Oncofertility: The Story of the Spermatogonial Stem Cell

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Sperm Cryopreservation

• Patients are now commonly requesting sperm extractions from terminally ill cancer patients

• 16 yo with lymphoma, intubated in ICU

• This is not the ideal time to think about banking…
Overview

• Cancer treatment and reproduction
• Focus on chemotherapy NOT XRT, surgery
• Spermatogonial stem cells, population health and the future
Background

• 20,000 reproductive age men treated annually

• Focus on Quality of Life after treatment

• ASCO Guidelines: Offer Sperm Banking!

• No such thing as “non-toxic” chemotherapy, many infertile after treatment (24% azoospermic)

Sperm Banking

- Can bank only 1 sperm and use for ICSI

- Lose 50-60% sperm with freeze-thaw, can freeze indefinitely

- Need >5M motile sperm post thaw for IUI

- OncoTESE $\rightarrow$ cryopreservation $\rightarrow$ IVF

Nangia et al. Fert Ster 2013, Hone et al. Hum Reprod 2004,
<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Oligozoospermia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular</td>
<td>28</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma</td>
<td>25</td>
</tr>
<tr>
<td>Leukemia</td>
<td>57</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>33</td>
</tr>
</tbody>
</table>

### TABLE 4

Predictors of men who can use intrauterine insemination based on total motile count $\geq 5 \times 10^6$.

<table>
<thead>
<tr>
<th>Group</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.00</td>
<td>1.001–1.001</td>
</tr>
<tr>
<td>Semen total motile sperm</td>
<td>1.00</td>
<td>1.000–1.000</td>
</tr>
<tr>
<td>Testicular cancer</td>
<td>0.84</td>
<td>0.732–0.959</td>
</tr>
</tbody>
</table>

Note: All other cancer diagnoses were found to be not statistically significant and were adjusted for in regression analysis.

“Onco-TESE”

• Testicular sperm extraction in an azoospermic men with cancer
  – Approximately 50% recovery rate
• Prepubertal boys prior to treatment
  – Cryopreservation of immature testicular tissue
  – Future use for stem cell spermatogonia?

Spermatogonial Stem Cells & Somatic Health

Epigenetics/Environment

Human SSC
Baseline de-novo mut. rate

Sperm
Population of 100M+ sperm Population wide effect?

Cancer Risk elevated
Ego

Increased: cancer, infertility, chemo
Same Phenotype?

Population-wide or SSC specific?

Familial? Progeny?
Congenital Anomalies in Offspring

- 2-3% incidence of congenital anomalies in general population
- Similar rates in offspring of cancer survivors
- No ↑ risk of de novo cancer in progeny

Healthy offspring can be expected

Li et al. JCI, 1979.
Green et al. JCO, 2009.
### Subfertile Cohort

<table>
<thead>
<tr>
<th>N</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>39,480</td>
<td>Relatives of subfertile individuals</td>
</tr>
<tr>
<td>274,190</td>
<td>Historical subfertile individuals (married, with complete fertility information born between 1910-1925, verified nulliparity)</td>
</tr>
<tr>
<td>10,614</td>
<td>Children of IVF/ICSI/IUI</td>
</tr>
<tr>
<td>50,000</td>
<td>Fertile controls (age and SES matched)</td>
</tr>
<tr>
<td>7,000</td>
<td>Biobank specimens (linked to UPDB)</td>
</tr>
</tbody>
</table>

- **BMI Data**: DLD
- **Medical Records and Vital Status**: UDOH
- **Family History Data**: GSU
- **Utah Population Database (UPDB)**
- **Cancer Diagnoses**: UCR, CDRI
- **Medical Records**: UUHSC, IH
- **Driver’s License Division**: DLD
- **Genealogical Society of Utah**: GSU
- **Cancer Data Registry of Idaho**: CDRI
- **SEER Utah Cancer Registry**: UCR

- Banking 15-20 testicular tissue specimens monthly and >50 sperm samples monthly
Cancer in relatives of infertile men

- 13k men with semen analysis and fertile controls with 80k siblings, 435k cousins.
- Oligozoospermia → 2x increased risk of any childhood cancer in sibs NOT cousins
- **Are people predisposed to cancer predisposed to infertility?**

Anderson et al, J. Urol, accepted
Are the sperm relevant?

Non-genetic Paternal Contribution to Embryo Quality and Offspring Health

Fertility?
Embryogenesis Quality?
Risk to offspring?
Lamarckian Evolution & Chemo

• Mice taught to fear an odor, both their offspring and the next generation are born fearing it.

• Gene for an olfactory receptor activated by the odor demethylated in the germ line
Germline stem cell therapy

- Testis biopsy
- Isolation of SSCs
- In vitro expansion of SSCs
- Correction of gene mutation
- SSC transplantation
- Chemotherapy/radiation treatment (optional)
- Patient (cancer or gene mutation)
- Sperm production

Kubato & Brinster 2006
Pluripotency versus Unipotency

Egg → Zygote → ES cells → PGCs

Unipotency (normally, only make sperm)

'Latent' Pluripotency (evidence: germ cell tumors, GPSCs)

Profile RNA, DNAme, nucleosomes (+chemical marking/mod)
Loss of germ cell identity after culture

Zheng et al., 2014
Mouse vs. human SSC development

Shinaohara, 2013; Valli et al., 2014
Novel Signaling Pathways in Human SSCs

<table>
<thead>
<tr>
<th>Ligands used for mouse culture</th>
<th>LIF</th>
<th>GDNF</th>
<th>FGF2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FZD3, FZD7, ROR2, LRP6, CSNK1A1, AXIN2, APC, GSK3B</td>
<td>LIFR, JAK1</td>
<td>GFRA1</td>
<td>FGFR1, FGFR2, FGFR3</td>
</tr>
<tr>
<td>DVL, RAC3, RAC2, DAAM1, RHOA, ROCK2, b-catenin</td>
<td>STAT3, Klf4, Axin2</td>
<td>PTEN, PI3K</td>
<td>MEKs, SMAD1/5/9, Etv5, Bcl6, FOXO, PI3K</td>
</tr>
<tr>
<td>higher expressed in SSEA4+ SSCs</td>
<td>higher expressed in KIT+ SSCs</td>
<td>highly expressed in both</td>
<td></td>
</tr>
</tbody>
</table>

Guo, Carrell, Hotaling & Cairns, submitted to Cell
Goals

1. Understand Identity, Pluripotency, and Differentiation of Human Spermatogonial Stem Cell (SSC) (measure gene expression, signaling, and chromatin)

2. Define human SSC culture, and use molecular and genetic tools to further Goals #1 and #3.

3. Understand how germ cell tumors form, and how infertility might be treated?

- Dmrt1
- Kit
- Kras
- Cdc27
Conclusions

• Cancer diagnosis → sperm banking offered
• There is no fertility friendly chemo
• Impact of chemo is likely transgenerational
• Future of the field lies in culturing spermatogonial stem cells