Transplantation of testicular stem cell suspensions and testicular grafting

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A different kind of medical school. A different kind of medicine.
A_{dark} spermatogonium

Spermatogonia Spermatocytes Spermatids

Stem Cell Differentiating Cell Meiotic Cells Postmeiotic Cells

mitotically inactive proliferating
Monkey Spermatogonia: Clonal expansion in whole mounts

Confocal Microscopy: BrdU and acrosin IHC

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

**Spermatogonia**
- Stem cell
- quiescent
- proliferating

**Spermatocytes**
- Differentiating Cells

**Spermatids**
- Meiotic Cells
- Postmeiotic Cells

DNA-damage ?

X-ray/chemo-sensitive
Male Infertility after Cancer Therapy

A Stem Cell Disease?
Testis development after cancer therapy during childhood

Immature Testis

Period of treatment

Sertoli-cell-only Syndrome
*(Side effect after onological therapy)*

Normal adult testis
Cynomolgus monkey testis after testicular X-irradiation

Focal Sertoli Cell Only: All or Nothing
Stem Cell Treatment Option I: Protection

1. Suppress stem cell turnover

2. Expand the stem cell population

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

3. Protect genetic integrity of spermatogonia

Mutation frequency declines during spermatogenesis in young mice but increases in old mice.
Evidence for selective advantage of pathogenic FGFR2 mutations in the male germ line.
Future Gonadal Protection Strategies

Cryopreservation

Oncological Patient
Future Gonadal Protection Strategies

Single Cell Suspension

Oncological Patient

Cryopreservation

Testis Tissue
Future Gonadal Protection Strategies

- Cryopreservation
- Single Cell Suspension
- Analysis of Tumour Cell Contamination
- Testis Tissue
- Oncological Patient
- Cell Sorting
- Autologous Germ Cell Transplantation
Future Gonadal Protection Strategies

- Cryopreservation
- Single Cell Suspension
  - Analysis of Tumour Cell Contamination
  - Cell Sorting
- Testis Tissue
  - Oncological Patient
  - Xenografting
  - Sperm
  - ICSI/IVF
- Autologous Germ Cell Transplantation
Undifferentiated Spermatogonia are stem cells: Potential for (re)colonization and (re)population

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

Ectopic Testicular Grafting
Transfer of the spermatogonium with its niche from the donor to an ectopic site in the recipient.
Stem Cell Treatment Option II: Germ Cell Transplantation

Aim: Extracorporeal storage and reinjection of target cells
Development of Germ Cell Transplantation

- **1994**  
  Spermatogenesis following male germ cell transplantation.  
  (Brinster and Zimmermann, PNAS 91: 11298)

- **1996**  
  Rat spermatogenesis in mouse testis.  
  (Clouthier et al., Nature 381: 418)

- **1996**  
  Reconstitution of spermatogenesis from frozen spermatogonial stem cells.  
  (Avarbock et al., Nat Med 2: 693)

- **1998**  
  Culture of mouse spermatogonial stem cells.  
  (Nagano et al., Tissue & Cell 30: 389)

- **1999**  
  Germ cell transfer into rat, bovine, monkey and human testes.  
  (Schlatt et al., Hum Reprod 14: 144)

- **2001**  
  Primate spermatogonial stem cells colonize mouse testes.  
  (Nagano et al., Biol Reprod 64: 1409)

- **2001**  
  Transgenic mice produced by retroviral transduction of male germ-line stem cells.  
  (Nagano et al. PNAS 98:13090)

- **2002**  
  Germ cell transplantation into X-irradiated monkey testes.  
  (Schlatt et al. Hum Reprod (17: 55)
Xenologous transplantation of primate spermatogonia

Baboon germ cell colonising mouse testes

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

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

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

No Colonisation

Colonisation
Germ Cell Infusion into the Monkey Testis

Microinjection of seminiferous tubules: difficult and inefficient

Injections into efferent ducts: surgically demanding and inefficient

Injections into the rete testis: Easy, efficient and reproducible

involuted recipient testis ultrasound guidance
Germ Cell Transfer into Rat, Bovine, Monkey and Human Testes
Schlatt et al., Hum Reprod 14: 144-150 (1999)
Testis Volume Following Irradiation and Germ Cell Transfer

Testis volume (ml)

- Right Testis
- Left Testis

Germ cell transfer

Irradiation

Testis volume (ml)

Days

Days

Days

Days
Individual testis volume 35 weeks after germ cell transfer

Testis volume (ml)
Histology of monkey testes 35 weeks after germ cell transfer

Right testis
Germ cell transfer

Left Testis
Saline Injection
Warning!!!

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

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

Procedure for Enrichment of Differentiating Spermatogonia

- Enzymatic digestion of testis tissue

- Testicular cell suspension

- Spermatogonium

- Tumour cell

- Magnetic cell separation

- Positive selection

- Negative selection

- Enrichment of spermatogonia

- Depletion of tumour cells

C-kit Immunoreactivity in Marmoset Testes
Magnetic Cell Sorting of Primate Spermatogonia

Human Testes

Marmoset Testes

<table>
<thead>
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<th>Experiment</th>
<th>MACS separation fraction</th>
<th>1C population (%)</th>
<th>2C population (%)</th>
<th>S-phase population (%)</th>
<th>4C population (%)</th>
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</table>

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

Aim: Extracorporeal storage and activation of testicular tissue

Stem cell plus niche
Xeno/Auto-grafting

Honaramooz et al.,
Nature 418: 778-781
(2002)

Sperm from neonatal mammalian testes grafted in mice.
Schlatt et al., Progeny from sperm obtained after ectopic grafting of neonatal mouse testes. Biol Reprod 2003 (in press)
Sperm morphology and oocyte-activating competence of sperm from testicular grafts

Histology of grafts from pig and goat testis

(All oocytes are from mice)
Xenografting of primate testicular tissue

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

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

Results
Monkey: Initiation of testis function
Man: Maintenance of tubular structure
No restoration of spermatogenesis
Stem Cell Treatment Option IV: Artificial Gametes

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


Embryonic Germ Cells in Testicular Cell Cultures

GFP-expression in mouse embryonic germ cells

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