Experimental Methods to Preserve and Restore Male Fertility

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Ovarian Club XII
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Anatomy of the Testis

Seminiferous tubule

Testis

Seminiferous tubule (cross section)
Fertility after Cancer

• Chemotherapy and radiation treatments for cancer or other conditions can cause permanent infertility

• Adult women and men can cryopreserve eggs, sperm or embryos, which can be used in the future to achieve pregnancy

• These options are not available to preadolescent boys and girls who are not producing mature eggs or sperm

• The five year survival rate for preadolescent cancer patients is 85% (SEER)

• Prepubertal boys have spermatogonial stem cells in their testes that are poised to initiate sperm production at puberty

• Centers world wide are preserving testicular tissue for boys and ovarian tissues for girls in anticipation that new reproductive technologies will be available for them in the future
Fertility Preservation Program in Pittsburgh
(https://fertilitypreservationpittsburgh.org/)

Cryopreserved since 2011
• Testicular tissue: 192 boys
• Ovarian tissue: 39 girls

Our Mission
• Educate Patients and Physicians
• Provide fertility preservation options
• Pioneer new technologies and translate them to the clinic
• Train the next generation of FP experts
Our National/International Impact

Pittsburgh Coordinating Center

US Coordinated Centers: Washington DC, Chicago, LA, Cincinnati, Mayo, Milwaukee

International Collaborators: Ben-Gurion University, Israel; Bio Cell, Jordan

Male patients (192 total)

Female patients (39 total)
Testicular Tissue Freezing

9-20 mm³ tissue pieces

Controlled rate slow freezing
Fertility Preservation for Boys with Cancer

A. Semen or TESE
- Semen analysis
- SSC Culture
- SSC transplantation
- IUI, IVF, ICSI

B. Testis biopsy
- Cell suspension
- De Novo Testicular Morphogenesis
- recover sperm
- ICSI
- Autologous grafting
- recover sperm
- ICSI
- Xenografting
- recover sperm
- ICSI
- Testicular tissue
- Tissue culture
- recover sperm
- ICSI

C. Somatic Tissue
- IPS cells
- Germ line stem cells (GSCs)
- GSC transplantation
- Intercourse
- Haploid germ cells
- ICSI
Monkey model of cancer survivorship

Hermann et al., *Stem Cells*, 2007

1. Biopsy
2. Chemotherapy
3. Cryopreservation
4. Semen analysis
Spermatogenic deficits
Autologous SSC Transplantation

1. Biopsy
2. Chemotherapy
3. Cryogenic Preservation
4. Auto Transplant
5. Mark donor cells with GFP
SSC transplantation (rodent vs primate)
Ultrasound-guided rete testis injection
Regeneration of Spermatogenesis

Hermann et al., *Cell Stem Cell*, 2012

**A**

![Graph showing sperm count over weeks relative to transplant](image)

- **SSC Transplantation**

**B**

<table>
<thead>
<tr>
<th>Weeks Post-Transplant</th>
<th>Controls</th>
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<td>M037 DNA</td>
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<td>Lentivirus</td>
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Hermann et al., *Cell Stem Cell*, 2012
Spermatogonial stem cell transplantation appears technically feasible in higher primates.
SSC Transplantation is Ready for the Clinic

• SSC transplantation in humans already happened
  ✓ Testicular cells suspensions frozen for 12 Hodgkin's patients (Radford et al., 1999)
  ✓ Transplant later performed on 7 of those patients (Brooke et al., 2001; Radford et al., 2003)
  ✓ Technology was in early stages in those days and had not been performed in any large animal species

• SSC transplantation is ready for the clinic now
  ✓ The technology is mature – proven in mice, rats, hamsters, dogs, cats, goats, sheep, pigs and monkeys
  ✓ We have the patients – more than 1000 so far worldwide (192 at Magee & affiliates)

• Optimal patient to transplant/Optimal age to transplant
  ✓ Patients receiving bone marrow transplant for benign disease
  ✓ Best time to transplant: during puberty
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Gassei and Orwig
Fertil Steril 2016
Autologous Grafting of Prepubertal Testis Tissue

Chemotherapy → Biopsy → Cryogenic Storage → Autologous Grafting
Post-meiotic germ cells in the adult Rhesus testis
Pre-Graft Histology
Autologous Grafting of Prepubertal Testis Tissue

- **Recipient**
  - ✓ Chemotherapy treated
  - ✓ Castrated

- **Grafts**
  - ✓ 4 tissues per site
  - ✓ 6 sites on back
  - ✓ 2 sites in scrotum

- **Experiments**
  - ✓ Fresh vs. Frozen
  - ✓ Back vs. Scrotum
  - ✓ Matrigel vs. Not
Testosterone

13-008 Testosterone Level

Testosterone ng/ml

Date


Autologous Grafting
Graft growth and appearance
Recovered Graft
(8-10 months post-graft)

Very Fibrotic
Complete spermatogenesis from autologous grafts
Preimplantation embryo development after ICSI

- ICSI: 11/10/2017
- Embryo transfer: 11/17/2017
- Preg. Confirmed: 12/15/2017
Grady

Graft-derived baby

Born April 16, 2018 by c-section

World’s first baby born from autologously grafted, frozen and thawed testicular tissue
Future Directions

- Developmental assessments of Grady
- Assess Grady’s fertility and health of her progeny
- Repeat experiment in animals with testes to model the prepubertal cancer survivor
- Repeat with human tissues
Conclusion

• Fertility preservation is a multi-disciplinary effort
• It does not always fits nicely in the IVF fertility clinic workflow (flexibility is required)
• Efficient communication is key
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Patients