Engineering Reproduction

Teresa K. Woodruff, Ph.D.
Thomas J. Watkins Memorial Professor and Vice Chair for Research
Department of Obstetrics and Gynecology
Chief, Division of Reproductive Science in Medicine
Northwestern University Feinberg School of Medicine
Preservation of Fertility After Cancer

- Life preserving treatments
  - Chemotherapy
  - Radiation
  - Surgery

- Can threaten fertility

Woodruff *Nature Medicine* 2009;
Gradishar, Smith, & Woodruff, *JAMA Oncol*, 2016
Options for Women
Tissue Transplant

Tissue Cryopreservation Preserves Function
Efficiency? Transfer of Disease?

Silber, St. Louis;
Suzuki, Japan; Anderson, Denmark;
Donnez, Belgium

onc.fertility.northwestern.edu
Fertility and Endocrine Needs of Pediatric Cancer Patients

Figure in: Cordeiro, Kim, Woodruff. *Cancer Treatment and the Ovary* (2015)
Patient Sample 4 y.o.; scale bar = 100 μm; National Physicians Cooperative
Laronda, et al. (2015) Biomaterials

Acute Lymphoblastic Leukemia Cells in ovarian cortical tissue
Fertility and Endocrine Needs of Pediatric Cancer Patients

- Follicle maturation
- High fidelity oocyte maturation
- Endocrine hormone production
- Pubertal transition
- Cyclical hormones to support systemic health
Perhaps the structural context matters to developmental competence
Encapsulated In Vitro Follicle Growth (eIVFG)

Lonnie Shea, Ph.D.

NICHD U54/P50
Woodruff and Shea, 2000-present
Encapsulated In Vitro Follicle Growth (eIVFG)

Two somatic cell compartments

DNA
StAR

DNA
Inhibin α

Theca-like

Granulosa cells

Follicles produce steroid hormones

Xu et al, Human Repro. 2009
In Vitro Ovulation
96% rupture (n=70)
In Vitro Ovulation and Luteinization

Skory, Xu, Shea and Woodruff, Human Reprod. 2015
Mechanisms of Ovulation

Skory, R, unpublished; Gates Foundation
eIVFG Produces MII Eggs and Supports Live Birth

NICHD P50


Shea and Woodruff, Tiss Eng 2006
Premise: Reproductive Hormones Impact Every Tissue of the Body
Problem: Petri Dishes Do Not
Potential: Health and Disease Models

- Genes
- Epigenetics
- Environment
- Drug Interactions
- Obesity
- Age
- Sex

Multiorgan, Multi-etiologic Disease; Drug Metabolism
Promise: Endocrinology in a Dish

EVATAR Biology and Microfluidic Technology
Personalized: Your Genes, Your Environment

Each of Us!
Shuo Xiao, Ph.D. Postdoc 2013-2016
Assistant Prof USC


NIEHS/ORWH/NCATS UH3
Discover Magazine, Top 100 Discoveries, 2017
Microfluidic Systems

**SOLO-MFP™**
- Single tissue

**DUET-MFP™**
- Two tissue interactions
- Recirculation optional

**QUINTET-MFP™**
- Up to 5 tissues
- Recirculation


NIEHS/ORWH/NCATS UH3
*Discover Magazine, Top 100 Discoveries, 2017*
Microfluidic Follicle Culture

Imaging

FemKube Image Integration: Mingyang Jiang
Dino-Lite AM4815ZTL
Solo-MFP™ Supports 28 Day Follicle Function
Microfluidic Follicle Culture


**NIEHS/ORWH/NCATS UH3**

Discover Magazine, Top 100 Discoveries, 2017
EVATAR – Ovarian Cycle in a Dish


NIEHS/ORWH/NCATS UH3

Discover Magazine, Top 100 Discoveries, 2017
Evatar™ Quintet-MFP™
Fallopian Tube Functioning

Xiao et al, Nature Commun, 2017

Discover Magazine, Top 100 Discoveries, 2017

NIEHS/ORWH/NCATS UH3
Quintet-MFP Supports Pregnancy-like Hormone Conditions

Xiao et al, Nature Commun, 2017
NIEHS/ORWH/NCATS UH3
Discover Magazine, Top 100 Discoveries, 2017
Encapsulated islets produce stable insulin levels across 21 days of culture and support normal ovarian endocrine patterning.
- Ovarian explants maintain structure throughout culture period without addition of exogenous insulin.
- Explants exhibit normal functional responses to endocrine patterning, including ovulation following hCG surge.
ADATAR and DudeKube

• **Goal:** Replicate the Male reproductive tract (MRT), testis and prostate *ex vivo*, including reproductive hormone signaling.

• **Clinical Utility:**
  – Testis Cancer Paradigms
  – Hypogonadism (low T)
  – Male Infertility
  – Toxicological Screening
  – Male Contraceptive Development
  – Benign Prostatic Hyperplasia
  – Prostatic Carcinoma

Maxwell Edmonds, Grad Student
EVATAR
Premise - Problem – Promise - Potential - Personalized

Personalized Drug Testing
Toxicology Testing
Signaling Pathways
Integrated Cell Biology

Device Mimicking Female Reproductive Cycle Could Aid Research, NPR
Meet Evatar: The Lab Model That Mimics the Female Reproductive System, New York Times
How to build a female reproductive system that fits in the palm of your hand, PBS News Hour
‘Organ On A Chip’ Re-creates the Female Menstrual Cycle, Discover Magazine
EVATAR Named Top Paper of 2017 by NIEHS,
Follicle maturation *in vitro* phenocopies *in vivo* development

Grow - Secrete Hormones - Differentiate
Egg Matures - Ovulatory Mechanics - Connections
Live, Healthy Births in Mice

**Architecture, Environment and Hormones**
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
What Makes a Good Egg?

Structure Informs Function

Duncan...Woodruff, *Aging Cell*, 2012

Hornick, Duncan, Marko, Woodruff, *JARG*, 2014
What Makes a Good Egg?

Kim, Bernhardt, Kong, Duncan, Que, Zhang

*Nature Chemistry, Nature Chemical Biology, Scientific Reports etc* (2009-present)

Tom O'Halloran, Ph.D.
Morrison Professor of Chemistry

Francesca Duncan, Ph.D.
Assistant Professor
Northwestern University

Hoi Chang Lee, Ph.D.
Postdoctoral Fellow
Woodruff Lab

NICHD, Ferring
The zinc spark is an inorganic signature of human egg activation

Francesca E. Duncan, Emily L. Que, Nan Zhang, Eve C. Feinberg, Thomas V. O’Halloran, Teresa K. Woodruff*  Scientific Reports, 2016

Zinc is only released from mature, health human eggs
MAJOR MOMENTS in Assisted Human Reproduction

Major Moments in Assisted Human Reproduction

Infertility is as old as humankind, as is the use of herbs, remedies, or rituals claimed to treat it. But the modern era of science-driven assisted human reproduction is less than a century old.

1953 First baby born using frozen sperm.
1978 Baby born via in vitro fertilization.
1980 Birth from a legal surrogate mother in America.
1984 Birth using a donated oocyte.
1984 Baby born as a result of a frozen embryo.
1990 Tests of "transplantation," a technique to help embryos attach to the uterine wall.
1992 Case of intracytoplasmic sperm injection, in which a single selected sperm is injected directly into an oocyte.
2003 Monkey birth from fresh, non-cryopreserved ovarian tissue.
2004 Human birth from cryopreserved ovarian tissue.
2015 "Stem-cell baby" born from an oocyte with mitochondria boosted using ovarian stem cells.

2016 Researchers find that when sperm enzymes fertilize the egg, there is a burst of naturally occurring zinc. These zinc “sparks” are brighter for healthier eggs, which could lead to more effective fertilization techniques.
What makes a good egg?

Chromosome number and Structure

Oocyte-specific gene expression

Zn exocytosis at fertilization
Fertility Needs in Pediatric Cancer Patients

Ovarian cortical tissue from NPC participants;
Scale bar = 50 um Laronda, et al. (2015) Biomaterials

Patient Sample 4 y.o.; scale bar = 100 μm; National Physicians Cooperative
Decellularized Ovary for Bio-active Scaffold

Monica Laronda, Ph.D.
Burroughs Wellcome Career Awardee
Assistant Professor, Dept Pediatrics, NU

Bioinspired design of structural ECM as organ scaffolds

0.3 g dECM (~1 Ovary) → 40 cm² TP <20 min. Fabrication

Decell’d Bovine Ovary

Powdered dECM

dECM “Ink”

Ovarian dECM “Tissue Paper”

Retains substantial porosity and texture

Jakus, Laronda, …Woodruff and Shah, *Advanced Materials* 2017
Bioinspired design of structural ECM as organ scaffolds

30° advancing angle  60° advancing angle  90° advancing angle


Discover Magazine, Top 100 Discoveries, 2017
Bioinspired Scaffold Design – Follicle Development and Oocyte Maturation

30°; D8

stroma / cytoskeleton / DNA


Discover Magazine, Top 100 Discoveries, 2017
Bioinspired Scaffold Design – Soft Tissue Transplant

Bioprosthetic Scaffold in bursa

- Fat
- Vessel
- GFP+ follicles
- Oviduct

PECAM DNA


Discover Magazine, Top 100 Discoveries, 2017
Bioprosthesis 3wks post-surgery

Primary follicle

Secondary follicles

Large follicles

CL

Uterine horn

strut

vessel

strut

Bioprosthesis 8wks post-surgery

Scale bar = 200 and 50 μm


Discover Magazine, Top 100 Discoveries, 2017
Live Birth from Ovarian Bioprothesetic Transplant

Transplant recipient (EGFP-) with EGFP+ pup


Discover Magazine, Top 100 Discoveries, 2017
Timeline of Discovery

1895
First ovarian graft transplant

1931
Human uterine transplant

1936
First modern penile implant

1941
First testis prosthesis

1978
Human birth from IVF
Human testis transplant

1984
Human birth from frozen embryo

1986
Successful cryopreservation of human oocytes

1988
Human testis transplant

1992
IVF by ICSI

2004
Human birth from ovarian tissue transplant
Human whole ovary transplant

2006
Cryopreservation of whole human ovary
Oncofertility field established
Live animal birth from follicles
grown in vitro within a biomaterial

2014
Engineered vagina transplant

2015
Human MII egg in vitro

2017
EVATAR BioBag
Functional 3D-printed ovarian bioprosthesis

William Tuttle Morris, M.D.
First ovary transplant (1895)

Roger Gosden, Ph.D.
Ovarian Tissue Cryopreservation

Kutluk Oktay, M.D.

Donnez, Belgium,
Suzuki, Japan;
Silber, St. Louis;
Anderson, Denmark
And more…

Gargus, Rogers, McKinnon, Edmonds, Woodruff, *Nature Biotechnology*, under revision
Engineering the Reproductive Axis

Follicle Maturation in a Dish

NUBorn & NUAge

Oncofertility Solutions

Engineered Reproductive Tract

Artificial Ovary

Decellularized Ovary

MFP-Ovulation
Oncofertility 2018

- Global Oncofertility Community
- Human MII Eggs from eIVFG
- Human Zinc Spark
- Ovarian Cycle in a Dish
- Ovarian (Transplant) Bioprosthetic
  - Live Birth in Mice

We seek solutions. We don’t seek - dare I say this - just scientific papers any more.

Steven Chu, March, 2009
Oncofertility 2018-2058

- Better Cancer Control and Tx
- Higher Selectivity of Patients
- Neo-adjuvant Fertoprotective
- In Vitro Follicle Maturation
- Designer Ovarian Bioprosthetics
- Epigenetic Regulation by Tissue
- Eliminate the Field

I have been taught that the way of progress is neither swift nor easy.
Marie Curie, 2021
Engineering That Enables Translation from Bench to Bedside
Funded by:
Oncofertility Consortium
NIH/NICHD: P50HD076188

Microfluidic Menstrual Cycle (Evatar)
NCATS/ORWH/NIEHS: UH3TR001207

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

Joanna Burdette, Ph.D.
Julie Kim, Ph.D.
Lonnie Shea, PhD,
Tom O’ Halloran, Ph.D,
John Marko, Ph.D.,
Ramille Shah, Ph.D.,
Francesca Duncan, Ph.D.
Shuo Xiao, Ph.D.
Hunter Rogers, M.S.
Draper Labs
Woodruff Lab Past and Present