Bioprosthetics: 3D printing as ovary restoration strategy

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Ovarian Club XII – December 2018 Hong Kong
Why pursue fertility preservation?

- American Society of Clinical Oncology & American Society for Reproductive Medicine & American Academy of Pediatrics recommend a **discussion about fertility preservation** for male and female patients undergoing gonadotoxic treatments.

- Infertility is one of the **primary concerns** of cancer survivors:
  - 75% of young adults who are childless at the time of diagnosis report a **desire to parent** in the future.
  - Cancer survivors report **PTSD symptoms** related to infertility as long as 10 years post-treatment.
  - Parents want **fertility preservation options presented** regardless of infertility risk/prognosis*.

Gonadotoxicity from Disease or Treatments

- **Childhood Cancer Survivors** are significantly more likely to be **infertile** or have **difficulty getting pregnant** than their siblings.

- **Genetic Causes for gonadal dysfunction:**
  - Turner Syndrome
  - Fragile X Syndrome
  - Germ cell tumor
  - Galactosemia

7 out of 8 children with cancer will survive.

Cancer.gov (0-19 y.o., rev: 1/13/15); Woodruff (2013) Nat Rev Endo

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Ovarian tissue cryopreservation (OTC)

- Ann & Robert H. Lurie Children’s Hospital: IRB-approved research protocol since 2010

- Study aims:
  - To cryopreserve gonadal tissue for patient’s own future use
  - Obtain research tissue biopsy (optional) and processing media
    - For optimizing freeze/thaw techniques
    - For developing technology for immature follicle maturation
    - For establishing pediatric-specific tissue processing techniques
  - Long term patient follow-up for timing and type of tissue and hormone restoration
Protocol Inclusion Criteria

- Pre or post pubertal individual
- Will undergo imminent surgery, chemotherapy or radiation therapy that has implications on future fertility and reproductive hormone potential:
  - any health condition or malignancy that requires removal of all or part of one or both ovaries,
  - whole abdomen or pelvic irradiation ≥10Gy in post-pubertal girls or ≥15Gy in pre-pubertal girls
  - total body irradiation, and
  - alkylating-intensive chemotherapy
    - cyclophosphamide cumulative dose ≥7.5 g/m²
    - any treatment regimen containing procarbazine
    - busulfan cumulative dose >600 mg/m²
    - alkylating chemotherapy conditioning prior to stem cell transplantation
    - combination of any alkylating agent with total body irradiation or whole abdomen or pelvic radiation
    - cranial radiation ≥30 Gy
    - summed alkylating agent dose score ≥3 (Green et al., 2009)
    - cyclophosphamide equivalent dose (CED) ≥ 4,000 mg/m² (Green et al., 2014)
- Patient may have newly diagnosed or relapsed disease.
Fertility Preservation: Child

Release of EGGS at Puberty

- Primordial Follicle
- Primary Follicle
- Secondary Follicle
- Early Antral Follicle
- Preovulatory Follicle
- Resumption of Meiosis
- Ovulation
- Meiosis Arrest at Metaphase II
- Corpus Luteum

National Physicians Cooperative Patient: 4 y.o. scale bar = 100 \( \mu \text{m} \)
Oocytes are a Finite Resource

## Sex Hormones

<table>
<thead>
<tr>
<th>System / Organ</th>
<th>Disease / Disorder Developed in POI or menopause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>Decreased bone mass, Osteoporosis</td>
</tr>
<tr>
<td>Muscle</td>
<td>Decreased mass, strength</td>
</tr>
<tr>
<td>Vascular</td>
<td>High blood pressure</td>
</tr>
<tr>
<td>Skin</td>
<td>Decreased laxity, wound healing</td>
</tr>
<tr>
<td>Brain</td>
<td>Decreased short and long-term memory</td>
</tr>
</tbody>
</table>

Ovarian tissue preservation

Unilateral Oophorectomy

- Remaining ovary compensates and maintains same level of hormones
- There is little difference in age at menopause
- Remaining ovary can release more eggs when stimulated for ART

Re-implanting post-pubertal ovarian tissue restores fertility & hormones.

- > 130 reported live births
- Hormone production up to 12 years, average 2 – 5 years
- Possibility of reintroducing cancer cells
- Need to improve transplant and *in vitro* maturation to restore function long-term in all patients

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3. Laronda, M. M. *et al.* *Biomaterials* 50, 20–29 (2015); Scale bar = 50 µm

*Acute lymphoblastic leukemia cells in ovarian cortical tissue*
Engineering an Ovary

Isolate desired cells; Remove cancer

Primordial Follicle

Oocyte granulosa

Support structure

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National Physicians Cooperative Patient: 4 y.o. scale bar = 100 µm
Matrices to graft ovarian follicles

- Telfer & Gosden (1990) Repro.
  - collagen-encapsulated pre-antral follicles
  - kidney capsule
  - embryos after stimulation and IVF

  - plasma clot-encapsulated primordial follicles
  - ovarian bursa (6-12 wks)
  - pups, no genetic verification from transplant, few small follicles left

  - fibrin(-HBP)-VEGF encapsulated ovarian tissue pieces OR primordial follicles
  - ovarian bursa (14-21 days)
  - pups

  - ovarian ECM scaffold primordial follicles & granulosa cells
  - kidney capsule (21 days)
  - antral follicles, few follicles left


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Decellularized Ovary

Primary ovarian cells cultured on ovarian ECM develop secretory blebs and follicle-like pores.

doi:10.1016/j.biomaterials.2015.01.051
Puberty was initiated in ovariectomized mice with ovary grafts.

- Initiated Puberty
- Supported Oocyte Differentiation
- Increasing Serum Estradiol
Development of Scaffold

Scaffold Criteria:

• Create a 3D feel
• Mechanically rigid to handle during implantation
• Open pores for nutrient flow, growth and ovulation
• Bioactive, bio-safe material

Gelatin scaffold with a microporous architecture

Different 3D printed advancing angles result in different pore design.

Intersecting (30°)  Intersecting (60°)  Grid (90°)

Follicles prefer more contact points.

Scale bar = 50 µm


Northwestern Medicine
Feinberg School of Medicine

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Follicles interact with scaffolds pockets


Vinnculin / dapi

Stroma / cytoskeleton / DNA

100 μm

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Follicles release MII eggs in vitro.

Bioprosthetic Ovary Transplant

- Both ovaries removed from adult mouse
- Rest of reproductive tract remains in tact
- 3D printed scaffold with seeded with follicles

Bioprosthesis 8wks post-surgery

Scale bar = 200 and 50 µm

Bioprosthesis 3wks post-surgery

Bioprosthetic ovaries develop functional vasculature.


Scale bar = 50 µm
Bioprosthetic ovary recipients reared healthy pups.

Implanted into Non-GREEN Ovariectomized Mouse

Bioprosthesis in bursa


GREEN pup from Ovary Implant
(- pup from different litter)

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Bioprosthetic ovary recipients reared healthy pups.

Females with Bioprosthetic Ovary:
- Cycling (vaginal histology)
- 3 had pups from transplant
- Supported pups with milk until wean
- Pups produced Grand-pups

Transplant recipient (EGFP-) with EGFP+ pup


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Feinberg School of Medicine
Next Steps: Compartmentalization

Bovine ovary

Next Steps: Compartmentalization

1. Define the differences between cortex and medulla
2. Observe folliculogenesis under altered environments
3. Modify ink / scaffold design to achieve desired outcome

- Slice ovaries
- Decellularize
- Create matrisome map

Northwestern Proteomics
Next Steps: Compartmentalization

- Printing different support cells
- Printing different structural proteins
- Printing different rigidity properties

stroma / cytoskeleton
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Artificial Ovary: patent application #15/545,175

LarondaLab.org

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In vitro Maturation of Small Follicles

- **Isolated Follicle Culture**
  - Growth of secondary follicles to healthy MII eggs

- **Cortical Strip Culture**
  - Maintain health of primordial follicles
  - UK/US group obtained MII eggs from cortical strip follicles

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