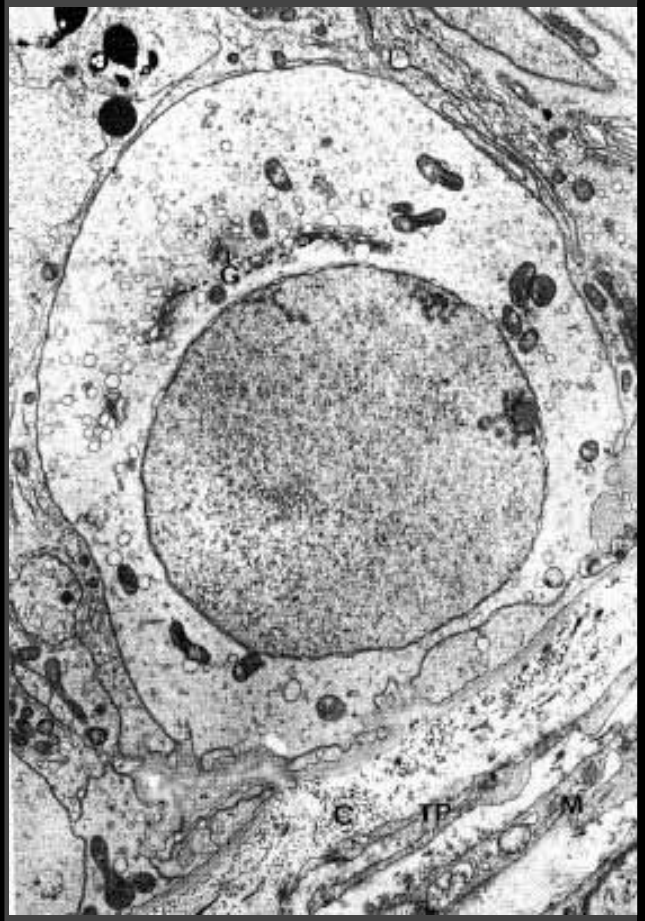
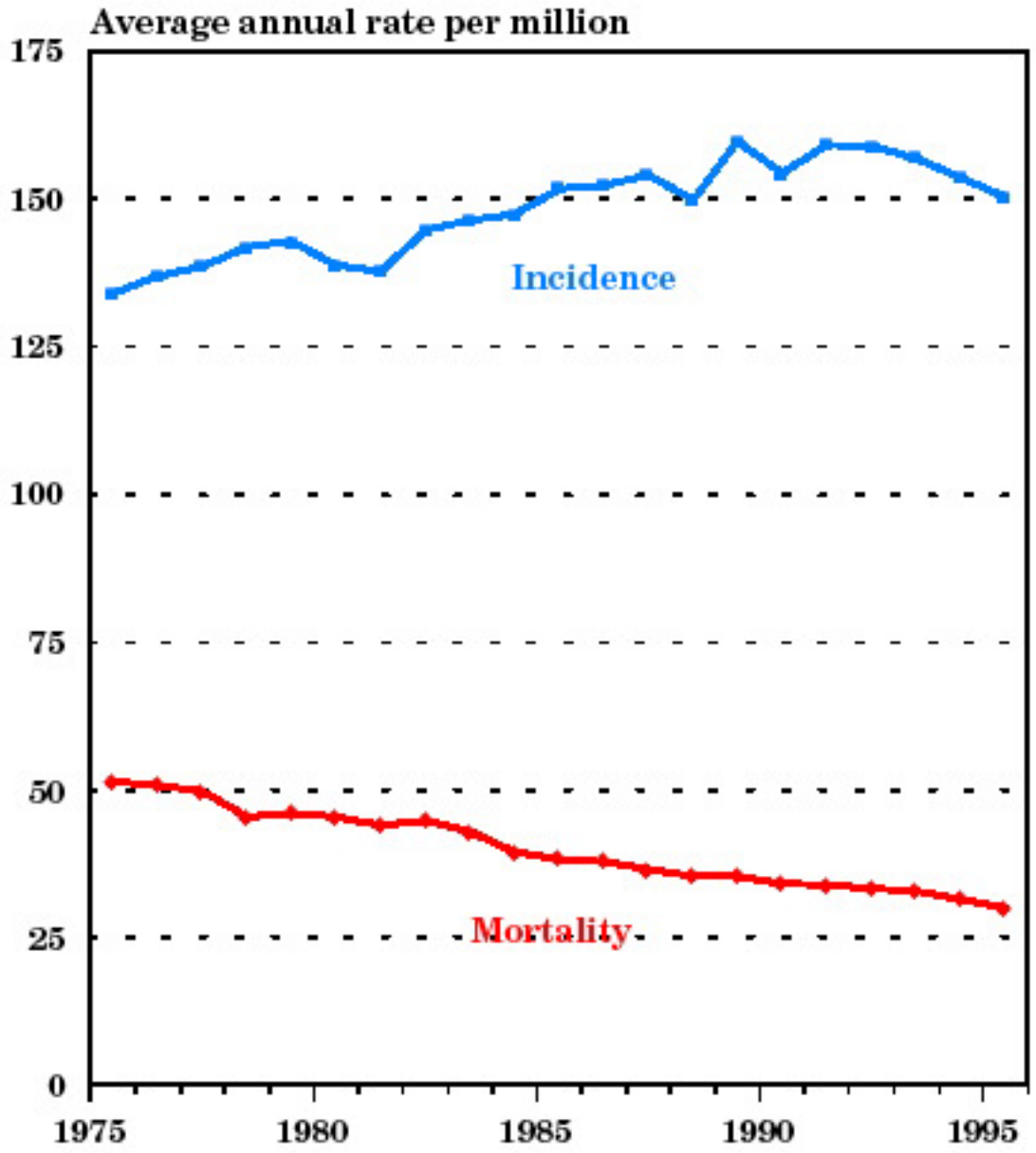


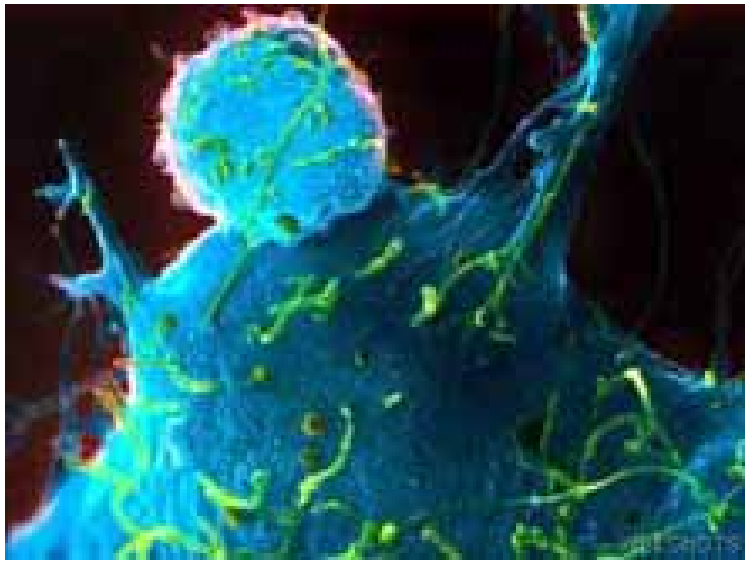
Translating testicular stem cell biology to the clinic

Prof. Dr. Herman Tournaye

Centre for Reproductive Medicine
Dutch-speaking Brussels Free University



*Adjusted to the 1970 US standard population



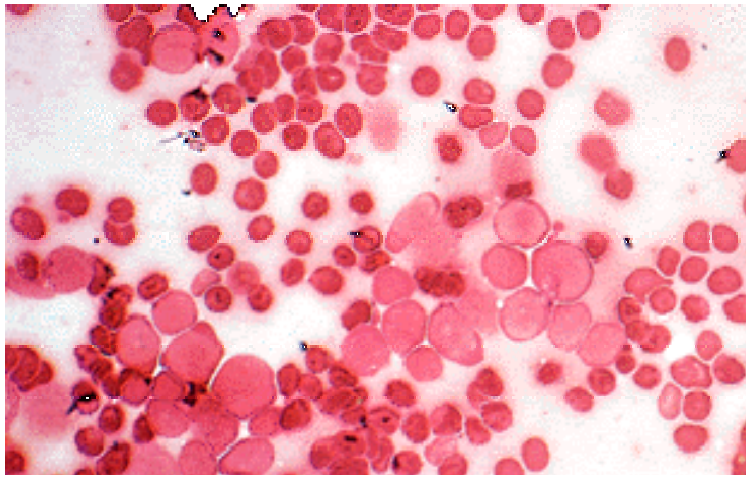


Figure XIV.1: Trends in childhood cancer age-adjusted* rates, all races, both sexes, age <20 SEER incidence & US mortality, 1975-95

Today, Childhood Cancers Are Mostly Treatable

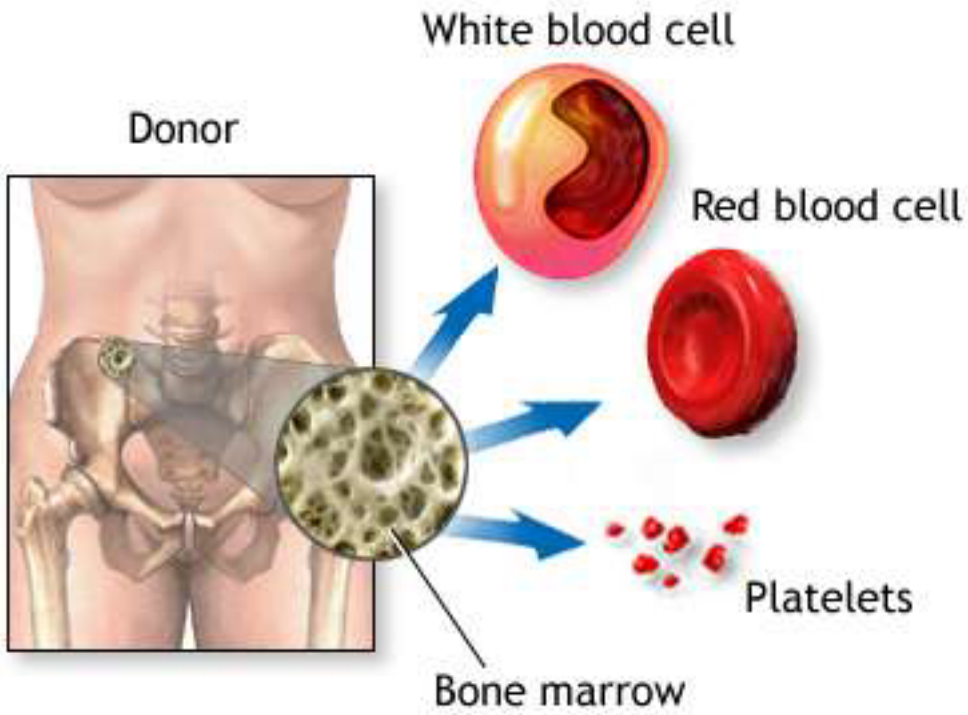
French Study Yields High Survival Rate for Children with Lymphoma, Leukemia

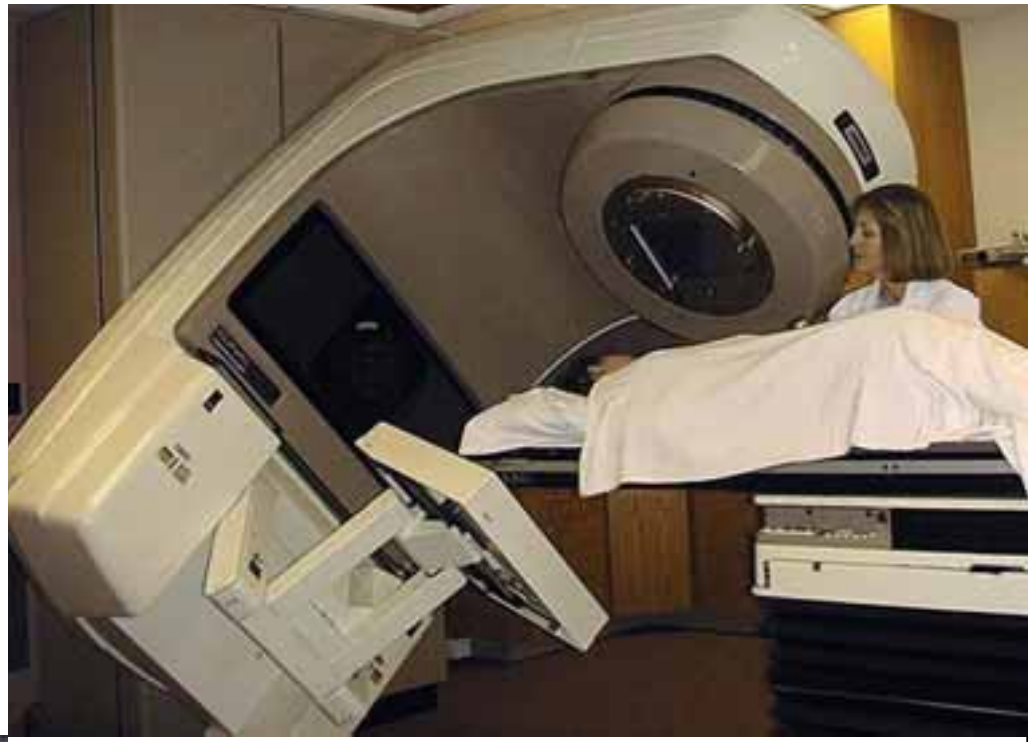
Article date: 2001/07/09

Once looked upon as a death sentence, today many childhood cancers are quite treatable, thanks to the use of effective combinations of anti-cancer drugs. A new French study in the journal *Blood* (Vol. 97, No. 11: 3370-3379) illustrates just that for patients with B-cell lymphoma and acute lymphoblastic leukemia (ALL).

Physicians were able to achieve complete remission in 97% of patients in the study — a remarkable but not really unexpected result, say experts.

According to Christine Patte, MD, pediatric oncologist at the Institut Gustave Roussy in Villejuif, France, the current study was designed based on the findings of three previous trials.


White blood cell
Donor
Red blood cell
Platelets
Bone marrow



Childhood cancer: the challenge

- childhoodcancer survivors aged 20-30:

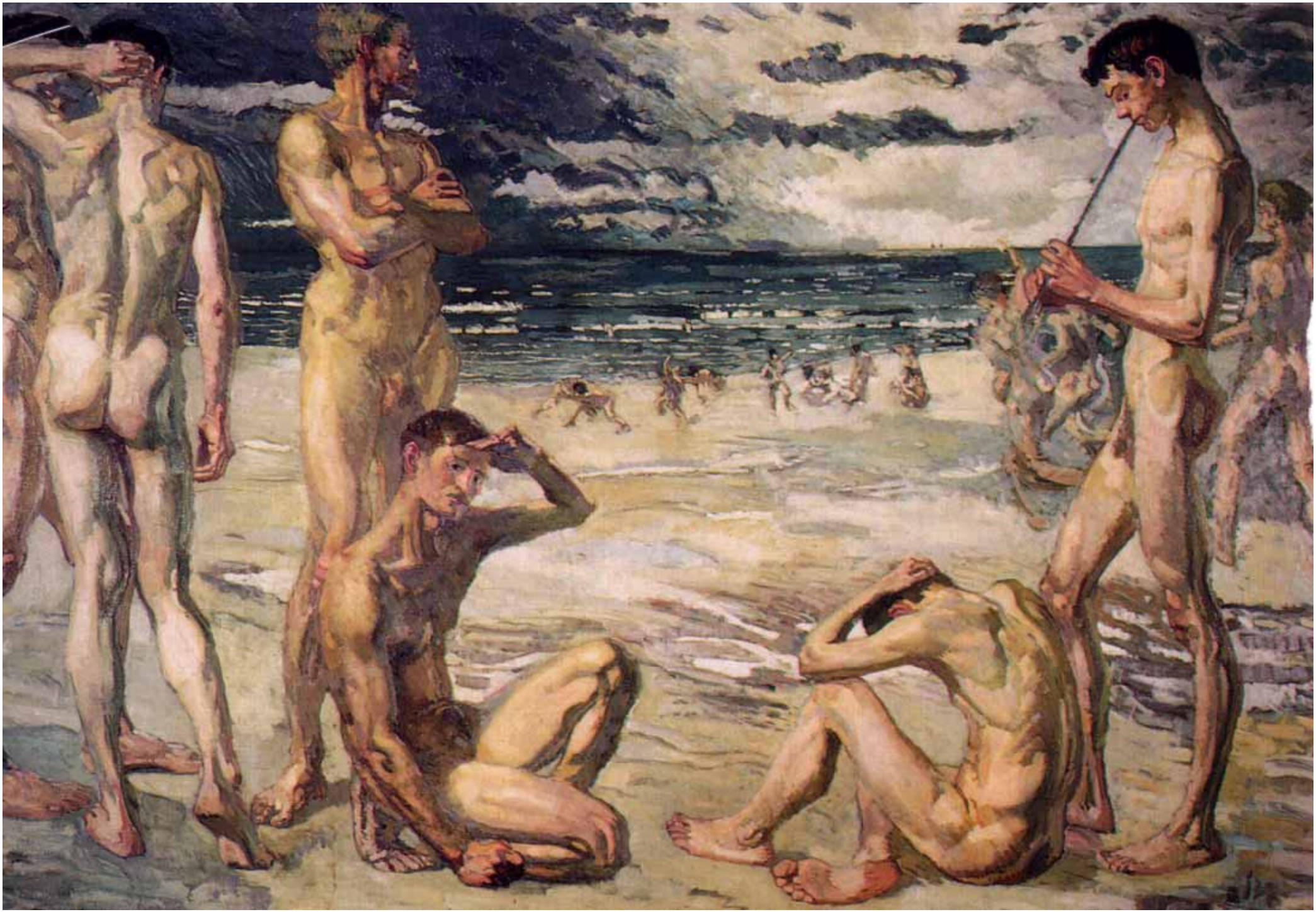
  UK: 1 in 1000

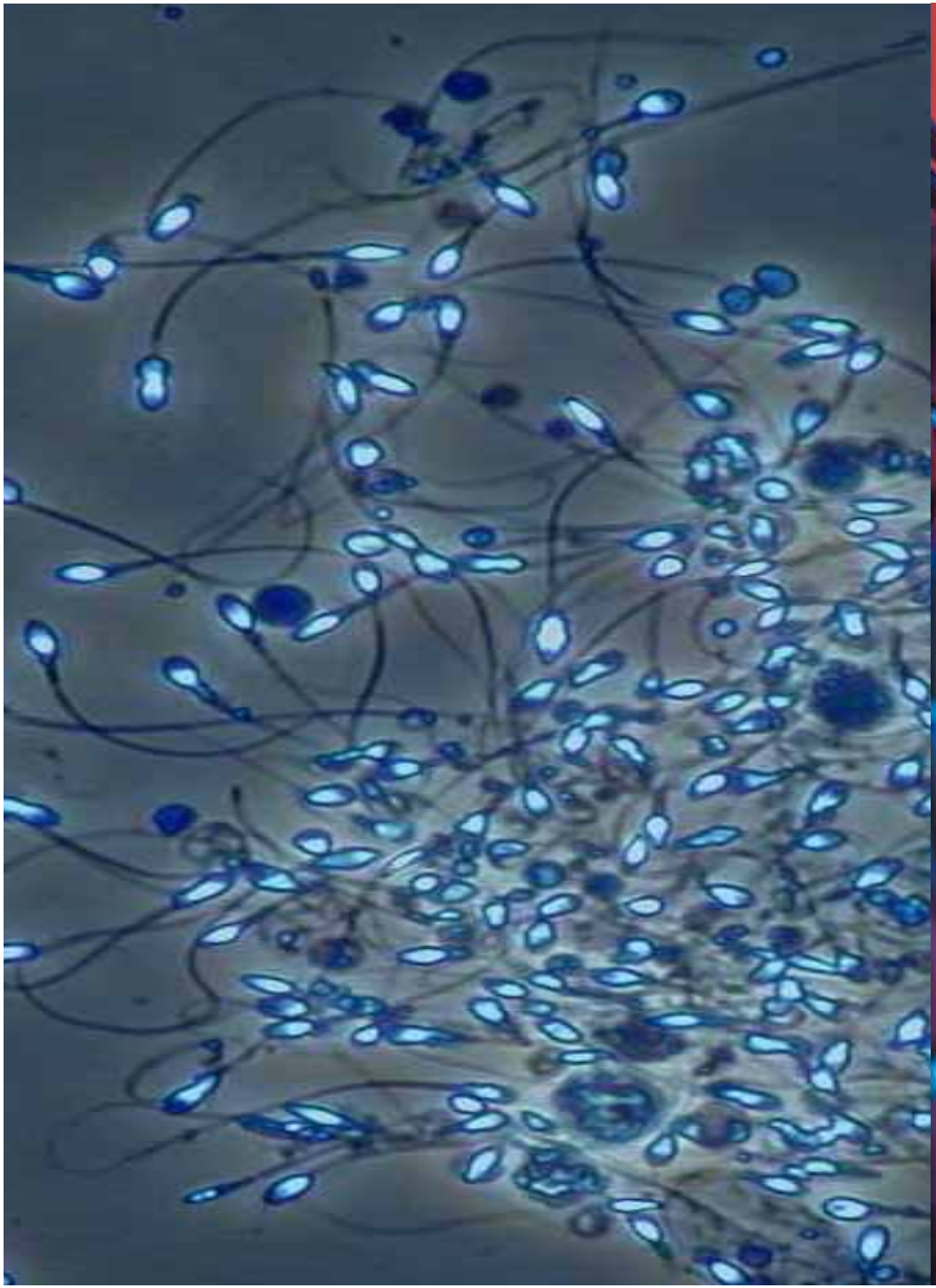
  (Hawkins & Stevens, 1996)

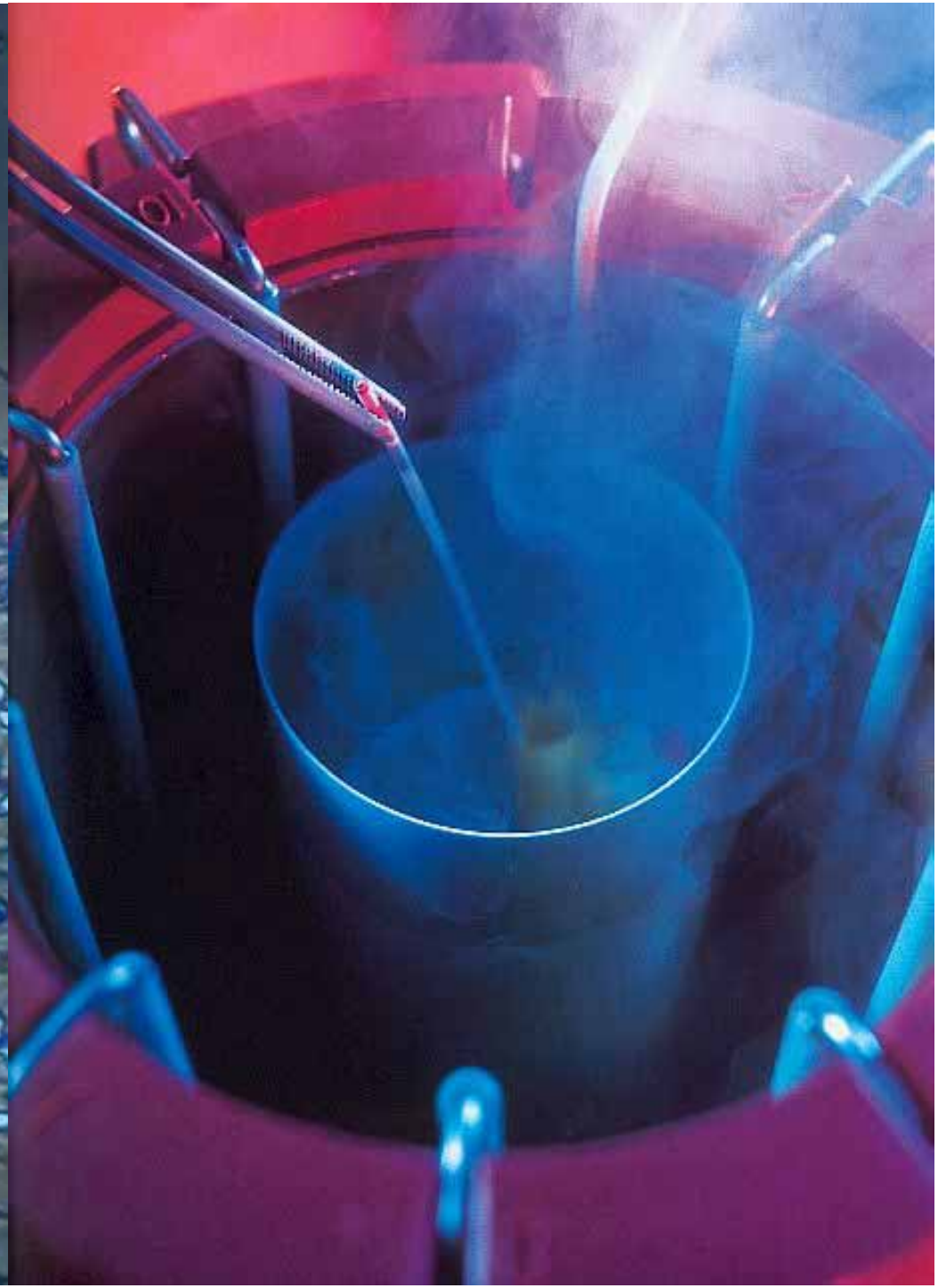
  US: by 2010 : 1 in 250 !

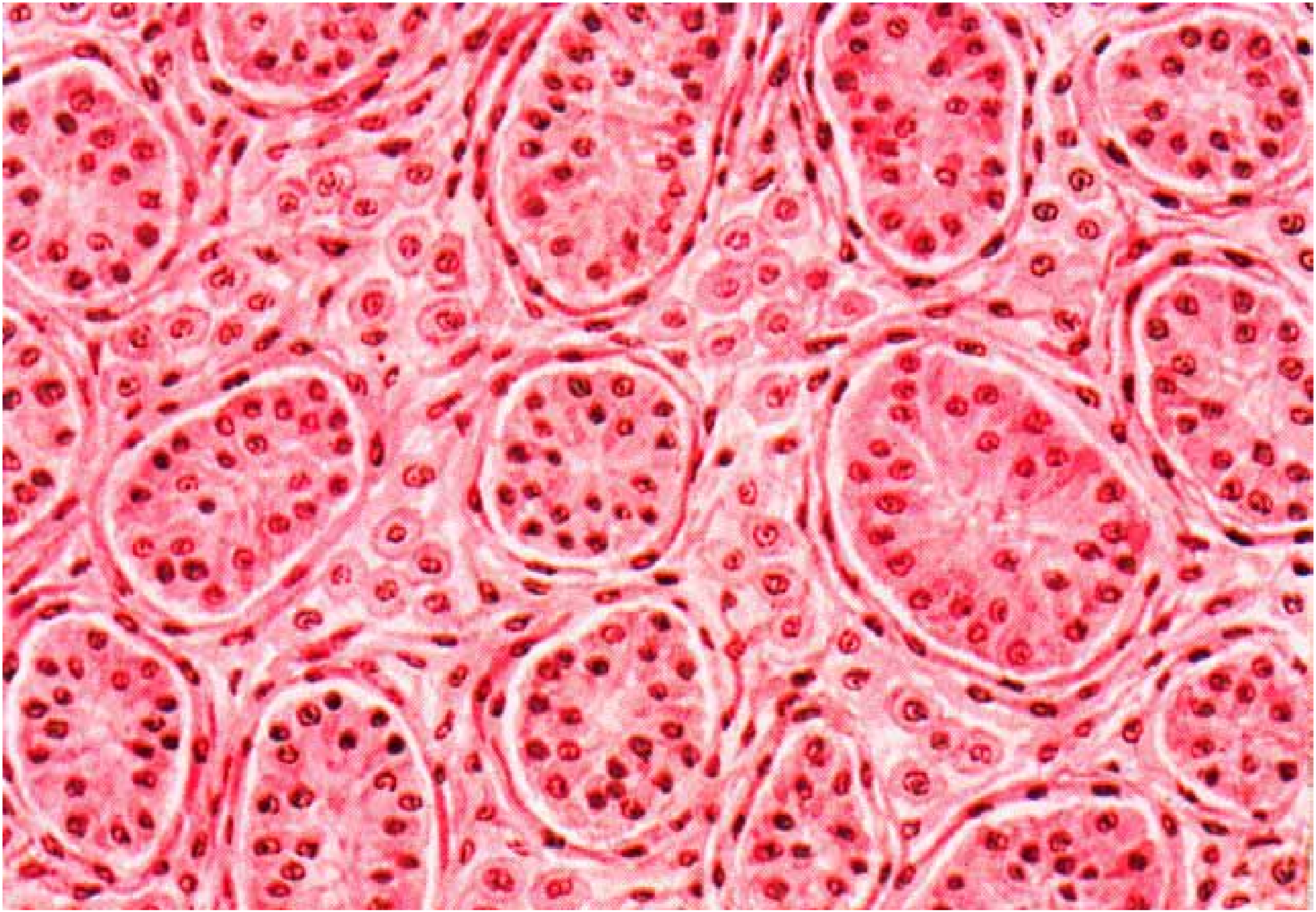
  (estimate from Bleyer 1990)


MAJOR ADVANCEMENTS IN THE TREATMENT OF
CHILDHOOD CANCER HAVE PAVED THE WAY FOR
INCREASING NUMBERS OF INDIVIDUALS TO
BECOME SURVIVORS OF CANCER.
HOWEVER, MANY WILL EXPERIENCE
NUMEROUS LONG-TERM EFFECTS
FROM THE DISEASE AND
TREATMENT THAT MAY
OCCUR MONTHS TO YEARS
FOLLOWING CESSATION
OF THERAPY.









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

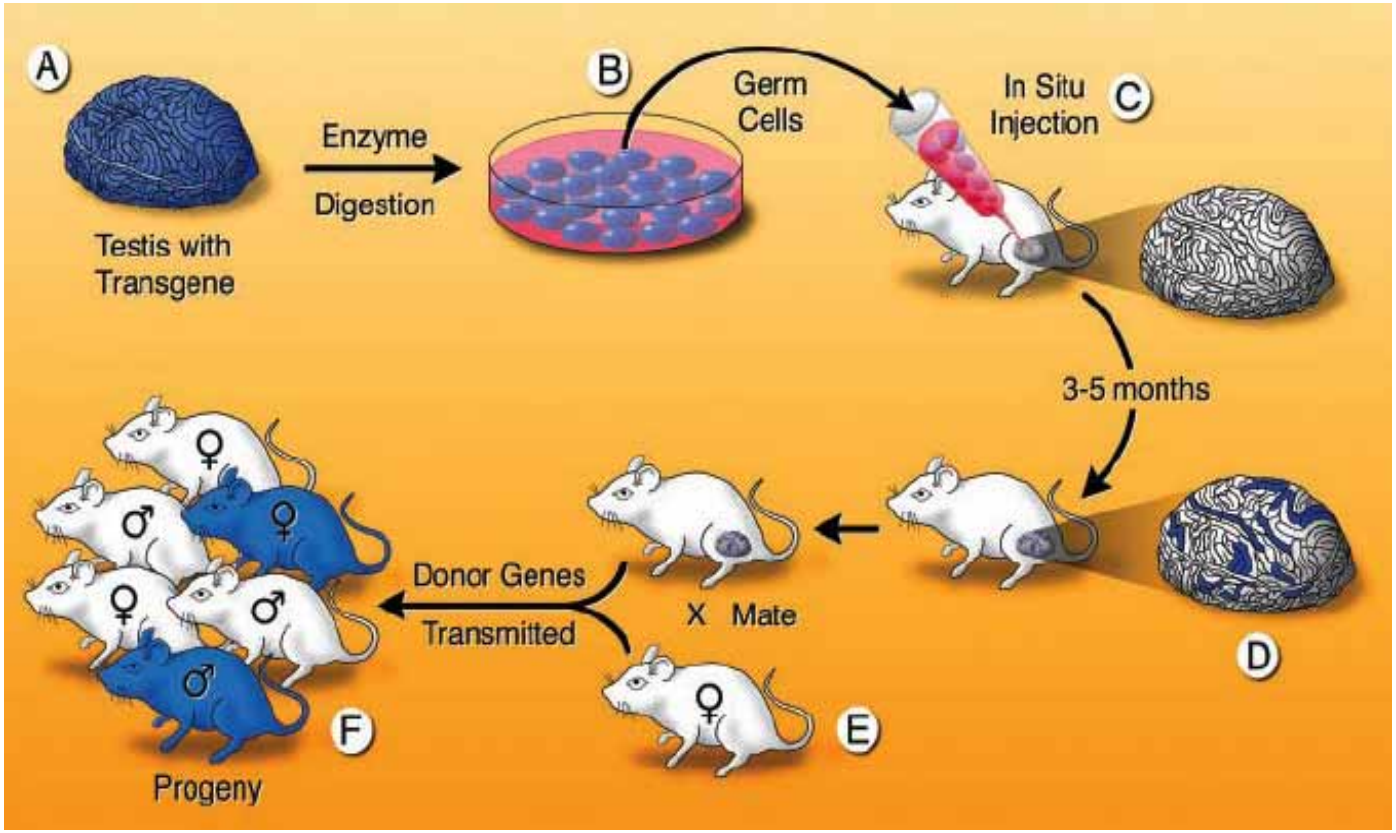
Spermatogenesis following male germ-cell transplantation

(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

Contributed by Ralph L. Brinster, August 11, 1994



Potential clinical applications

- *Prevention of (acquired) infertility in boys*

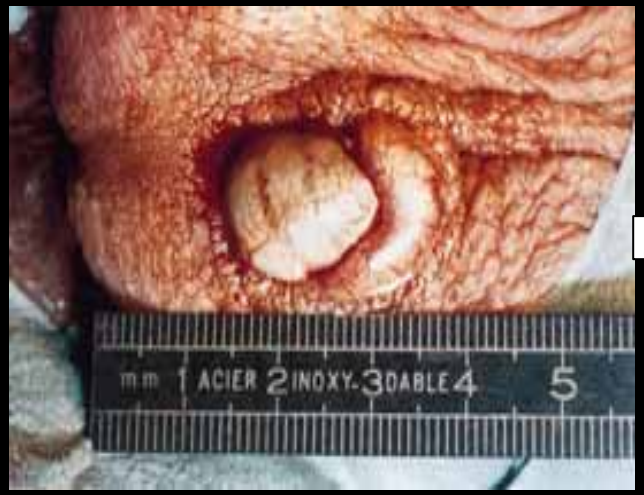
  Brinster et al. 1994

- *Treatment of (acquired) male infertility*

  Radford and Liebermann 1999

- *Treatment of (congenital) male infertility*

  Short et al. 1998; Ogawa et al. 2000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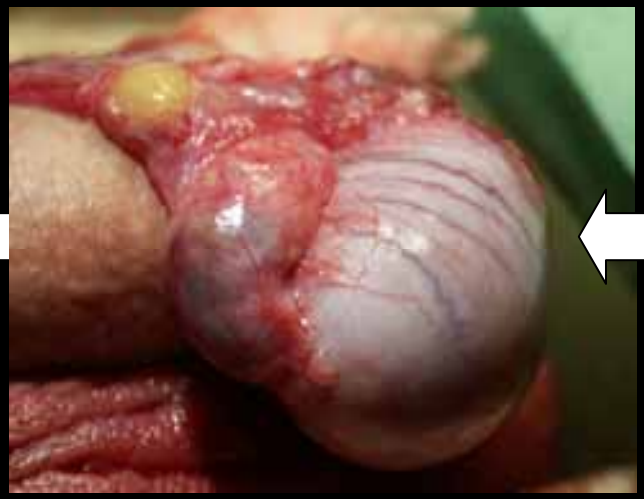

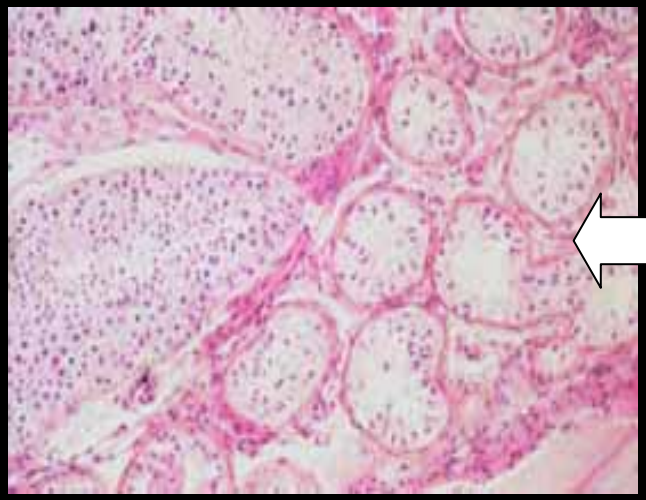

Fertility after treatment for cancer

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

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

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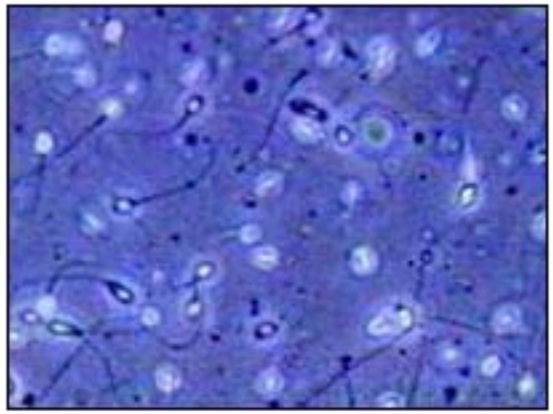
Testicle transplant makes sperm

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.

Dr Nikalaos Sofikitis, from the Laboratory for Molecular Urology in Ioannina, Greece, recruited 22 men for a pioneering trial into the technique

'I survived cancer'

Guides to treatment

- Mixed-sex human embryo created
- Public reassured on IVF safety
- Surrogate mothers happy in role
- UK bottom of Euro-IVF league
- Boy babies increase future risk
- Air-dried sperm 'stored at home'
- Womb transplant baby soon
- Testicle transplant makes sperm
- Aborted foetus could provide eggs
- Hormone link to lesbianism

Guides to cancer

- Have the scientists gone too far?

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TOP HEALTH STORIES NOW

- Tooth decay 'rampant in children'
- Fraud crackdown saves NHS £500m
- Diabetes cell transplant success
- Fruit 'helps prevent eye disease'

Transplantation of male germ line stem cells restores fertility in infertile mice

TAKEHIKO OGAWA, INA DOBRINSKI, MARY R. AVARBOCK & RALPH L. BRINSTER

Transplantation of stem cells from an infertile testis to a normal testis, preferably devoid of competing endogenous spermatogenesis, could provide the necessary environment and allow *in situ* differentiation of stem cells to occur. The possibility of obtaining offspring from males with oligo- and azoospermia has improved considerably with the development of assisted reproductive techniques, and testis cell transplantation should allow additional progress in this area.

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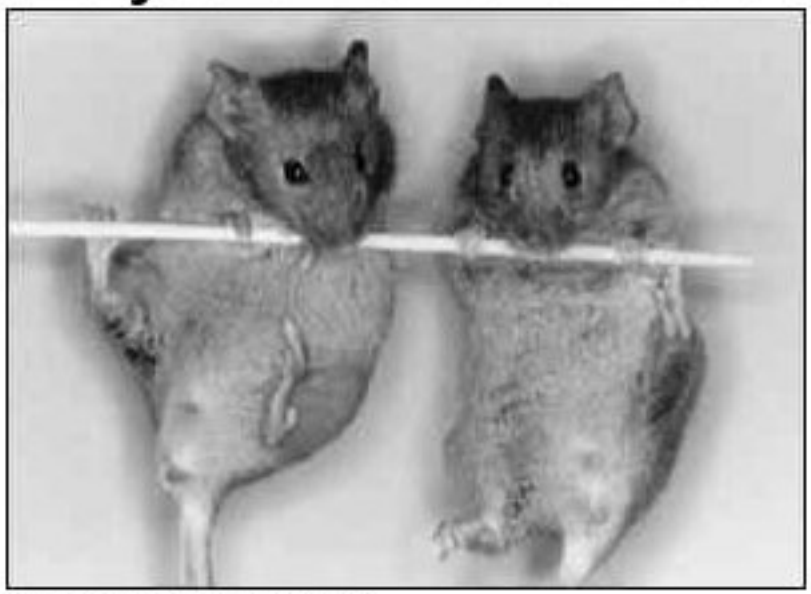
Thursday, January 29, 1998 Published at 01:01 GMT

Sci/Tech

Are you a man or a mouse?



Helping cure human infertility?

A leading fertility expert plans to produce human sperm using mice.

Roger Short, of the Royal Women's Hospital in Melbourne, Australia, hopes to transplant the germ cells that develop into sperm from human testes into a mouse.

Relevant Stories

20 Jan 98 | Sci/Tech
Malaria vaccine made from mouse milk

23 Dec 97 | Sci/Tech
Mice may help crack BSE mystery

Internet Links

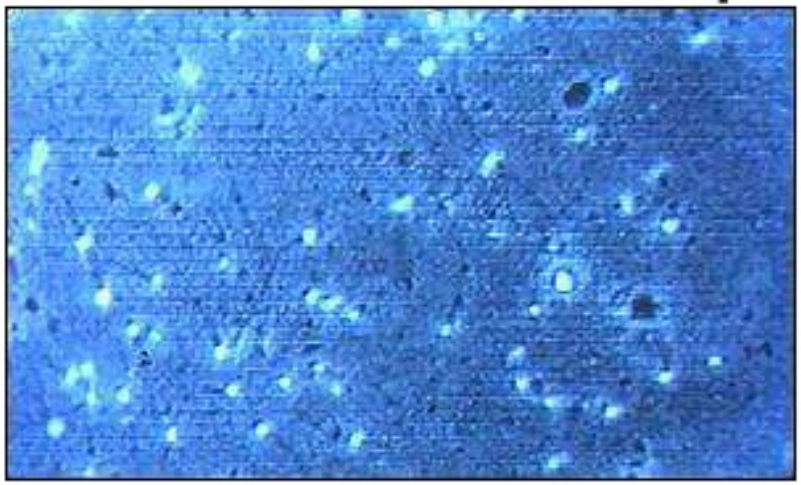
Nature

New Scientist, the magazine which published the story

Wednesday, February 3, 1999 Published at 14:32 GMT

Sci/Tech

Rodents make human sperm



The research is directed at male fertility problems

Researchers in Japan claim to have made rats and mice produce human sperm.

Nikolaos Sofikitis explains his research

The team at the Tottori University in Japan, say they implanted the human cells responsible for producing sperm - spermatogonia - into rat and mice testes in August 1998.

Five months later human sperm was detected in the animals.

Sci/Tech Contents

Relevant Stories

20 Jan 99 | Health
Chemical tricksters may revolutionise contraception

18 Jan 99 | Health
Sperm treatment trial urged

15 Dec 98 | Health
Genetic concerns over fertility treatment

15 Dec 98 | Health
Controversy over fertility wonder technique

29 Jan 98 | Sci/Tech
Are you a man or a mouse?

Internet Links

Tottori University

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In this section

Prerequisites for clinical application

- *Technically feasable*
  - *transplantation protocol*
  - *cryopreservation protocol*
- *Reproductive efficiency*
- *Reproductive safety*

Prerequisites for clinical application

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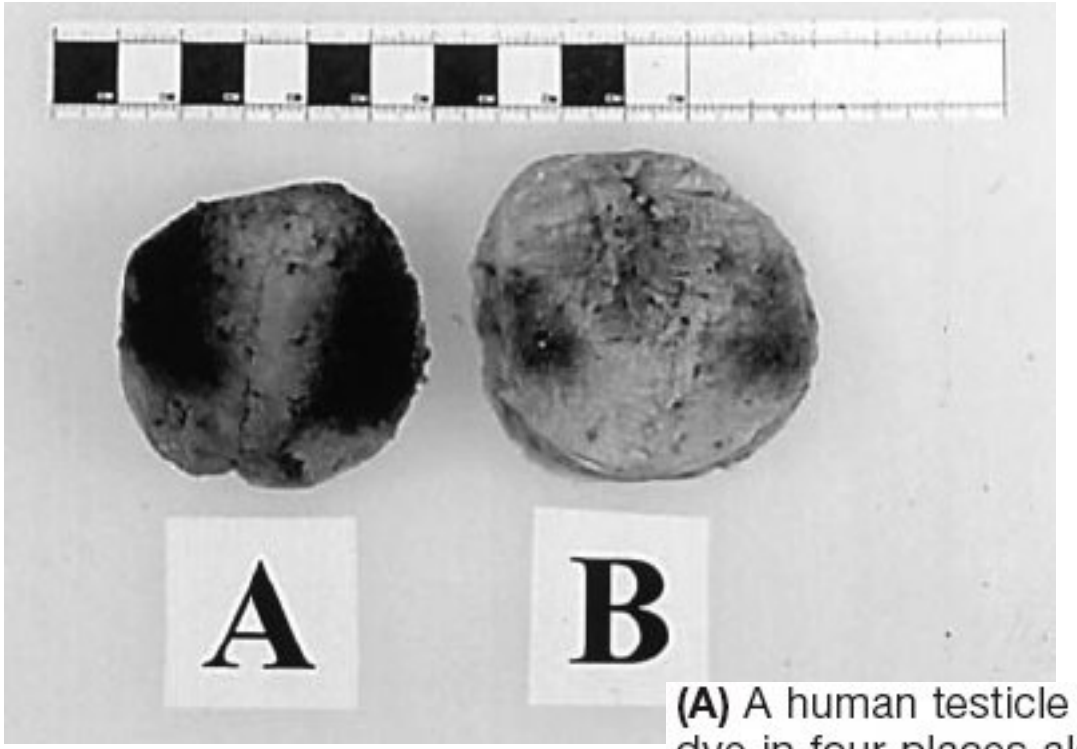
FERTILITY AND STERILITY®
VOL. 75, NO. 2, FEBRUARY 2001

Isolation of germ cells from human testicular tissue for low temperature storage and autotransplantation

Philip F. Brook, Ph.D.,[a] John A. Radford, M.D.,[b] Steven M. Shalet, M.D.,[c] Adrian D. Joyce, M.D.,[d] and Roger G. Gosden, D.Sc.[a]

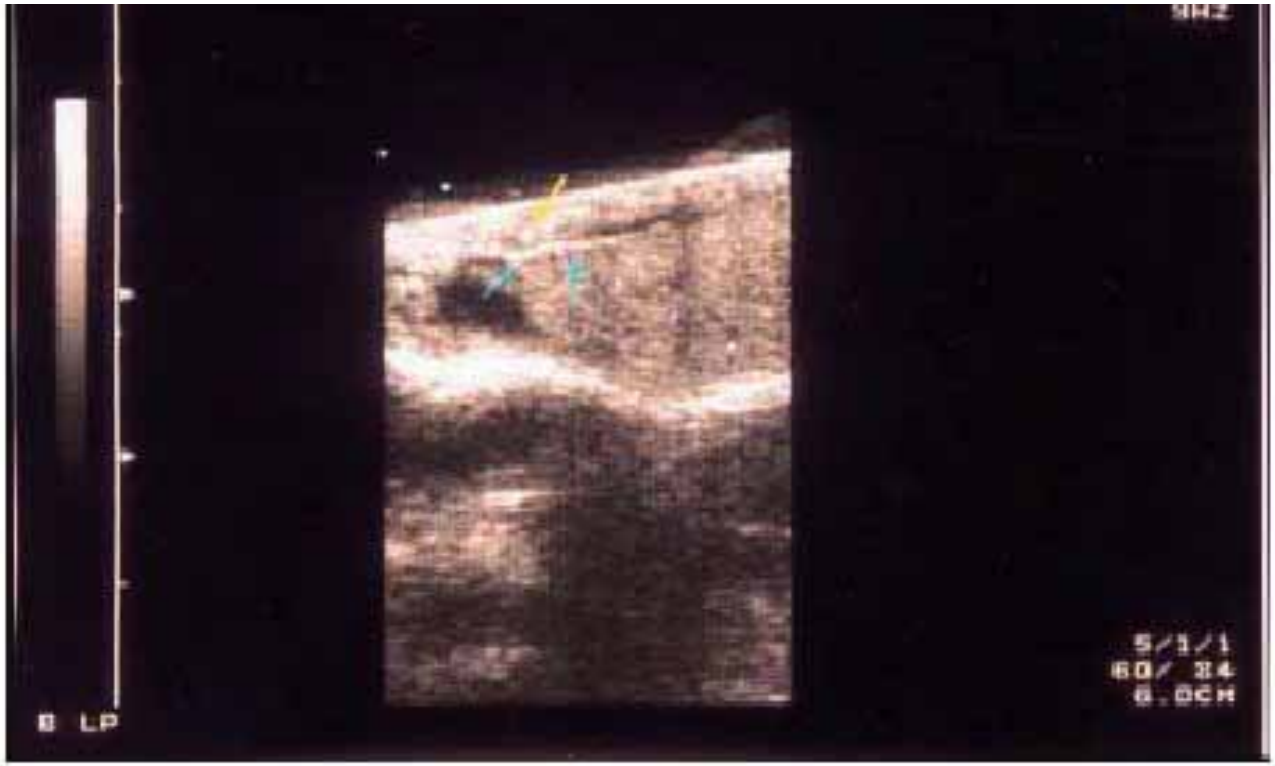


(A) A human testicle that had been injected with trypan blue dye in four places along the length of the rete testis. **(B)** An organ that received only a single injection of the same volume.

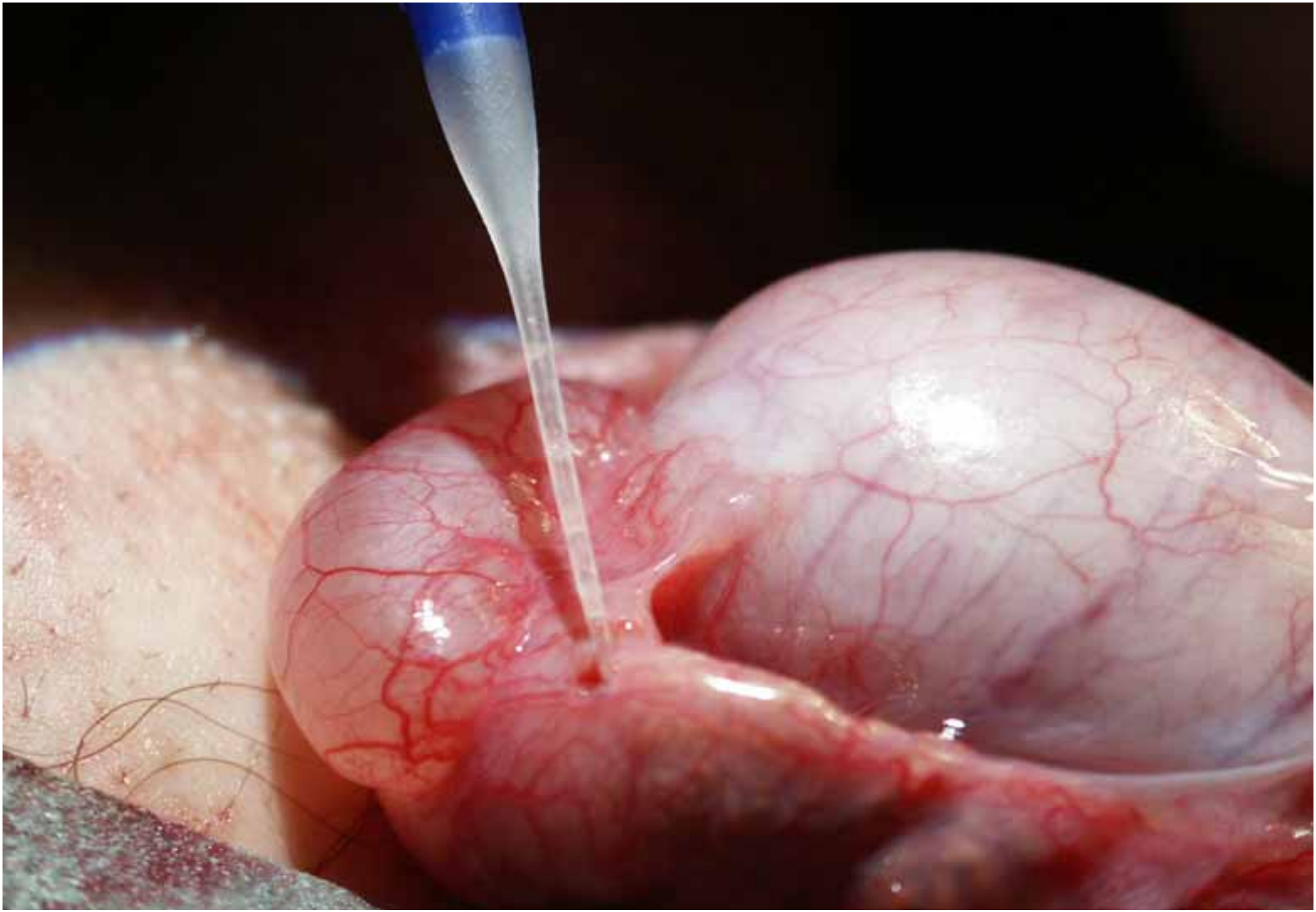
The role of ultrasonographically guided puncture of the human rete testis in the therapeutic management of nonobstructive azoospermia*

ANDROLOGIA **35,** 85–92 (2003)

A. Kaponis[1], D. Yiannakis[2], K. Tsoukanelis[2], D. Tsalikis[2], D. Tsabalas[2], D. Baltogiannis[2], X. Giannakopoulos[2], M. Schrader[3], I. Georgiou[2], Y. Yamamoto[1], N. Kanakas[1,2], I. Miyagawa[1], D. Loutradis[1], S. Touloupidis[4] and N. Sofikitis[1,2]



Ultrasonographically guided puncture of the rete testis (blue arrows). The tip of the needle (yellow arrow) approaches the rete testis.

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Human Reproduction Vol.18, No.12 pp. 2660–2667, 2003

Restoration of fertility in infertile mice by transplantation of cryopreserved male germline stem cells

M.Kanatsu-Shinohara[1], N.Ogonuki[2], K.Inoue[2], A.Ogura[2], S.Toyokuni[3] and T.Shinohara[1,4]

[1]Horizontal Medical Research Organization, [3]Department of Pathology and Biology of Diseases, Graduate School of Medicine, Kyoto University and [2]The Institute of Physical and Chemical Research (RIKEN), Bioresource Center, Ibaraki, Japan

Table III. Spermatogenesis after transplantation of freeze–thawed testis cells

Recipient animal	Recipient age	No. of recipients	No. of fertile recipients (%)	% tubules with spermatogenesis[a]	No. of epididymides with spermatozoa (%)
W	Pup	8	4 (50)	54.5 ± 8.9	7 (87.5)
W	Adult	9	1 (11)	38.4 ± 6.3	5 (55.6)
Busulfan	Adult	12	0 (0)	ND	ND

Prerequisites for clinical application

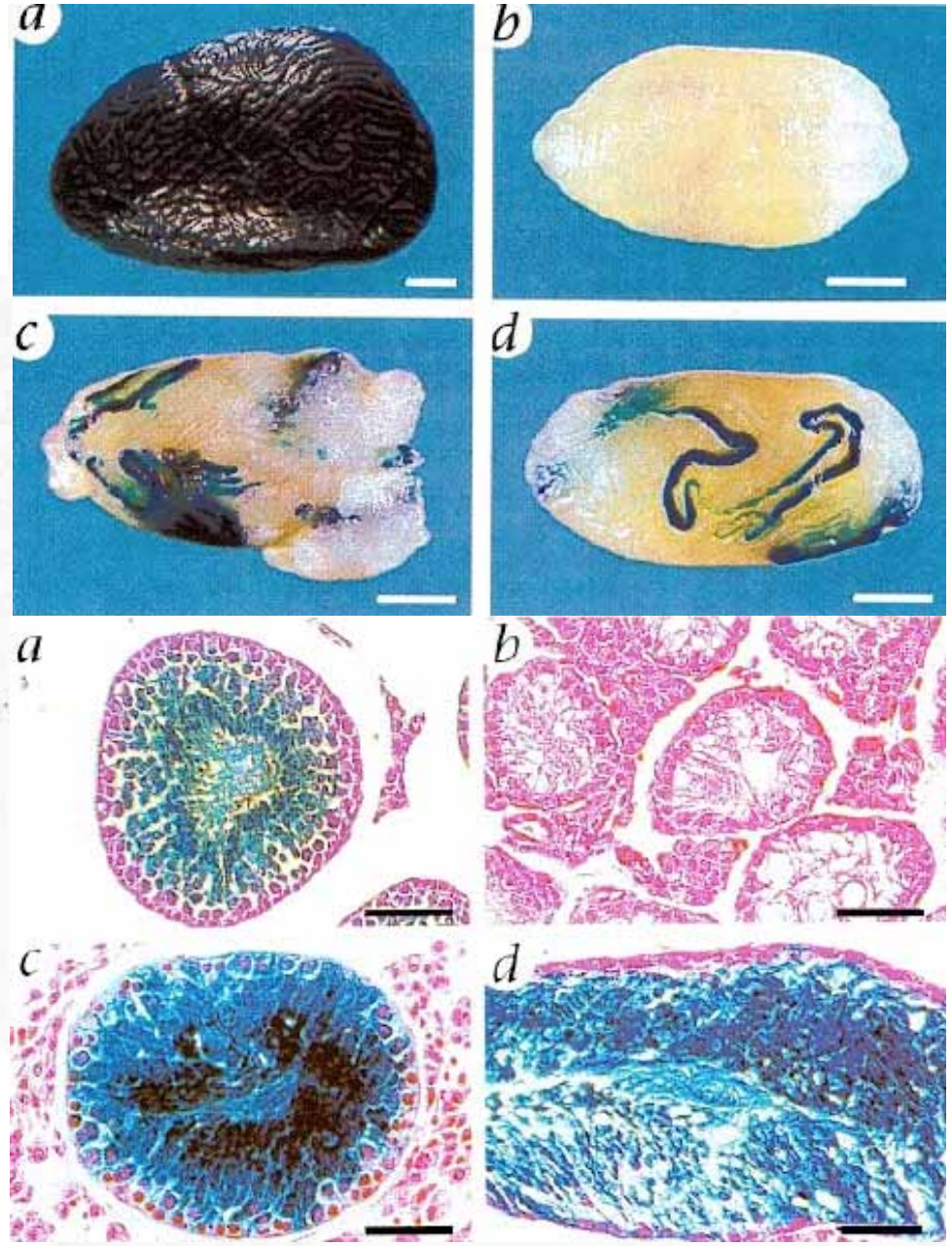
- *Technically feasable*
  - *transplantation protocol*
  - *cryopreservation protocol*
- *Reproductive efficiency*
- *Reproductive safety*

NATURE MEDICINE, VOLUME 2, NUMBER 6, JUNE 1996

Reconstitution of spermatogenesis from frozen spermatogonial stem cells

MARY R. AVARBOCK, CLAYTON J. BRINSTER & RALPH L. BRINSTER

Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA
Correspondence should be addressed to R.L.B.



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VOL. 75, NO. 2, FEBRUARY 2001

Isolation of germ cells from human testicular tissue for low temperature storage and autotransplantation

Philip F. Brook, Ph.D.,[a] John A. Radford, M.D.,[b] Steven M. Shalet, M.D.,[c] Adrian D. Joyce, M.D.,[d] and Roger G. Gosden, D.Sc.[a]

Viability of isolated cells from human testes after cryopreservation: comparison of four cryoprotective agents.

Cryoprotective agents (1.5M)	Mean % viability (range)[a]
Glycerol	54 (52–57)
DMSO	54 (51–57)
1,2-propanediol	58 (55–59)
Ethylene glycol	52 (51–53)

[a] $P > .05$.

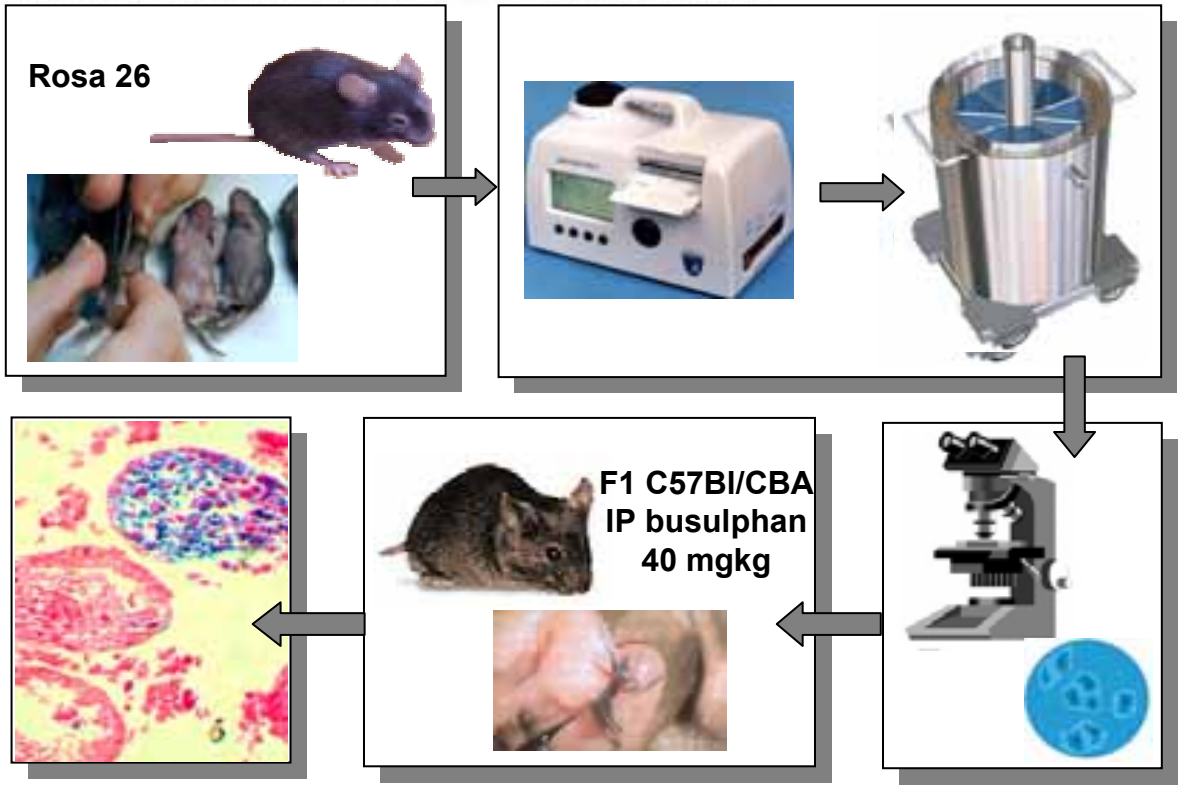
Brook. Isolation of male germ cells. Fertil Steril 2001.

Human Reproduction Vol.19, No.4 pp. 948–953, 2004

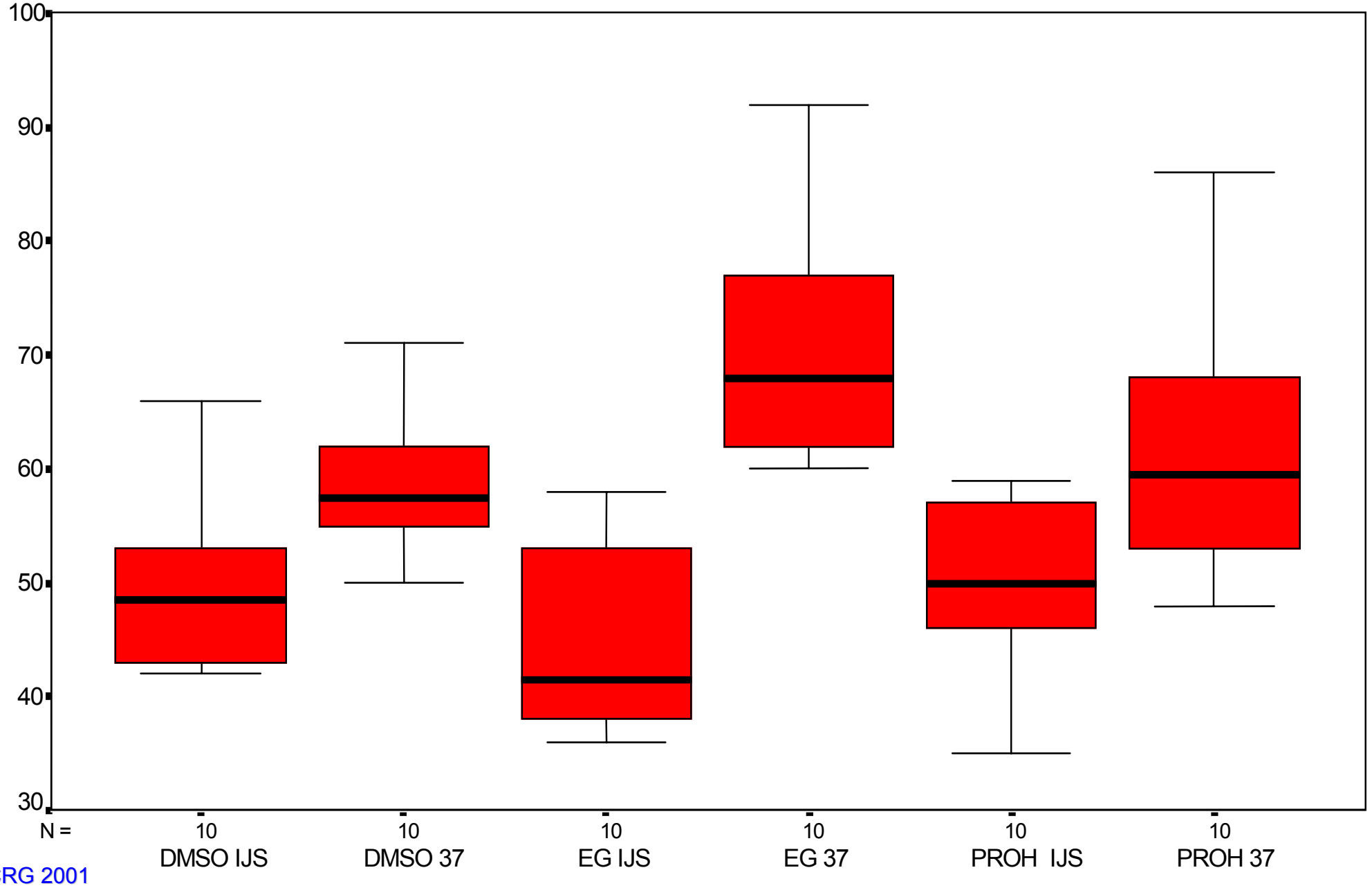
Recovery, survival and functional evaluation by transplantation of frozen–thawed mouse germ cells

V.Frederickx[1,2], A.Michiels[1], E.Goossens[1], G.De Block[1], A.C.Van Steirteghem[1] and H.Tournaye[1]

[1]Centre for Reproductive Medicine and Research Laboratories for Reproductive Medicine, University Hospital and Medical School, Vrije Universiteit Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium



cryopreservation of cell suspensions



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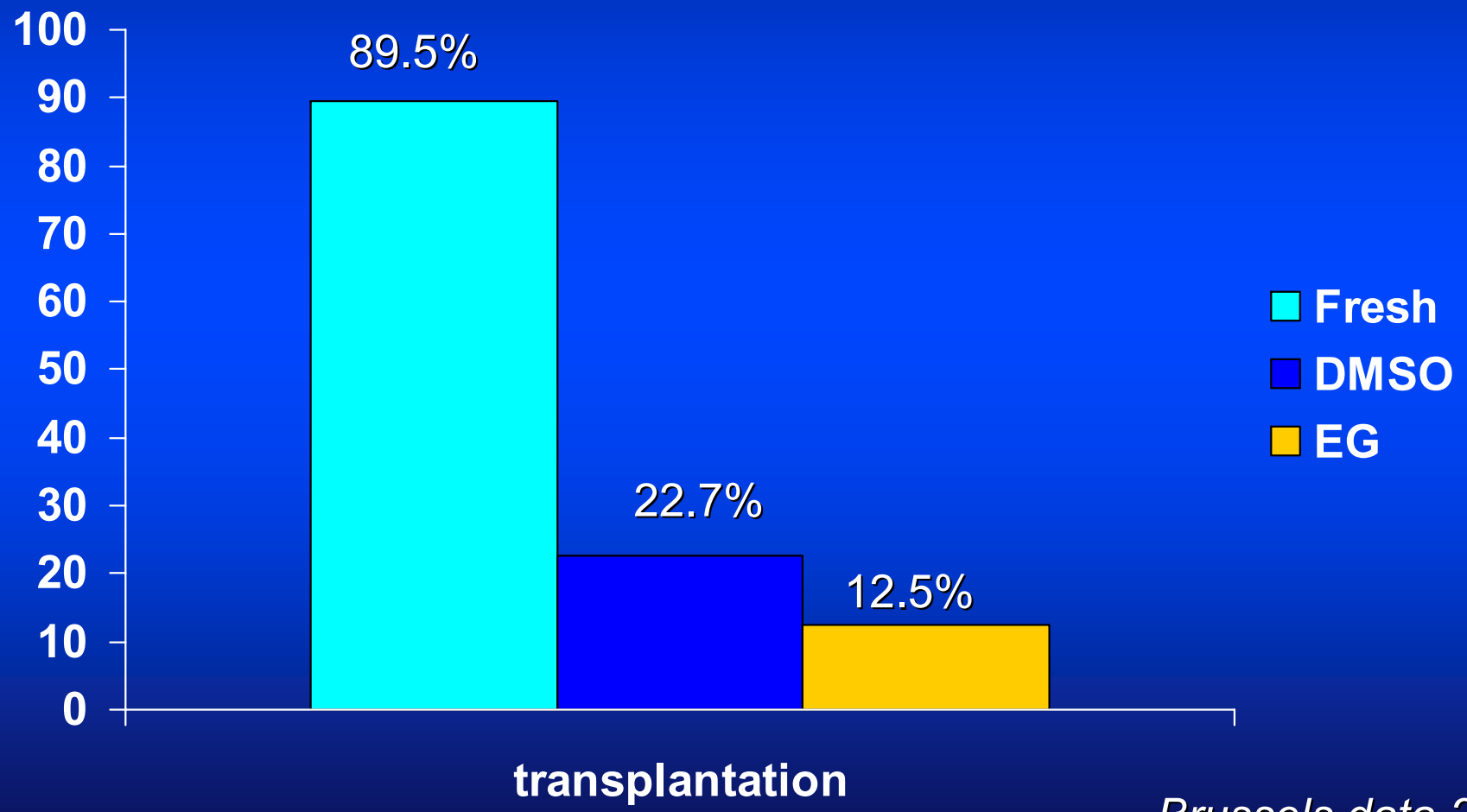
Transplantation after cryopreservation



Brussels data 2004

Human Reproduction Vol.18, No.12 pp. 2660–2667, 2003

Restoration of fertility in infertile mice by transplantation of cryopreserved male germline stem cells

M.Kanatsu-Shinohara[1], N.Ogonuki[2], K.Inoue[2], A.Ogura[2], S.Toyokuni[3] and T.Shinohara[1,4]

[1]Horizontal Medical Research Organization, [3]Department of Pathology and Biology of Diseases, Graduate School of Medicine, Kyoto University and [2]The Institute of Physical and Chemical Research (RIKEN), Bioresource Center, Ibaraki, Japan

Table II. Comparison of stem cell activity between freeze–thawed and fresh stem cells

Type of cells transplanted	W recipients		Busulfan-treated recipients	
	No. of testes injected	No. of colonies	No. of testes injected	No. of colonies
Frozen	11	62.1 ± 21.7	8	45.9 ± 16.2
Fresh	11	5.3 ± 2.2	8	9.0 ± 3.2

Values are mean ± SEM.

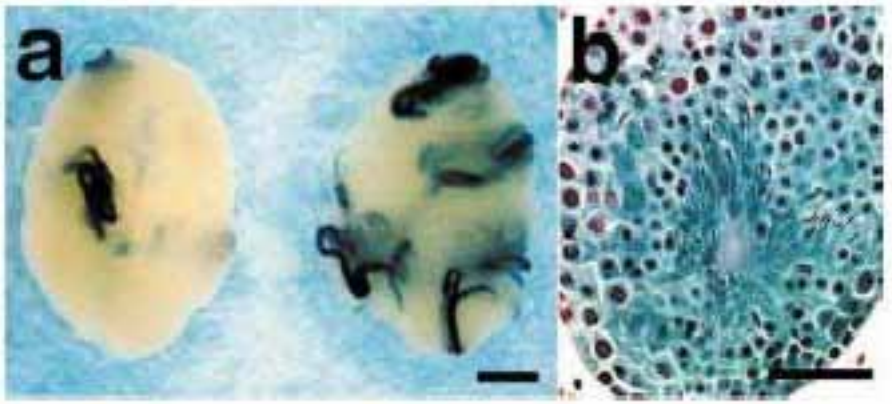


Figure 1. Comparison of the stem cell activities of fresh and freeze–thawed ROSA testis cells after transplantation into infertile W recipient mice.

Prerequisites for clinical application

- *Technically feasable*
  - *transplantation protocol*
  - *cryopreservation protocol*
- *Reproductive efficiency*
- *Reproductive safety*

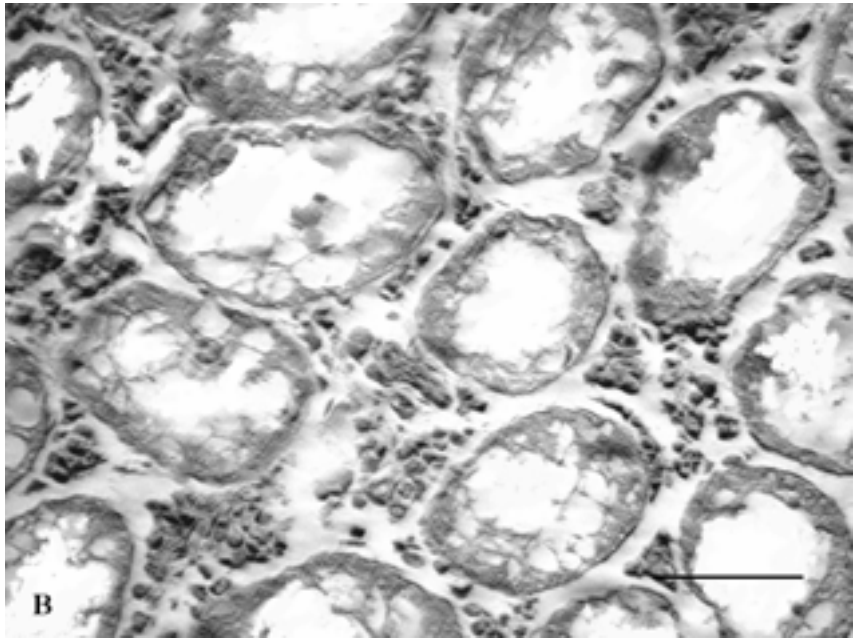
Human Reproduction Vol.18, No.9 pp.1–7, 2003 DOI: 10.1093/humrep/deg360

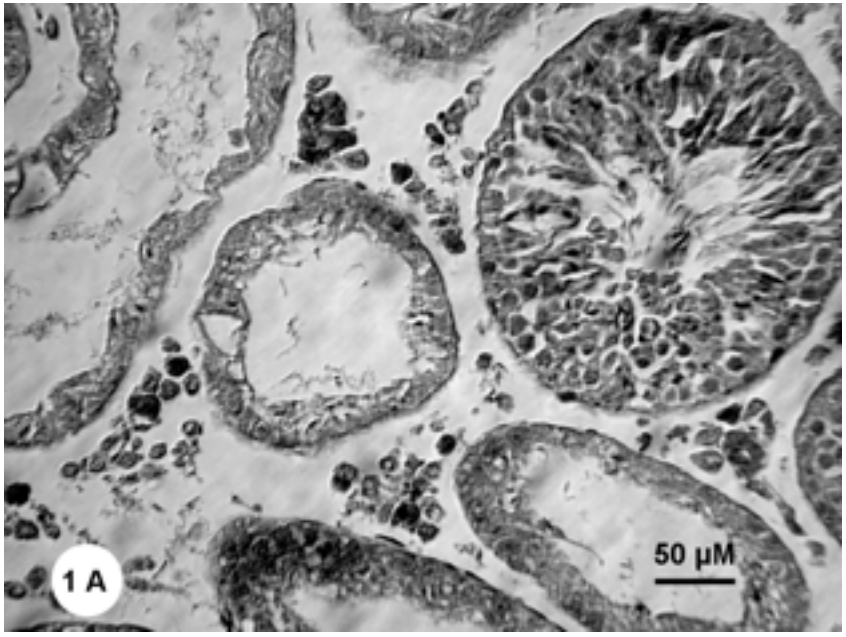
Reproductive capacity of sperm obtained after germ cell transplantation in a mouse model

E.Goossens[1], V.Frederickx, G. DeBlock, A.C.Van Steirteghem and H.Tournaye

Research Centre for Reproduction and Genetics, Faculty of Medicine and Pharmacy, Dutch-speaking Brussels Free University, Laarbeeklaan 101, 1090 Brussels, Belgium

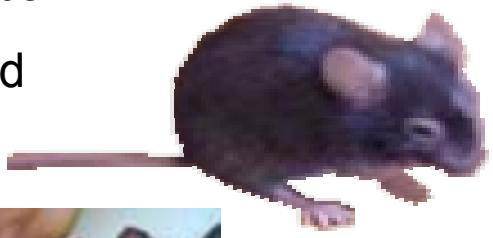
[1]To whom correspondence should be addressed.





C57Bl xCba F1

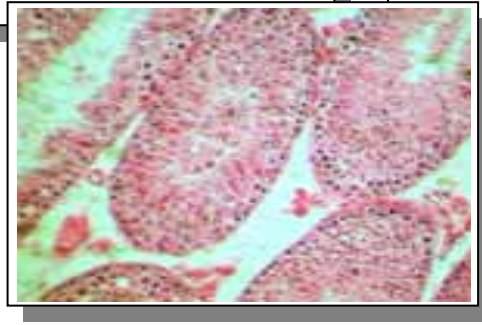
cryptorchid



W/W^v

6 wks

X

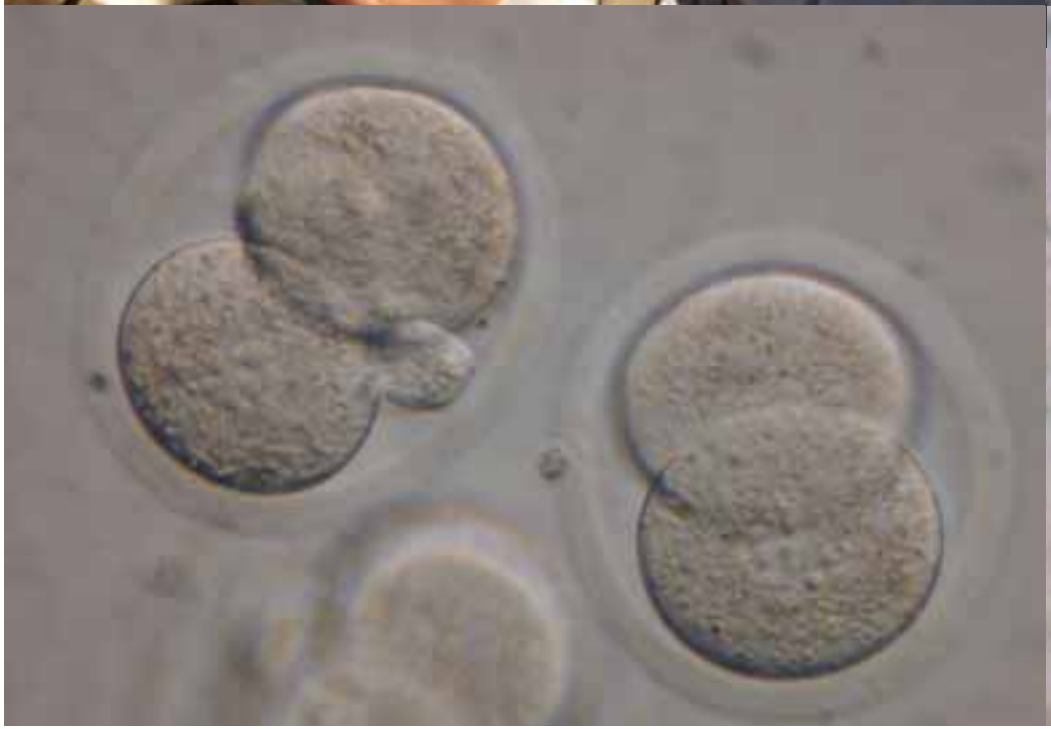


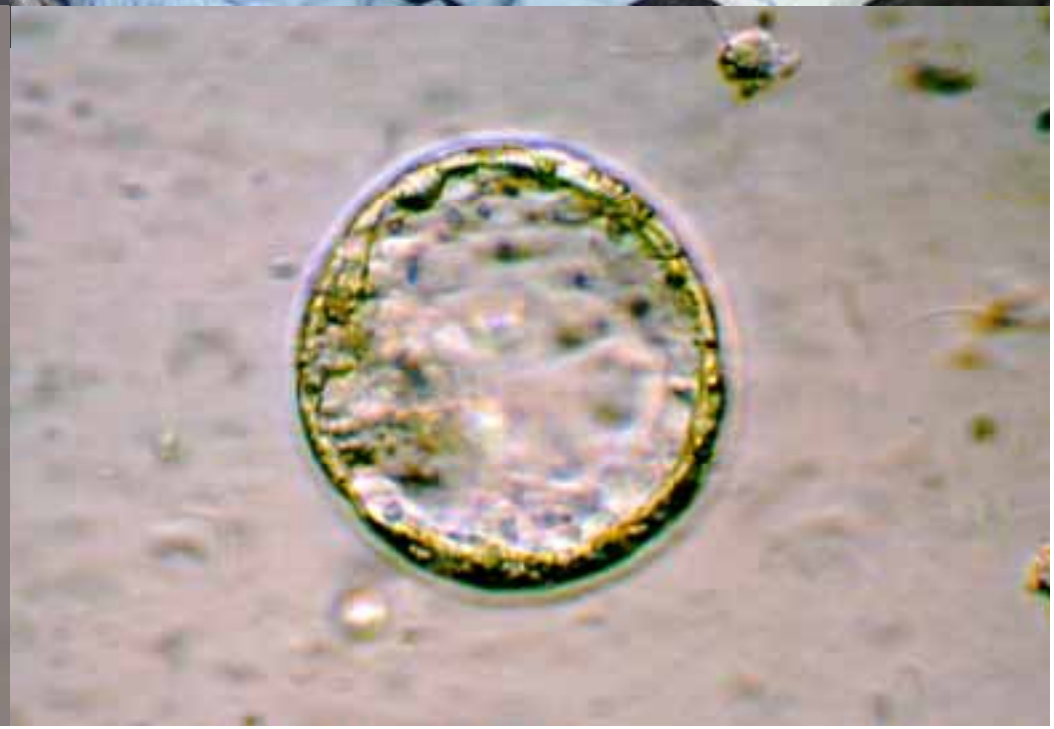
X

Table I. Natural mating with transplanted and control mice

Origin of sperm	No. of females	No. of plugs	Pregnancy rate (%)[a]	No. of fetuses	No. of aborted fetuses
Transplanted mice	23	17	6 (35)[b]	17	1
Control mice	15	10	9 (90)[b]	76	6

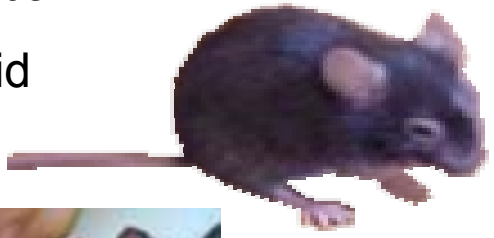
[a]Pregnancy rate = (no. of pregnancies/no. of plugs)×100.
[b]$P = 0.006$.





C57Bl xCba F1

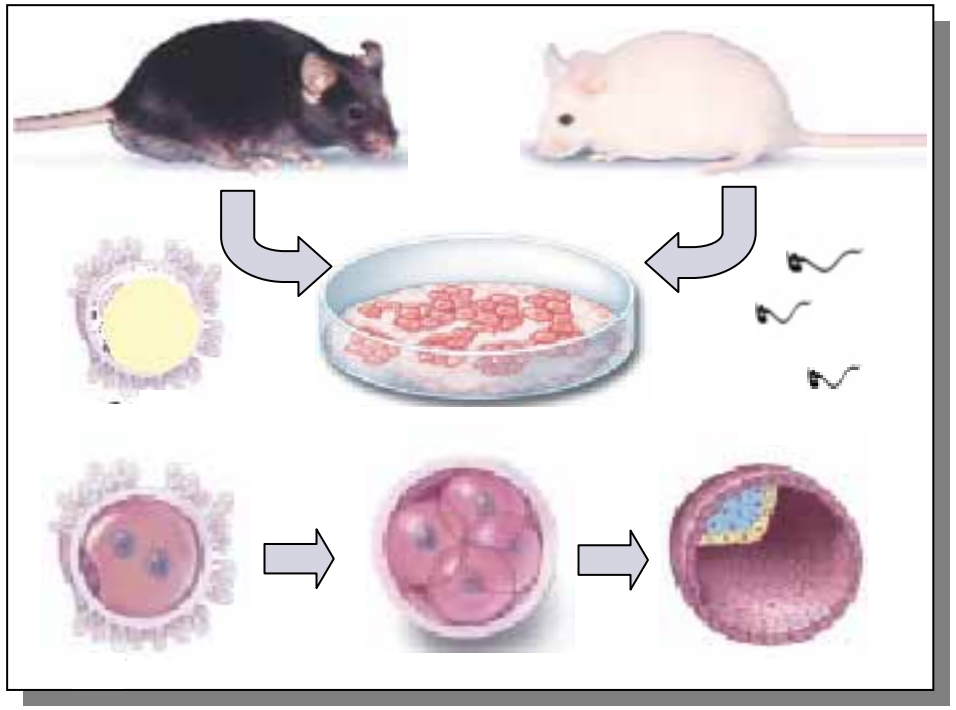
cryptorchid

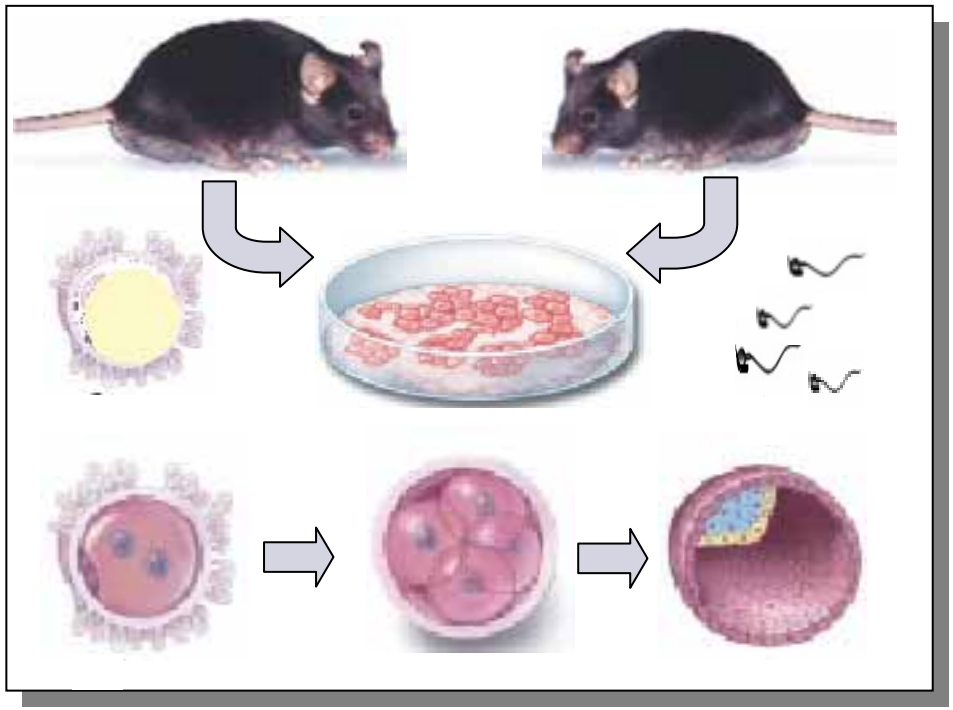


W/W^{v}

6 wks

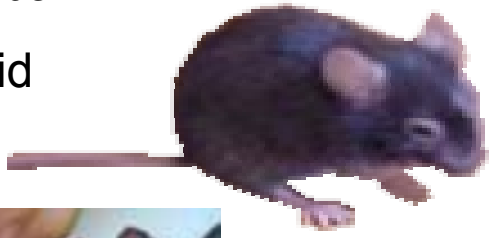
mouse IVF





C57Bl xCba F1

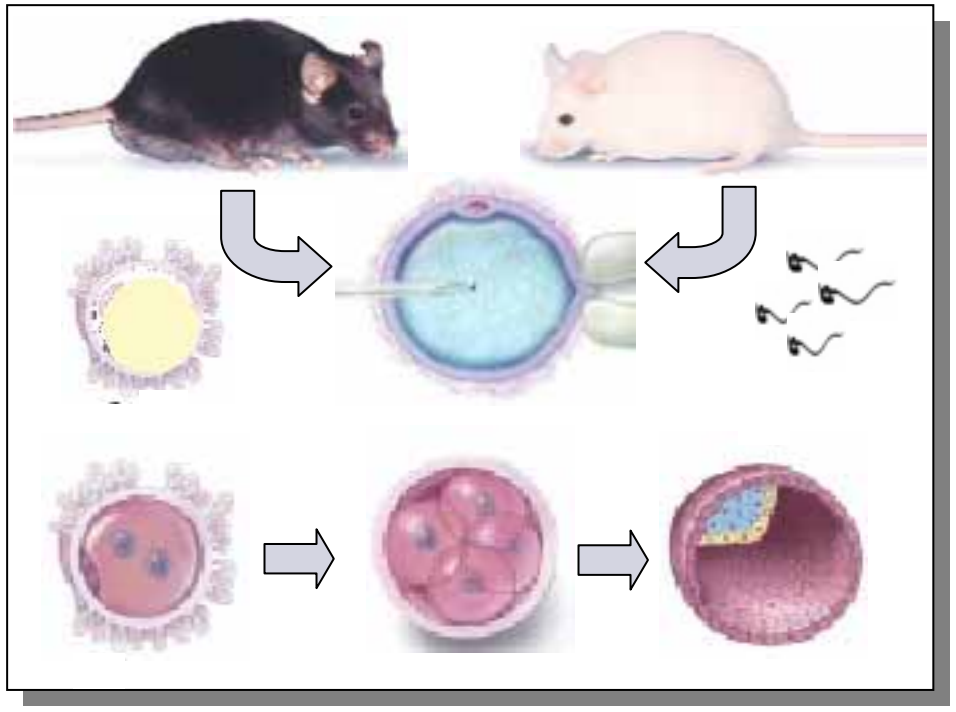
cryptorchid



W/W^{v}

6 wks

mouse ICSI



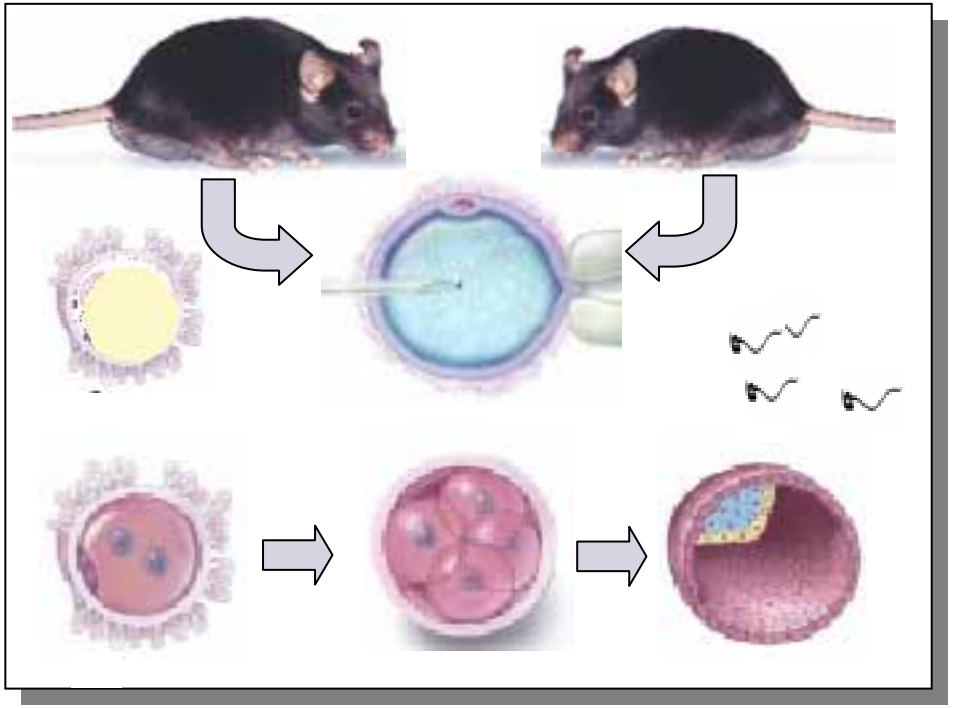


Table II. IVF with epididymal sperm obtained from transplanted and control mice

Origin of sperm	No. of exp.	No. of oocytes	Fertilization rate[a] (%)	Developmental rate[b] (%)
Transplanted mice	5	154	88 (57)[c]	24 (27)[c]
Control mice	5	195	155 (79)[c]	88 (57)[c]

[a]Fertilization rate = (no. of 2-cell embryos/no. of survived oocytes)×100.
[b]Developmental rate = (no. of blastocysts on day 5/no. of 2-cell embryos)×100.
[c]$P < 0.00001$ by Fisher's exact test.

Table III. ICSI with epididymal sperm obtained from transplanted and control mice

Origin of sperm	No. of exp.	No. of oocytes	Survival rate (%)	Fertilization rate[a] (%)	Developmental rate[b] (%)
Transplanted mice	5	187	83 (44)	57 (69)	17 (30)
Control mice	5	112	62 (55)	38 (61)	14 (37)

[a]Fertilization rate = (no. of 2-cell embryos/no. of survived oocytes)×100.
[b]Developmental rate = (no. of blastocysts/no. of 2-cell embryos)×100.

Increasing stem cell numbers in mice

- *use of multiple testes*
- *artificial cryptorchidism : 23 x enrichment*

  Shinohara et al. 2000
- *vitamin A deficiency: 23 x enrichment*

  Mc Lean et al. 2002
- *MACS: 7 x enrichment*
- *FACS: 166 x enrichment*

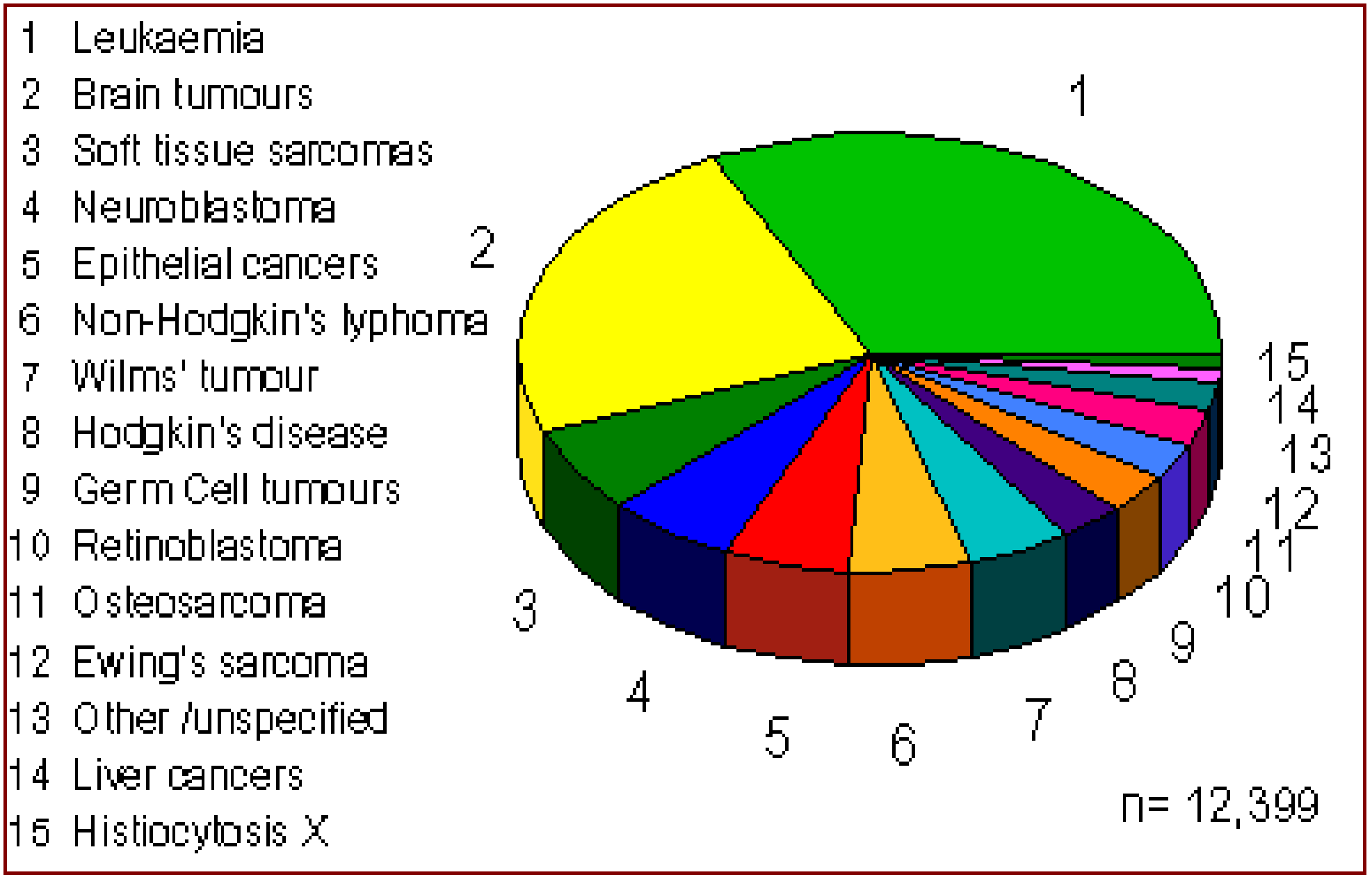
  Shinohara et al. 2000

Increasing stem cell numbers in man

- *use of testis instead of biopsy ?*
- *in-vitro culture ?*
- *MACS and or FACS ?*

Prerequisites for clinical application

- *Technically feasable*
  - *transplantation protocol*
  - *cryopreservation protocol*
- *Reproductive efficiency*
- *Reproductive safety*


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

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

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

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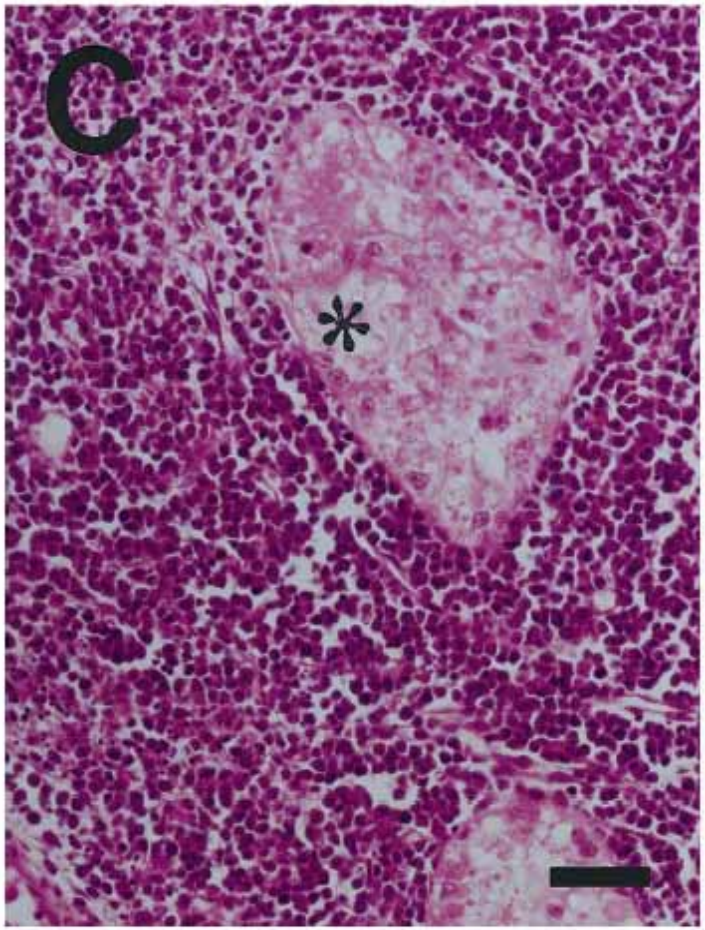
ABSTRACT

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.



Spermatogonial stem cell enrichment by multiparameter selection of mouse testis cells

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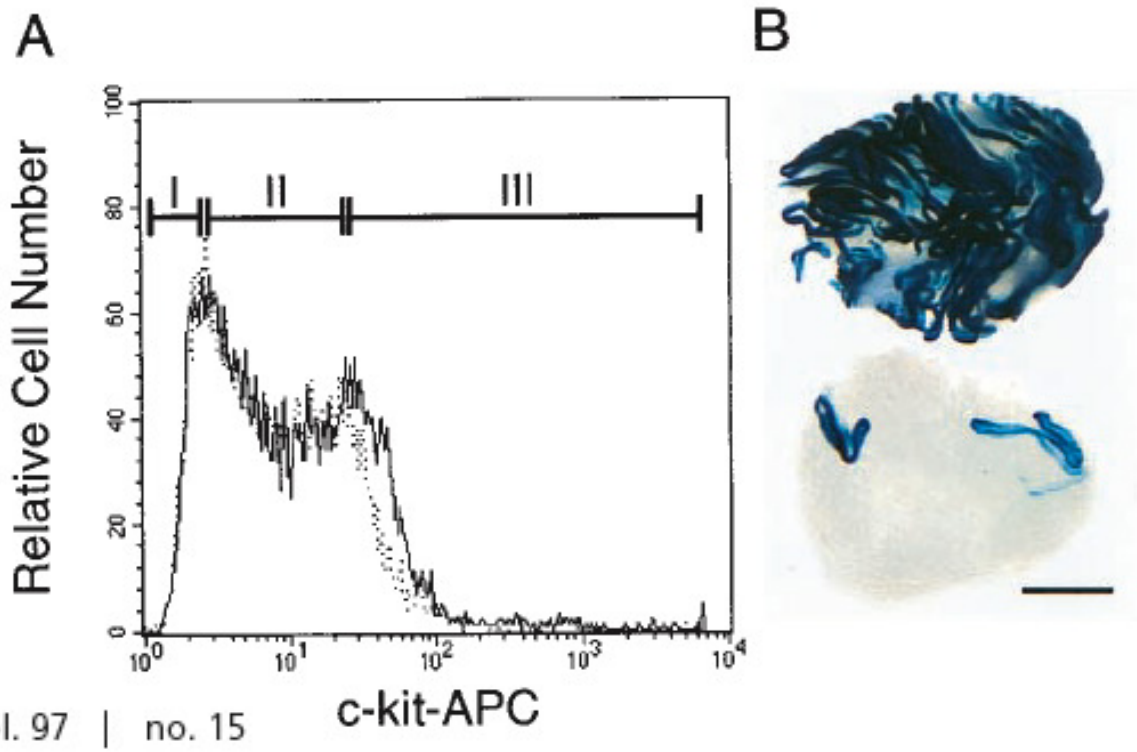
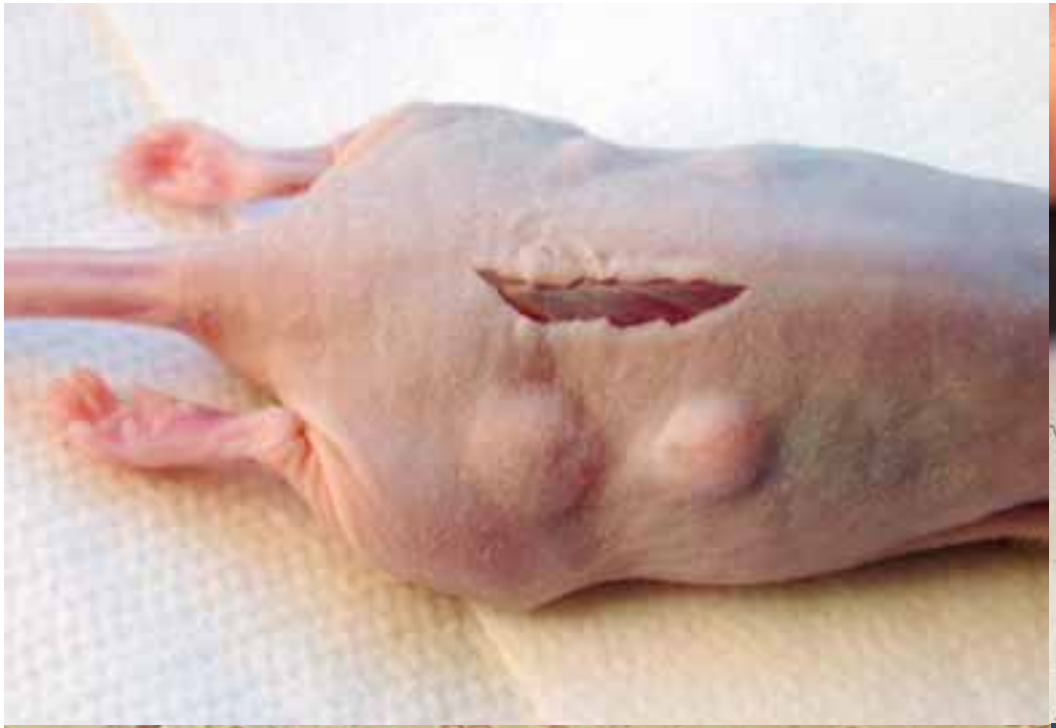
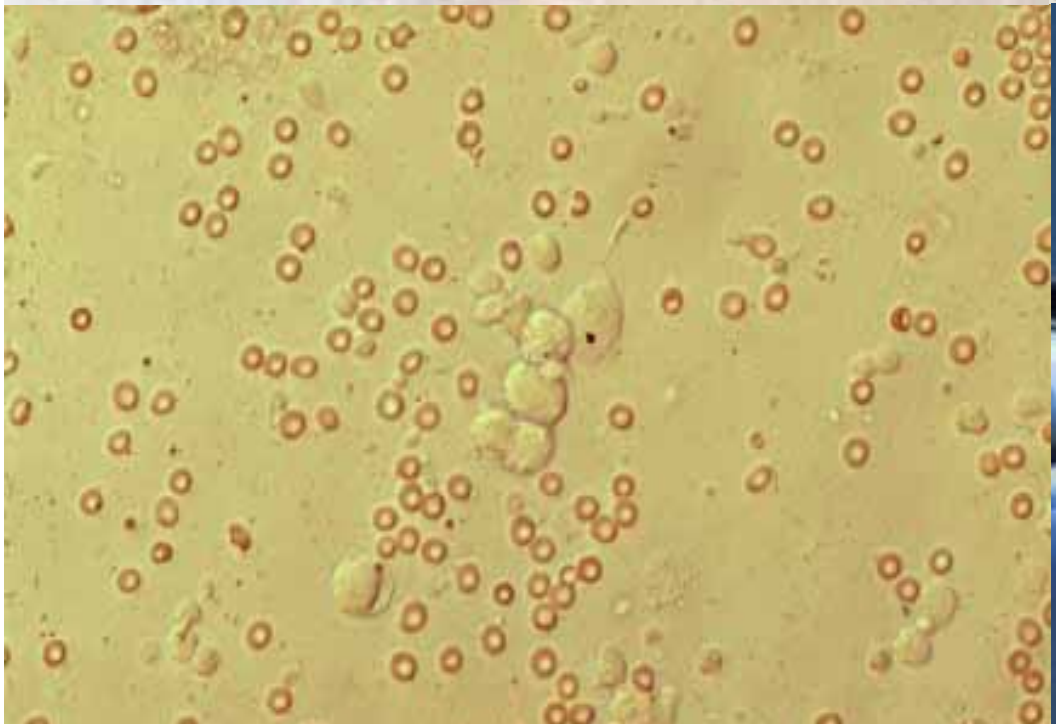
Contributed by R. L. Brinster, June 2, 2000







Clinical application at CRM Brussels

Counselling

- *indication: role oncologist and pediatrician*
- *experimental procedure: feasability ?*
- *safety: contamination with malignant cells*
- *need testisbiopsy or orchidectomy*
- *informed consent both parents*

Brussels' testicular stem cell transplantation project

Veerle Frederickx, MSc.

Ellen Goossens, MSc.

Gert De Block, MT.

André Van Steirteghem, MD. PhD.

Herman Tournaye, MD. PhD.