Oncofertility: Fertility Preservation and Restoration Progress Toward an Ovarian Bioprosthesis
Fertility Needs in Pediatric Cancer Patients

Patient Sample 4 y.o.; scale bar = 100 μm; National Physicians Cooperative

Ovarian cortical tissue from NPC participants;
Scale bar = 50 μm Laronda, et al. (2015) Biomaterials
Fertility Needs in Pediatric Cancer Patients

- Follicle maturation
- High fidelity oocyte maturation
- Endocrine hormone production
- Pubertal transition
- Cyclical hormones to support systemic health
Encapsulated In Vitro Follicle Growth (eIVFG)

Lonnie Shea, Ph.D.

Shea and Woodruff, 2000-present
Follicles have different cell types

Follicles produce steroid hormones

Xu et al, Tiss. Eng. 2006
Xu et al, Human Repro. 2009
In Vitro Ovulation and Luteinization

Skory, Xu, Shea and Woodruff, Human Reprod. 2015
96% rupture (n=70)
In Vitro Ovulation and Luteinization

Skory, R, unpublished
In Vitro Ovulation and Luteinization

Steroidogenic Enzymes

Gonadotropin Receptors

Inha Transcription Factors

Skory, R, unpublished
Mechanisms of Ovulation

Skory, R, unpublished
Follicular and Luteal Phase Hormone Secretion over 28 Day Cycle - EVATAR

Draper Laboratories, Cambridge, MA

Shuo Xiao, Ph.D.

Xiao et al, *unpublished*
eIVFG Supports Live Birth and EVATAR Ovulates and Produces MII eggs

Shea and Woodruff, Tiss Eng 2006

Xiao, unpublished
Human Tissue for Long Term Storage and Research

exploring and expanding options for the reproductive future of cancer survivors
44 patients; 65 follicles; 4 MII

National Center for Translational Research in Infertility – NICHD P50

Fundamental Roles of Hormones, Architecture and Environment in Follicle Development

- **Biomaterial Environment**
  (alginate, fibrin-alginate IPN, PEG)

- **Animal Environment**
  (age, metabolic status, and weight studies)

- **Physical Environment**
  (rigidity, PCOS, and primordial follicles)

- **Cellular Environment**
  (stroma/theca cells and other follicles)

- **In vitro Environment**
  (translation to human)

- **Human Environment**
  (policy, law, religion)

Shea and Woodruff, 2000-present
What Makes a Good Egg?

Duncan…Woodruff, Aging Cell, 2012
Hornick, Duncan, Marko, Woodruff, JARG, 2014
Tingen…Woodruff, Science 2013
What Makes a Good Egg?

Tom O’Halloran, Ph.D.
Morrison Professor of Chemistry
Northwestern University

Kim, Bernhardt, Kong, Duncan, Que, Zhang, Chang
Fertility Needs in Pediatric Cancer Patients

✓ Follicle maturation
✓ High fidelity oocyte maturation
✓ Endocrine hormone production
  • Pubertal transition
  • Cyclical hormones to support systemic health
Components of an Engineered Ovary

Specialized cells: Granulosa/Theca Cells & Oocytes
isolated or cultured from patient tissue, derived from human stem cells

Bioactive Scaffold
patient, donor, engineered

Laronda, et al. (2013) Stem Cell Research & Therapy;
Laronda, et al. (2014) JARG
In vivo follicle maturation
(retrieved for in vitro fertilization)

Cryopreserve ovarian tissue (in 5 mm cubes)
Isolate follicles
Encapsulated in non-degradable material

Shikanov, Woodruff, Shea, Tiss Eng. 2014
Degradable Hydrogel-Follicle Transplant Minimizes Cancer Transplant (results in live birth)

Kniazeva...Woodruff, Jeruss, Shea, Sci Rep. 2015
Decellularized Ovary for Bio-active Scaffold

Monica Laronda, Ph.D.
Burroughs Wellcome Career Awardee

Decellularized Ovary for Bio-active Scaffold

Laronda, et al. (2015) Biomaterials
Puberty Was Initiated in Ovariectomized Mice with Recellularized Grafts


Scale bar = 500 μm; 50 μm

>30 day transplant
Bioinspired Scaffold Design

Hormones – Architecture - Environment

- oocyte
- granulosa
- theca

Scaffold Criteria:
- Mechanically rigid to handle during transplant
- Mimic a 3D architecture
- Open pores for nutrient flow, growth and ovulation
- Bioactive, bio-safe material

Gelatin scaffold with a microporous architecture material

Follicle on Ovary Paper

Sutured to soft tissues

3D Printing by A. Rutz (Shah Lab)
Bioinspired Scaffold Design

Calcein / Rhodamine

Vinculin / DAPI

Scale bar = 50 μm

Rutz & Laronda, unpublished
Bioinspired Scaffold Design – Follicle Development and Oocyte Maturation

30º; D8

stroma / cytoskeleton / DNA

Scale bar = 50 μm

Rutz & Laronda, unpublished
Bioinspired Scaffold Design – Soft Tissue Transplant

Bioprosthesis in bursa

fat

vessel

GFP+ follicles

oviduct

PECAM  DNA

Rutz & Laronda, unpublished
Bioinspired Scaffold Design – Soft Tissue Transplant

Bioprosthesis 3wks post-surgery

Bioprosthesis 8wks post-surgery

Scale bar = 200 and 50 μm

Rutz & Laronda, unpublished
Live Birth from Ovarian Bioprothesetic Transplant

(first soft organ transplant)

Transplant recipient (EGFP-) with EGFP+ pup

Rutz & Laronda, unpublished
✓ Follicle maturation
✓ High fidelity oocyte maturation
✓ Endocrine hormone production
✓ Pubertal transition
✓ Cyclical hormones to support systemic health
Chemotherapy and Radiation Target Cancer and Other Susceptible Sites

Cancer cells

Germ cells

So-youn Kim, Ph.D.
Research Assistant Professor
Northwestern University
DNA damage response in Mammalian Oocytes

- **TAp63-independent apoptosis**
  - Primordial Germ Cells
  - Germ Cell Cluster
  - Meiosis

- **TAp63-dependent apoptotic pathway**
  - PCG Specification, Migration and Colonization
  - Sex Determination
  - Recombination (DNA breaks by Spo11)

- **TAp63-independent apoptosis**
  - Oogonia

**Prophase arrest**

**Germ Cell Loss and Survival**

**Follicle Assembly**

**Follicle Activation**

**Ovarian Reserve**

**Chemotherapy**

**Radiation Therapy**

**Apoptotic Pathway**

- ATM, ATR
- CHK
- c-Abl
- TAp63
- JNK, p38
- 14-3-3
- PUMA
- NOXA
- BAX
- BAK

- **KU55933, BEZ**
- **Chk2 inhibitor**
- **Imatinib**

- **JNK, p38 inhibitors**

**Low ATM and Chk ATR (unknown)**

**Resumption of Meiosis**

**Ovulation of Metaphase II oocyte**

**DNA damage response in Mammalian Oocytes**

- Primordial Germ Cells
- Oogonia
- Germ Cell Cluster
- Meiosis
- Recombination (DNA breaks by Spo11)
- PCG Specification, Migration and Colonization
- Sex Determination
- ATM, ATR
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**Chemotherapy**

**Radiation Therapy**

**Apoptotic Pathway**

**Low ATM and Chk ATR (unknown)**

**Resumption of Meiosis**

**Ovulation of Metaphase II oocyte**
Summary of *In vitro* Mitigation Test

<table>
<thead>
<tr>
<th>Targets</th>
<th>Inhibitors</th>
<th>Protection from CDDP</th>
<th>Protection from Radiation</th>
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<tr>
<td>ABL kinase</td>
<td>Imatinib</td>
<td>Yes</td>
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<tr>
<td>GNF2</td>
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<td>Nilotinib</td>
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<td>Chk2</td>
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<td>MK-2206</td>
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<td>p53</td>
<td>Pifithrin-u</td>
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Oncofertility 2036

- Better Cancer Control and Tx
- Higher Selectivity of Patients
- Neo-adjuvant Fertoprotectives
- In Vitro Follicle Maturation
- Designer Ovarian Bioprosthetics
- Eliminate the Field
Thank you!

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Oncofertility Consortium
NIH/NICHD: P50HD076188

Microfluidic Menstrual Cycle
NCATS/ORWH/NIEHS: UH3TR001207

Zinc Spark
NIGMS: R01GM115848; Keck Foundation; Argonne National Laboratories

Visit us at:
http://woodrufflab.org/
http://oncofertility.northwestern.edu/

Woodruff Lab, 2015

Lonnie Shea, PhD, Tom O’ Halloran, Ph.D., John Marko, Ph.D., Ramille Shah, Ph.D.
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