Spermatogonial stem cells: Hope or hype?

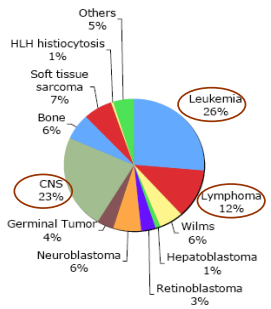
An update on spermatogonial stem cell banking and transplantation

Universitair Ziekenhuis Brussel
Vrije Universiteit Brussel

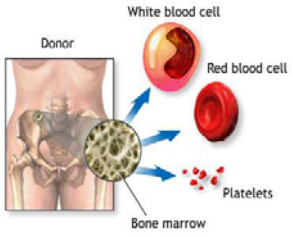
Ellen Goossens, PhD

Why ?

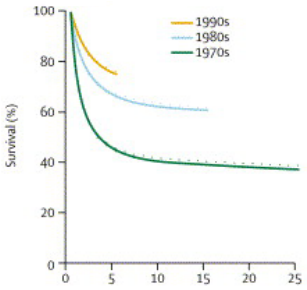
Why ?



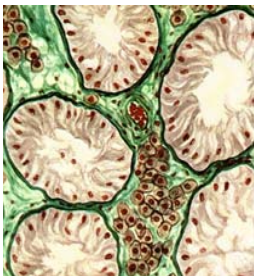
Why ?

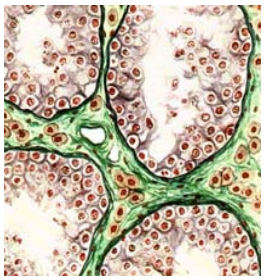

White blood cell
Donor
Red blood cell
Platelets
Bone marrow

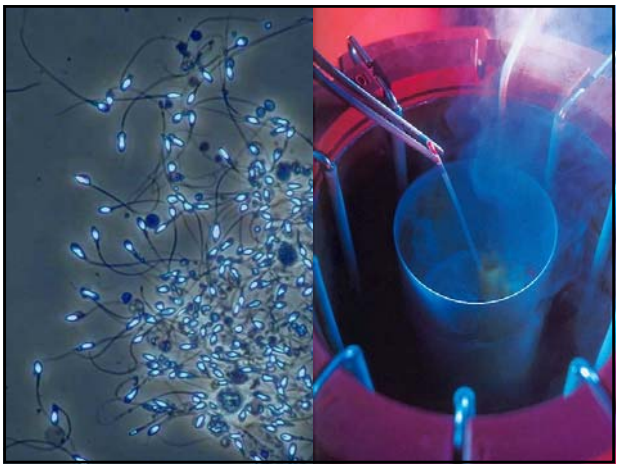
Why ?

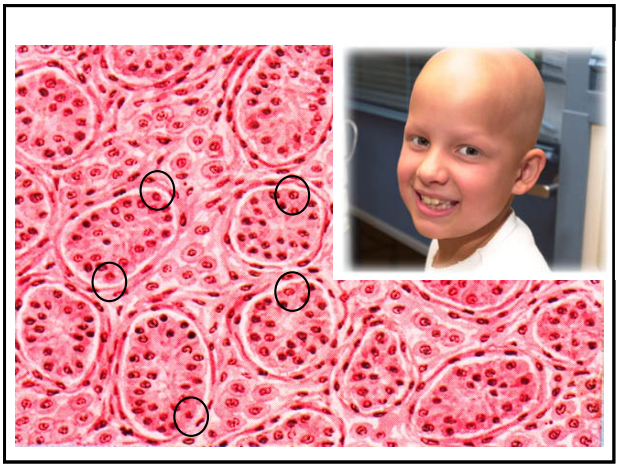

100
80
60
40
20
0
Survival (%)
0
5
10
15
20
25
1990s
1980s
1970s

Why ?

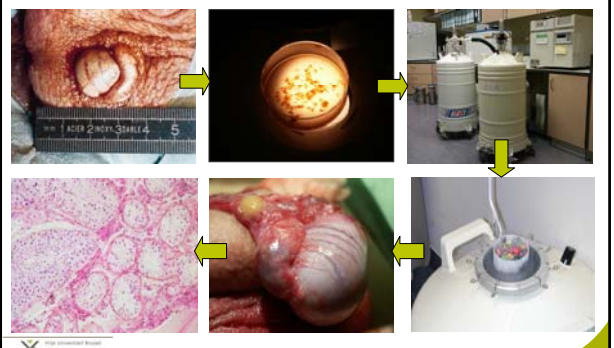








How ?



Step by step

1. Long term storage
   - → Cryopreservation
   - → Culture

(2. Malignant cell removal)

3. Transplantation protocol

4. Efficiency of reproduction

5. Safety of reproduction

Step by step

1. Long term storage
   - → Cryopreservation
   - → Culture

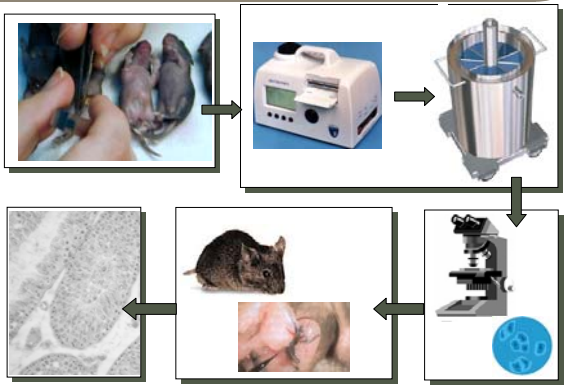
(2. Malignant cell removal)

3. Transplantation protocol

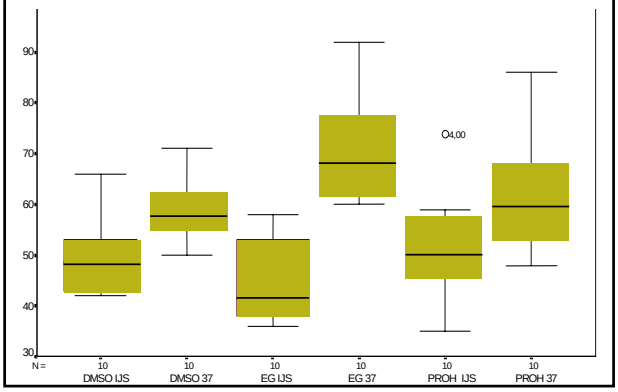
4. Efficiency of reproduction

5. Safety of reproduction

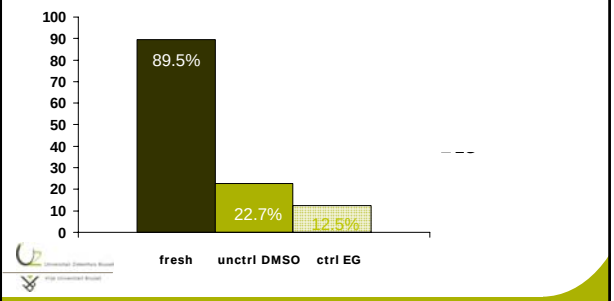
Cryopreservation of cell suspensions



Cryopreservation of cell suspensions

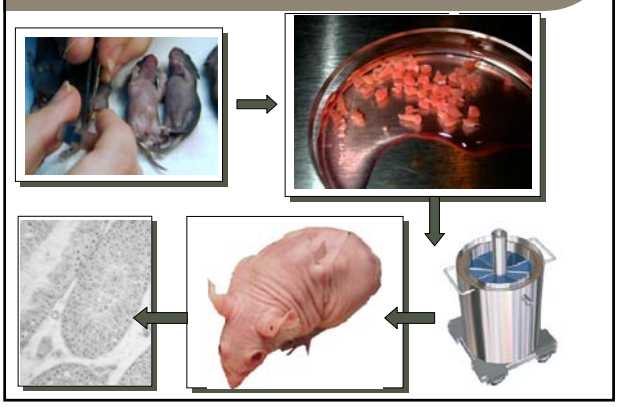


Cryopreservation of cell suspensions



14 ESHRE Campus Andrology 16 November 2007

Cryopreservation of tissue



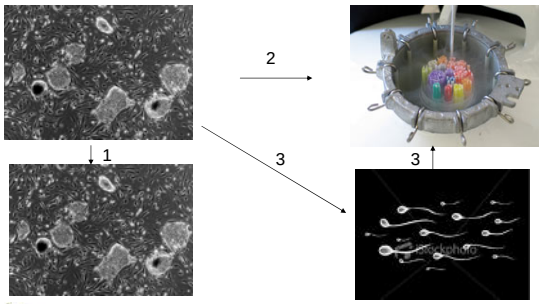
Cryopreservation of tissue

	No. of grafts	Sclerosis / atrophy	Most advanced cell stage		No. of tubules anaysed	No. spz containing tubules	No. of damaged tubules
			spg	spz			
Fresh	28	0 (0%)	-	28 (100%)	1538	354 (23%)	603 (39%)
EG	14	3 (21%)	-	11 (79%)	547	176 (32%)	231 (42%)
DMSO	14	1 (7%)	2 (14%)	11 (79%)	949	308 (32%)	302 (32%)

Conclusion

- DMSO is the cryoprotectant of choice for banking spermatogonial stem cells
- Tissue cryopreservation seems to result in better fertility preservation
- The protocol needs to be improved to achieve a better maintainance of tissue architecture and spermatogonial stem cell function

Culture



Step by step

1. Long term storage
   - → Cryopreservation
   - → Culture

(2. Malignant cell removal)

3. Transplantation protocol

4. Efficiency of reproduction

5. Safety of reproduction

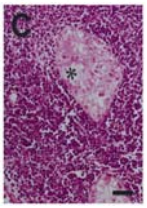
Malignant cell removal

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

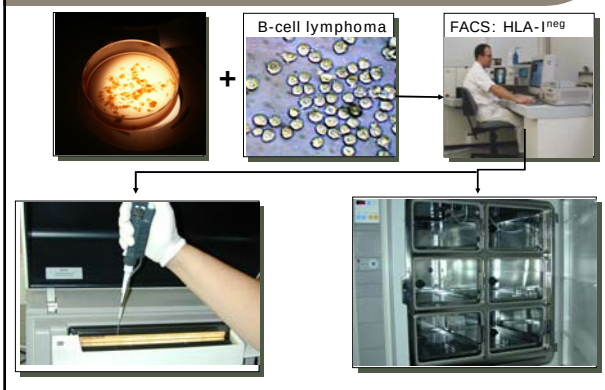
Kirsi Jahnukainen,[2] Mi Hou, Cecilia Petersen, Brian Setchell, and Olle Söder

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 4-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.



Malignant cell removal



Malignant cell removal

	HLA class 1 positivity (%)		Tumour growth in culture (%)		PCR
Patiënt	Before FACS	After FACS	[illegible]	[illegible]	[illegible]
1	12,03	0,09	[illegible]	[illegible]	[illegible]
2	7,72	0,25	[illegible]	[illegible]	[illegible]
3	16,31	2,26	[illegible]	[illegible]	[illegible]
4	5,79	0,49	[illegible]	[illegible]	[illegible]
5	17,14	0,88	[illegible]	[illegible]	[illegible]

Conclusion

- FACS for HLA-I alone is not effecient enough in decontaminating the cell suspension
- Additional markers will be necessary
- Other decontamination strategies need to be explored

Step by step

1. Long term storage
   - → Cryopreservation
   - → Culture

(2. Malignant cell removal)

3. Transplantation protocol

4. Efficiency of reproduction

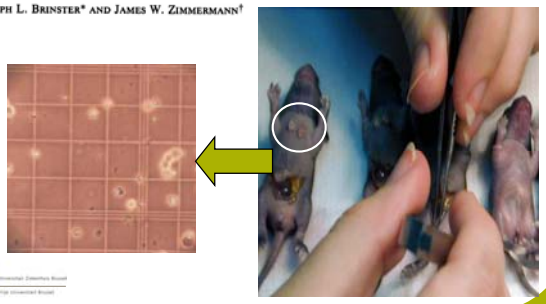
5. Safety of reproduction

Spermatogonial stem cell transplantation

Spermatogenesis following male germ-cell transplantation

(spermatogonia/stem cells/testes/transgenic mice)

RALPH L. BRINSTER* AND JAMES W. ZIMMERMANN†

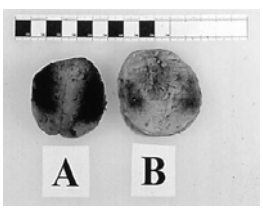


3-10 µl (~20 stem cells)

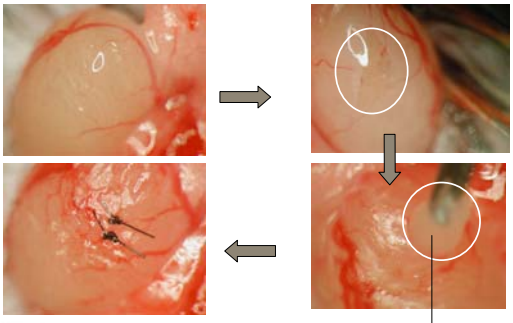
Spermatogonial stem cell transplantation

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]



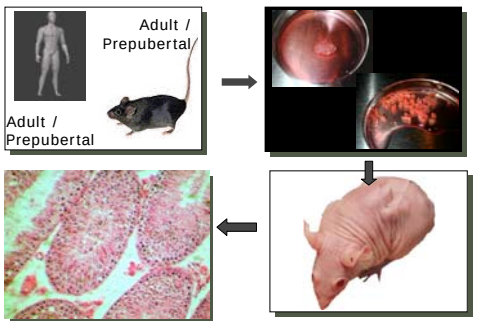
Intratesticular grafting



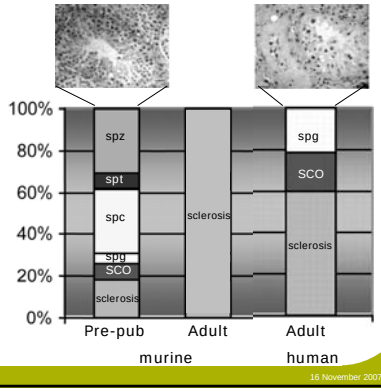
Intratesticular grafting vs SSCT

	N° of transplantations	N° of testes with donor spermatogenesis (%)	Total colony lenght/testis (mm)
SSCT	9	5 (55)	41.3
Intratesticular grafting	16	16 (100)	125.3
[illegible]	7	7 (100)	98.8

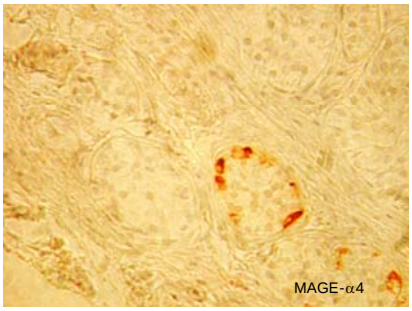
Ectopic (xeno)grafting



(Xeno)grafting



Prepubertal human to mouse



Conclusion

- SSCT and intratesticular grafting are both resulting in reconstitution of a feasible degree of spermatogenesis
- Intratesticular grafting is easier to perform
- Since xenografting holds a risk for zoonosis, it should only be considered for detecting malignant cells in the testis tissue

Step by step

1. Long term storage
   - → Cryopreservation
   - → Culture

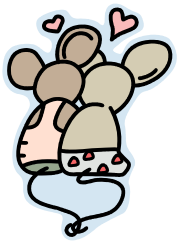
(2. Malignant cell removal)

3. Transplantation protocol

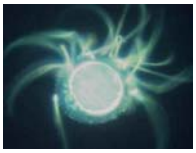
4. Efficiency of reproduction

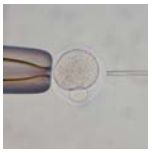
5. Safety of reproduction

Efficiency



In-vivo conception





IVF ICSI

Assisted reproduction

In-vivo conception

	No. of females with a vaginal plug	Pregnancy rate [%]	No. of foetuses
After transplantation	17	6 (35)	17 (2.8)
Fertile controls	10	9 (90)	76 (8.4)

P<0.01 *P*<0.01

Offspring

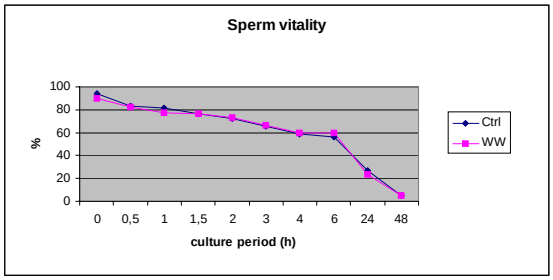
Litter size	Second generation (mean)	Third generation (mean)
After SSCT	5-11 (8.0)	6-8 (7.2)
Fertile controls	5-11 (7.6)	4-8 (5.3)

In-vitro fertilisation

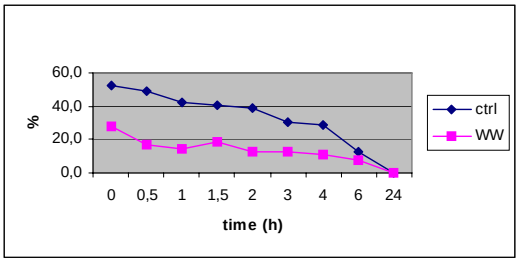
	No. of oocytes	Fertilisation rate (%)	Blastocyst rate (%)
After transplantation	154	88 (57)	24 (27)
Fertile controls	195	155 (79)	88 (57)

P<0.00001 *P*<0.00001

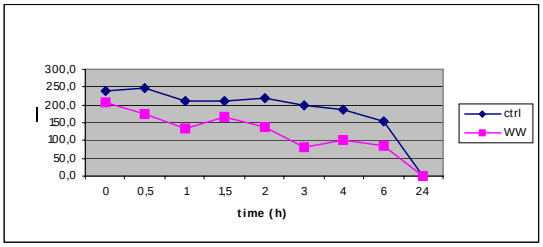
Vitality



Motility semen sample



Motility individual sperm cell



ICSI

	No. of oocytes	Fertilisation rate (%)	Blastocyst rate (%)
After transplantatie	187	57 (69)	17 (30)
Fertile controls	112	38 (61)	14 (37)

Step by step

1. Long term storage
   - → Cryopreservation
   - → Culture

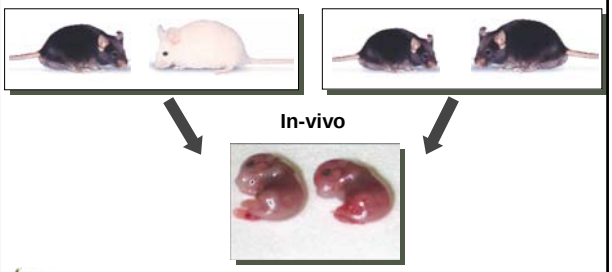
(2. Malignant cell removal)

3. Transplantation protocol

4. Efficiency of reproduction

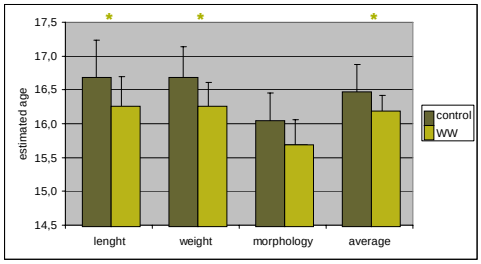
5. Safety of reproduction

Safety



Safety

*P<0.01



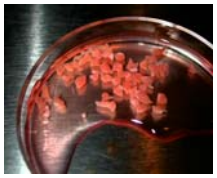
Hope or hype?

Although a number of questions remain unanswered and several aspects of spermtogonial stem cell banking and transplantation need to be improved, we believe in the applications which are associated with spermatogonial stem cells.

HOPE

Clinical cryopreservation program

Keros et al., 2007



Tissue pieces (1-10 mm³)

Cryoprotectant: Hank's Balanced Salt Solution + 5% DMSO + 5% HSA

Clinical experience

Requests: 15

Accepted	: 8	sickle-cell anemia (4) leukemia (3) idiopathic aplastic anemia (1)
Refused	: 1	leukemia (pre-treated)
Parents declined after counseling	: 6	leukemia (4) sickle-cell anemia (2)

Transplantation group