



Engineering Reproduction


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Northwestern University Feinberg School of Medicine

 Eunice Kennedy Shriver National Institute
of Child Health and Human Development
Health research throughout the lifespan

 National Center
for Advancing
Translational Sciences

 National Institute of Environmental Health Sciences
Your Environment. Your Health.

NIH Office of Research on Women's Health (ORWH)

Preservation of Fertility After Cancer

- Life preserving treatments
 - Chemotherapy
 - Radiation
 - Surgery
- **Can threaten fertility**



Jeruss & Woodruff **N Engl J Med** 2009;
Woodruff **Nature Medicine** 2009;
Woodruff **Nature Rev Clin Oncol**, 2010;
Woodruff, **Nature Rev Endocrinol**, 2013;
DeVos, Smitz, & Woodruff, **Lancet**, 2014;
Gradishar, Smith, & Woodruff, **JAMA Oncol**, 2016

Options for Women Tissue Transplant



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

Tissue Cryopreservation Preserves Function
Efficiency? Transfer of Disease?

Fertility and Endocrine Needs of Pediatric Cancer Patients

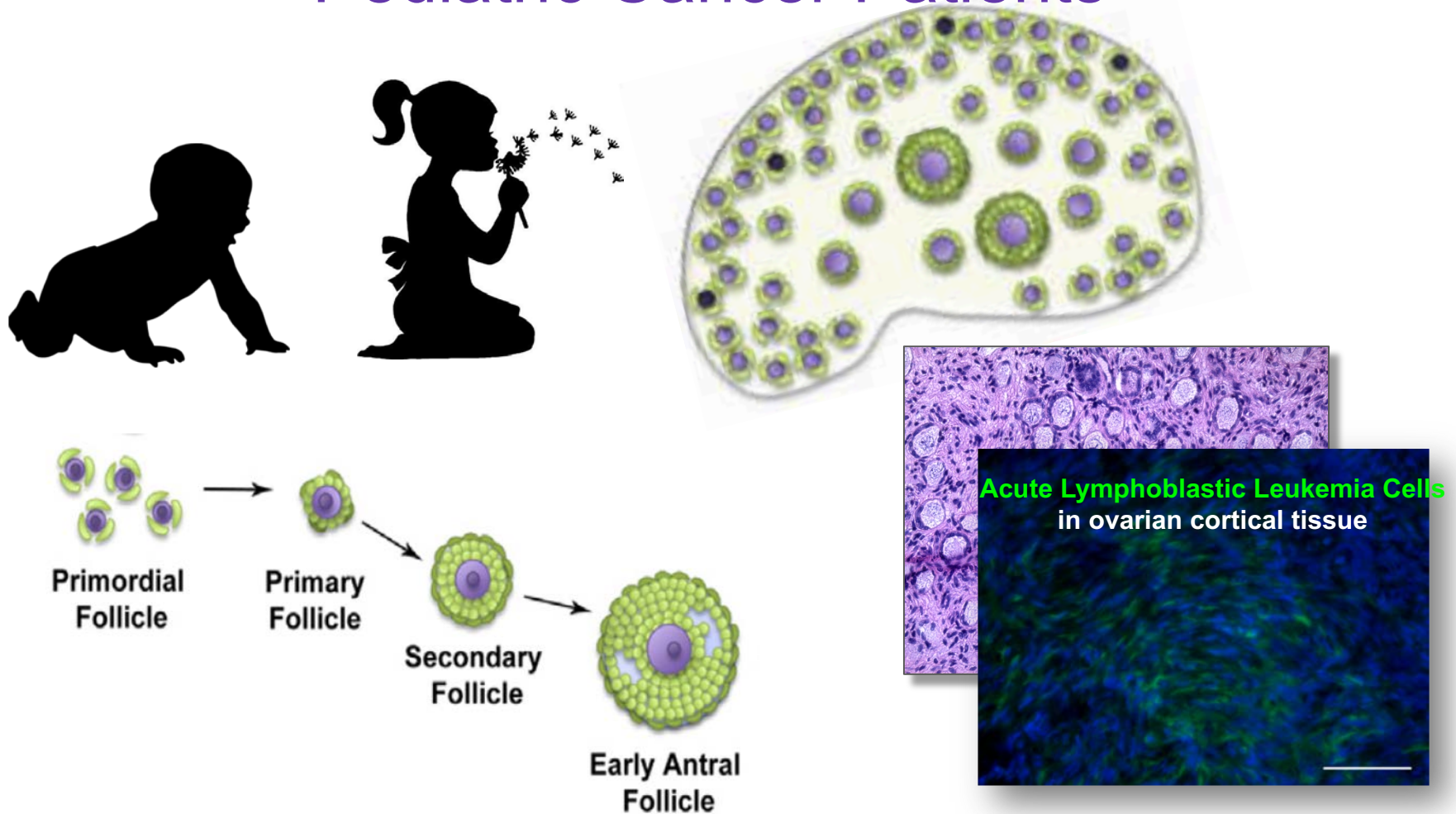
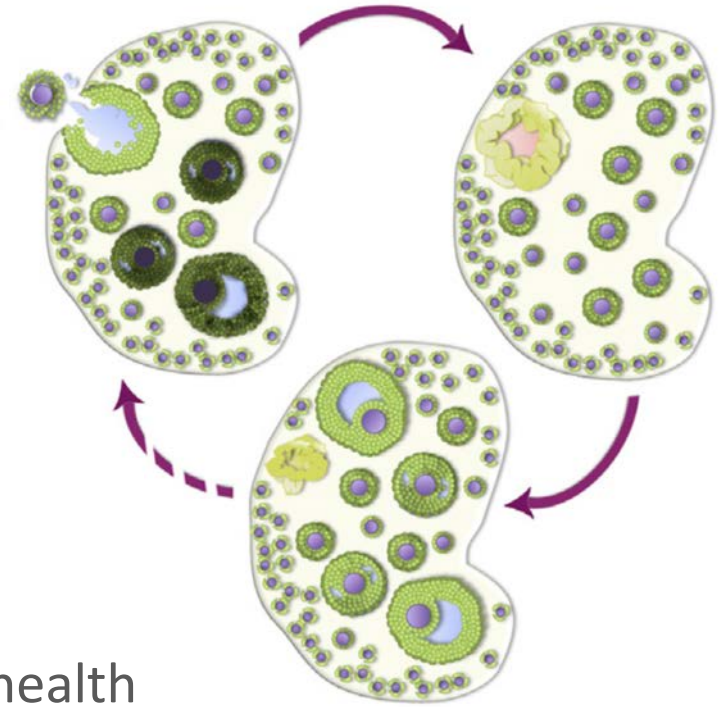


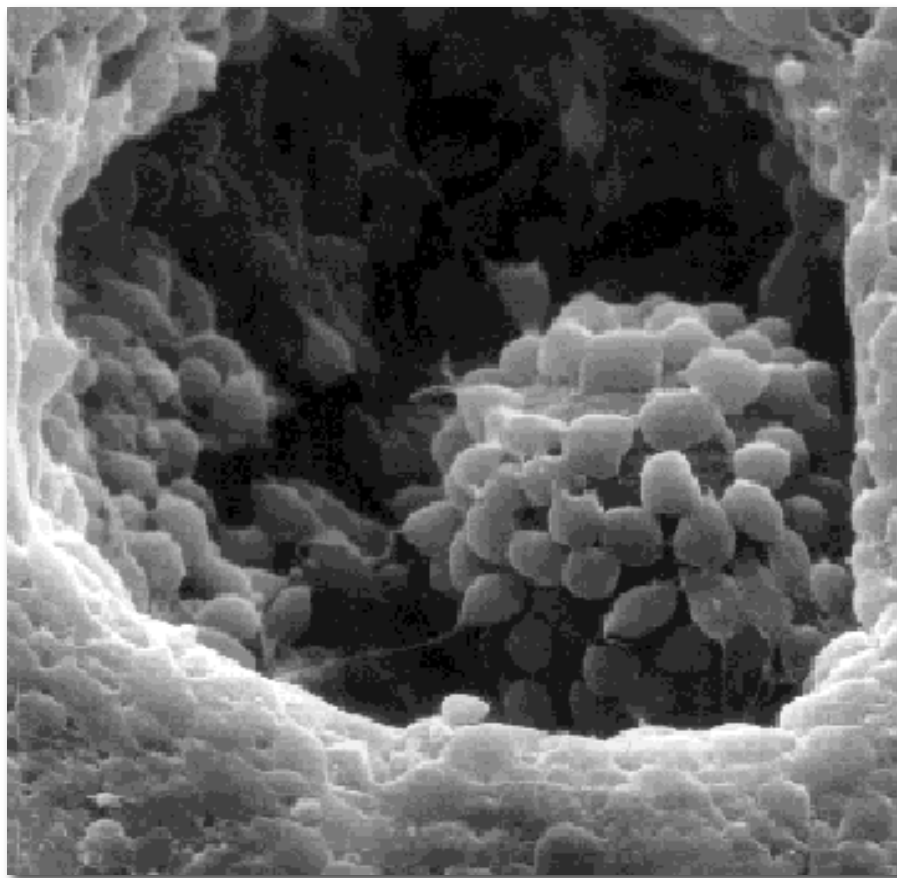
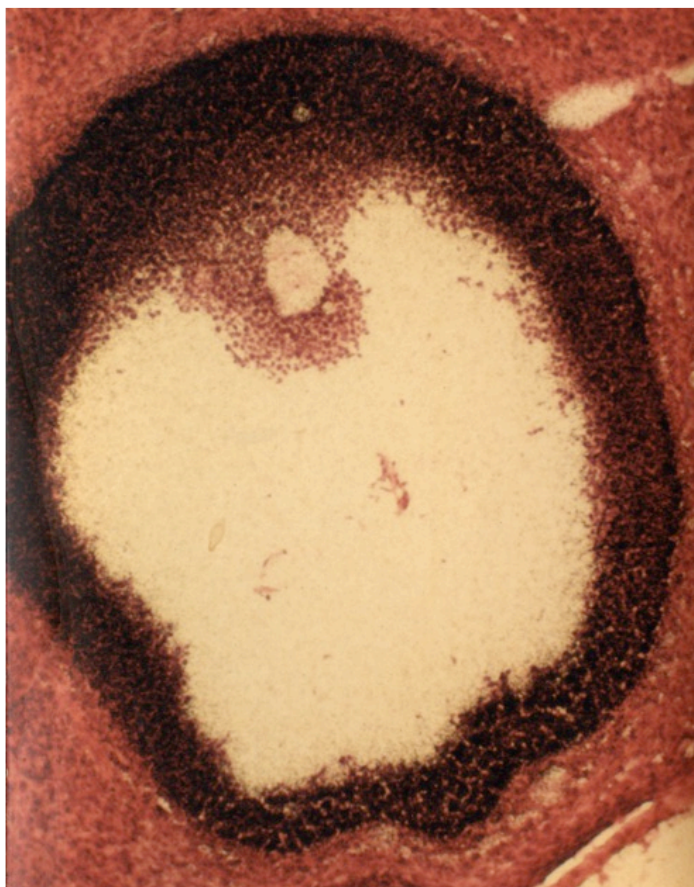
Figure in: Cordeiro, Kim, Woodruff. *Cancer Treatment and the Ovary* (2015)
Patient Sample 4 y.o.; scale bar = 100 μ m; National Physicians Cooperative
Laronda, et al. (2015) Biomaterials

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



Woodruff, D'Agostino, Schwartz and Mayo, *Science*, 1988

NATIONAL
PHYSICIANS
COOPERATIVE

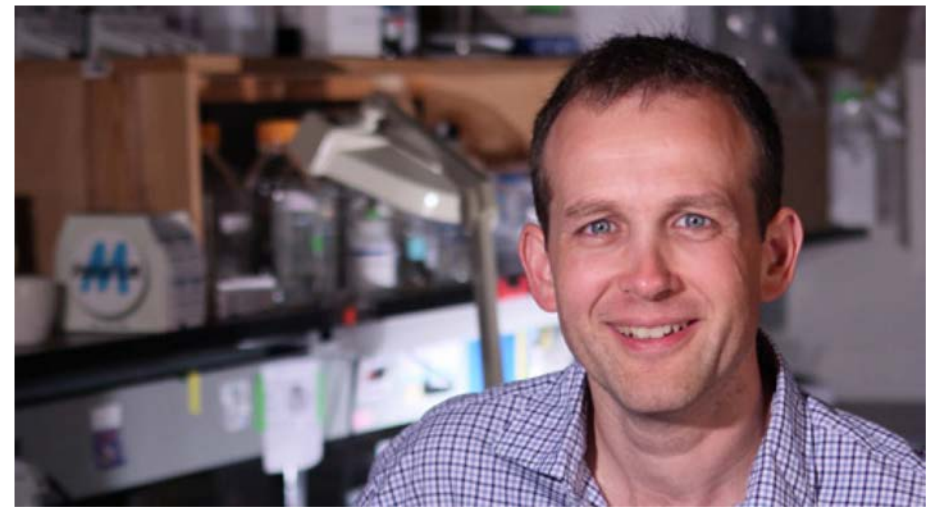
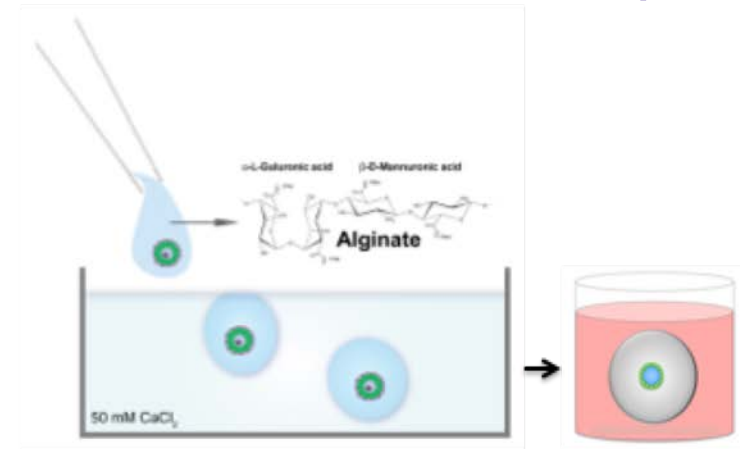
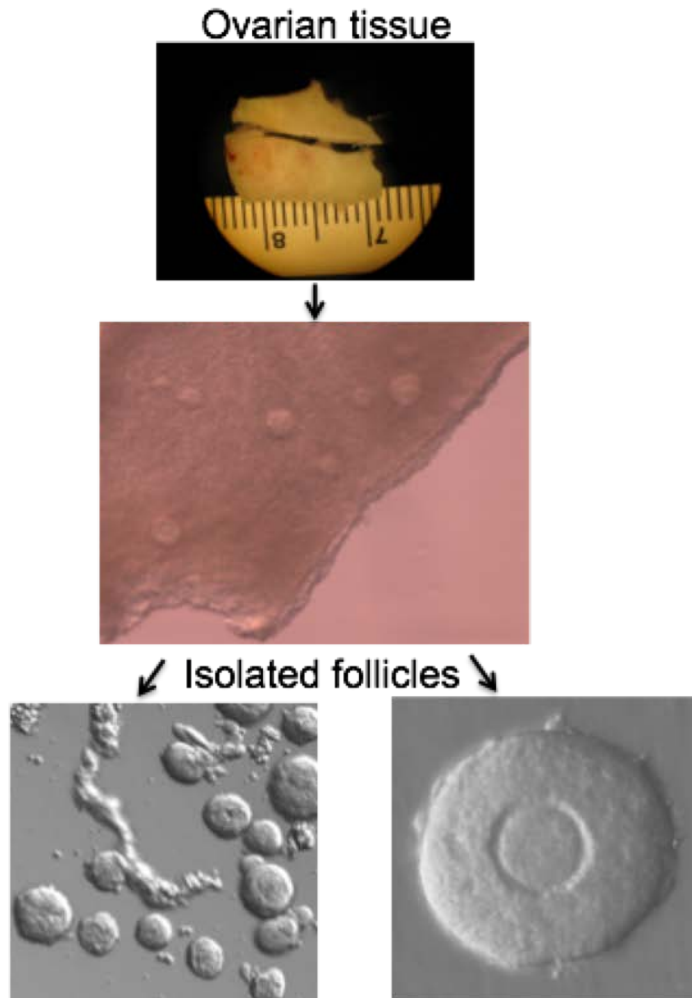


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Oncofertility[®]
Consortium

www.oncofertility.northwestern.edu

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ONCOFERTILITY
NETWORK

Encapsulated In Vitro Follicle Growth (eIVFG)



Lonnie Shea, Ph.D.

NICHD U54/P50

Woodruff and Shea, 2000-present

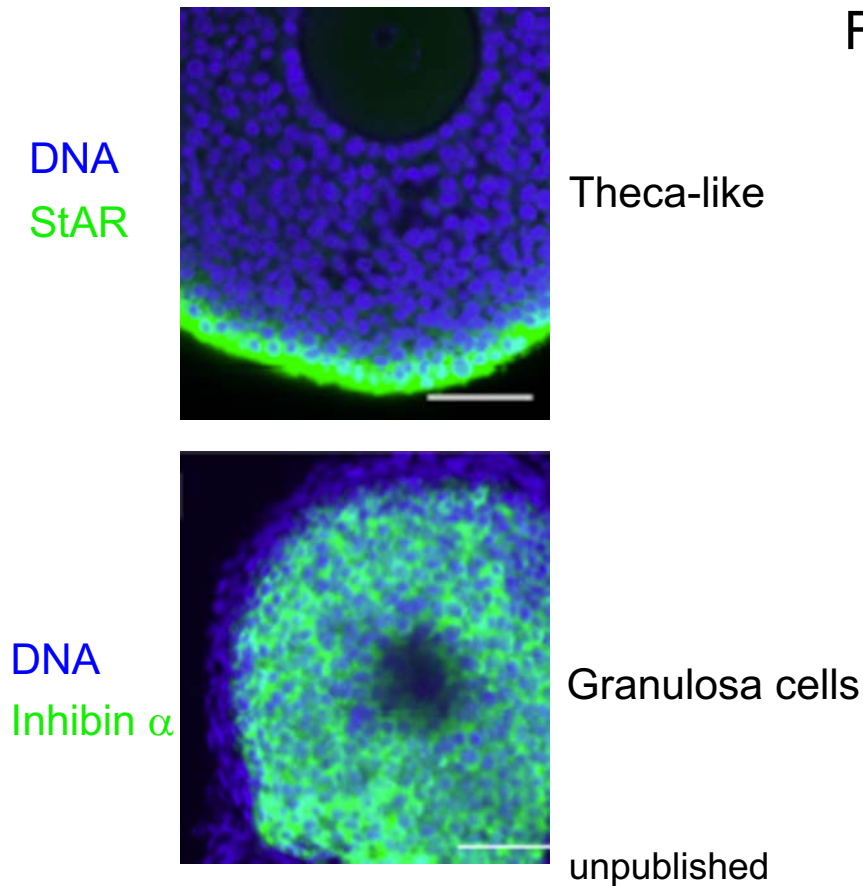
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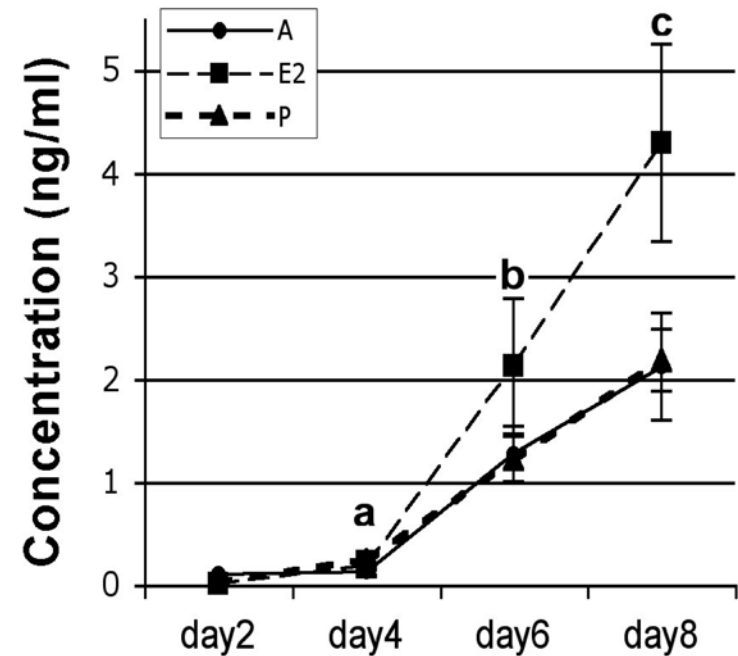
GLOBAL
ONCOFERTILITY
NETWORK

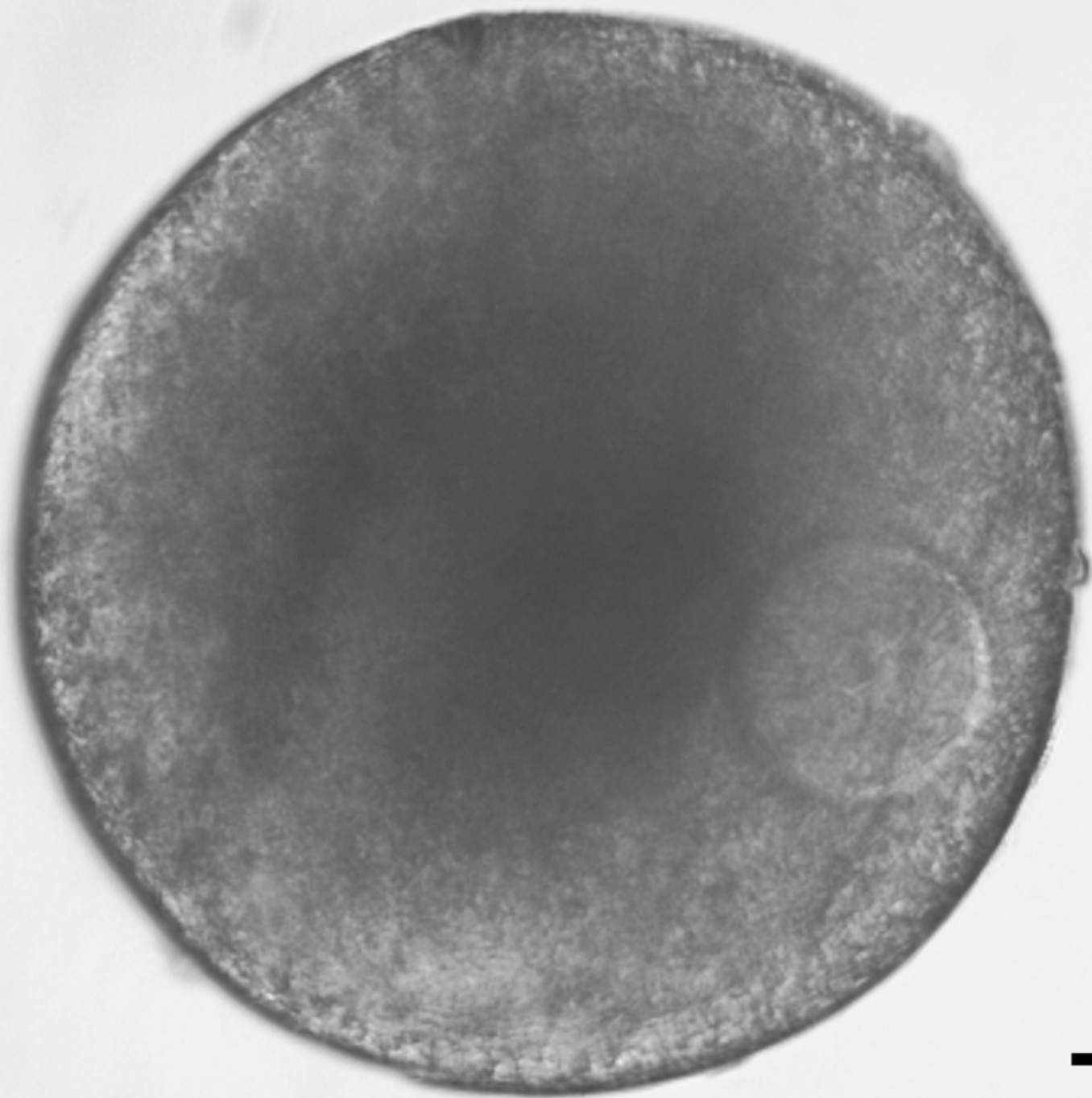
Encapsulated In Vitro Follicle Growth (eIVFG)

Two somatic cell compartments

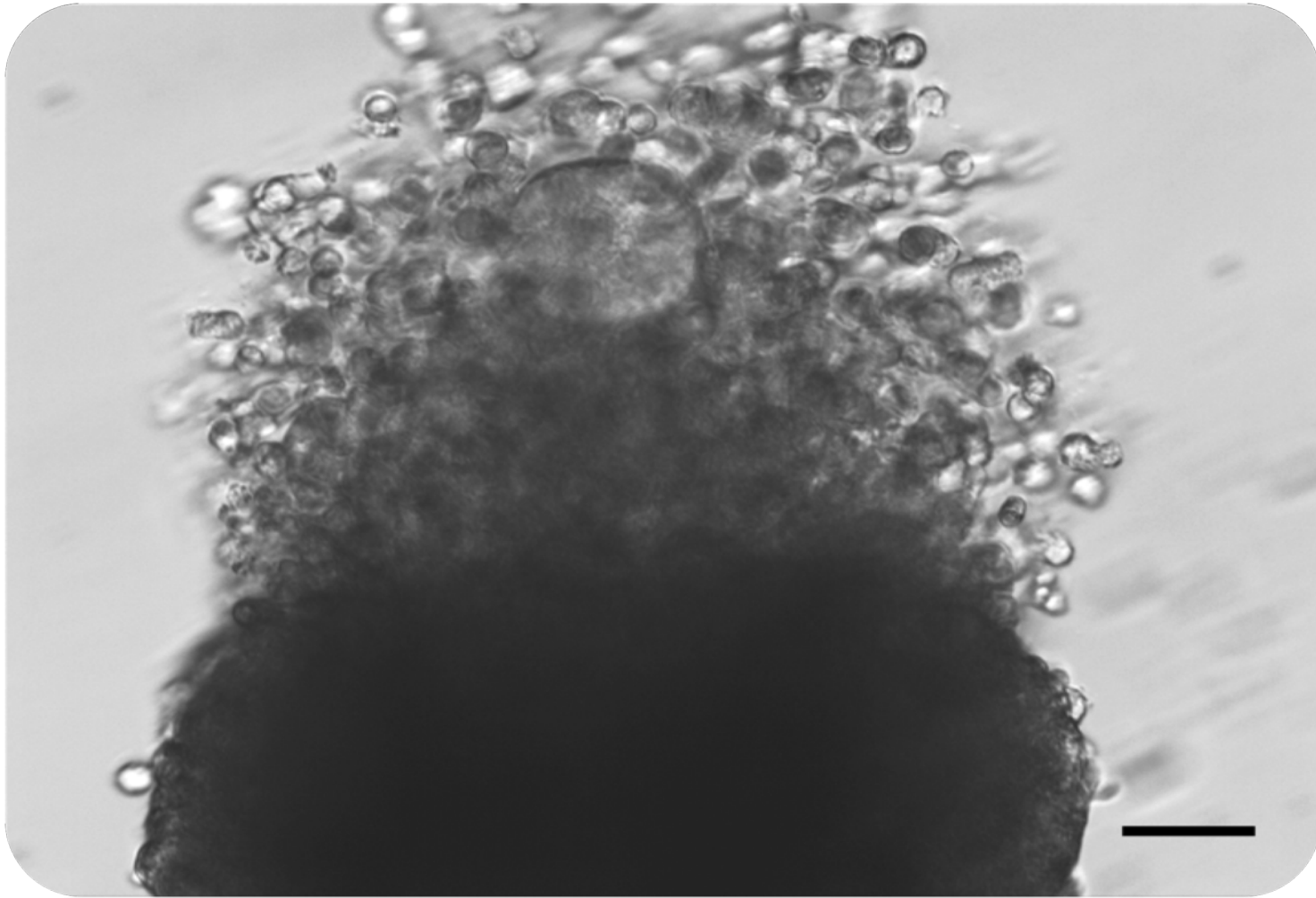


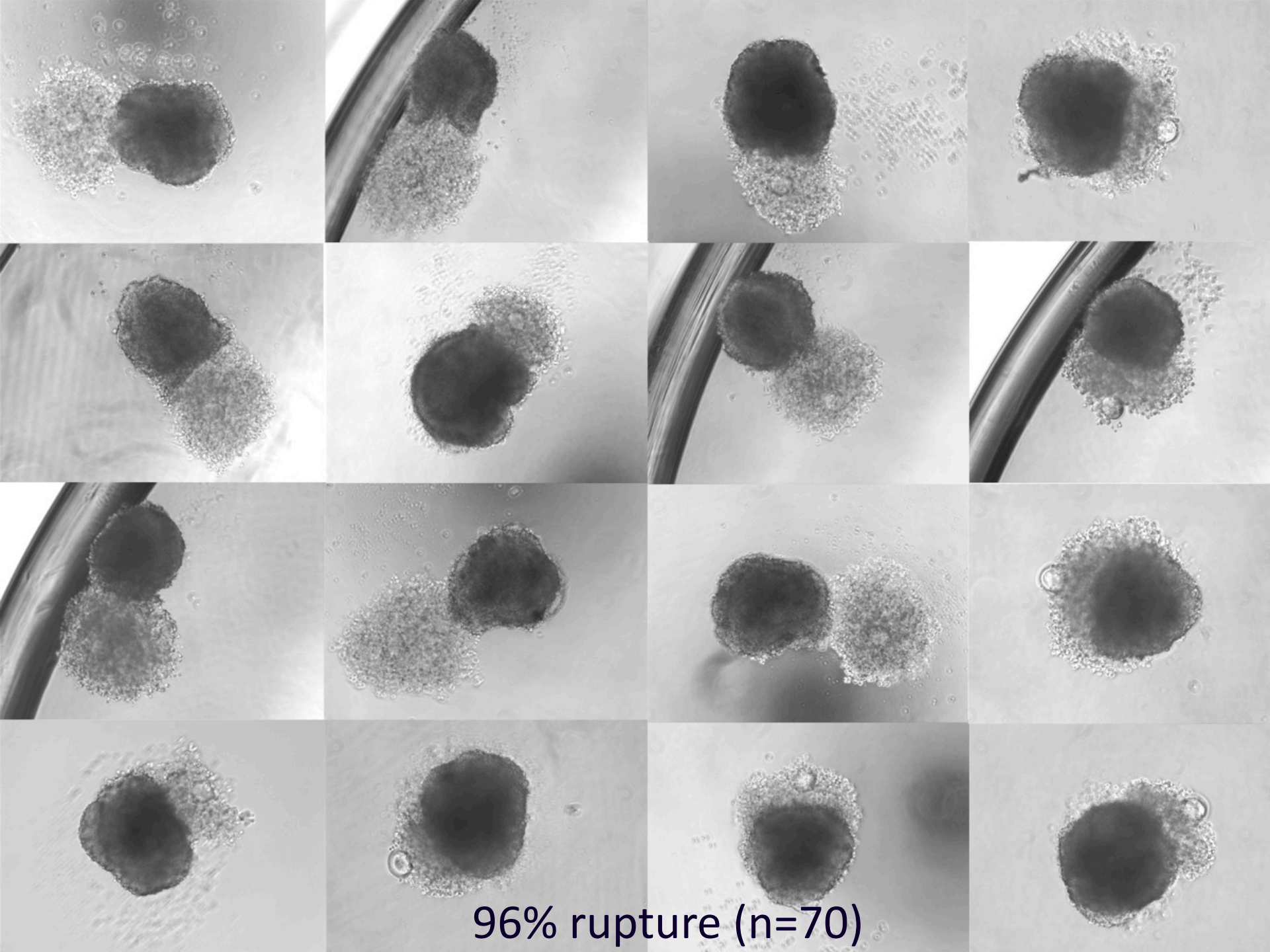
Follicles produce steroid hormones





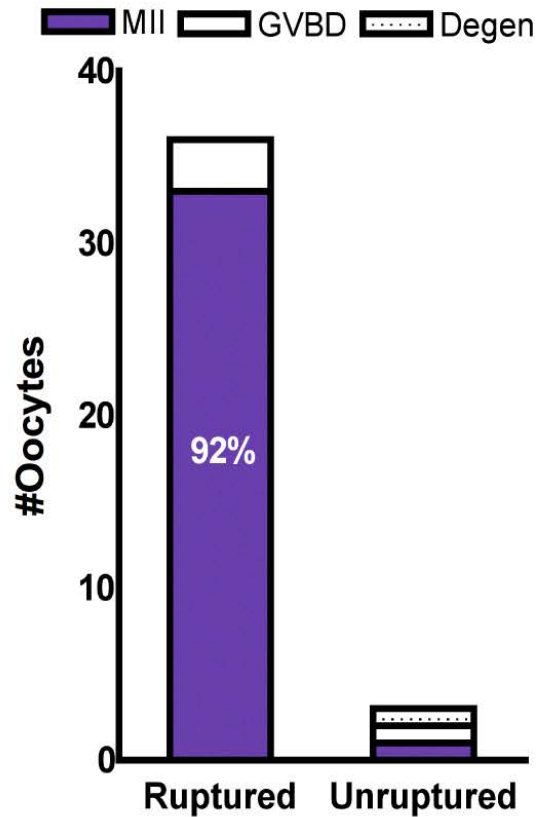
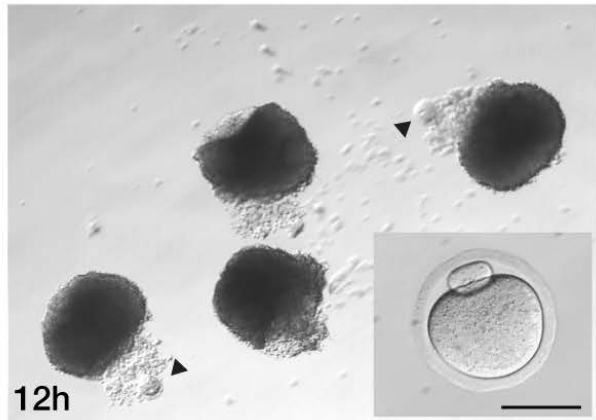
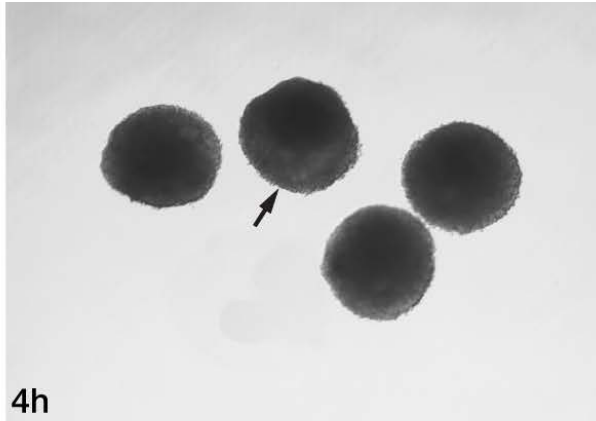
In Vitro Ovulation



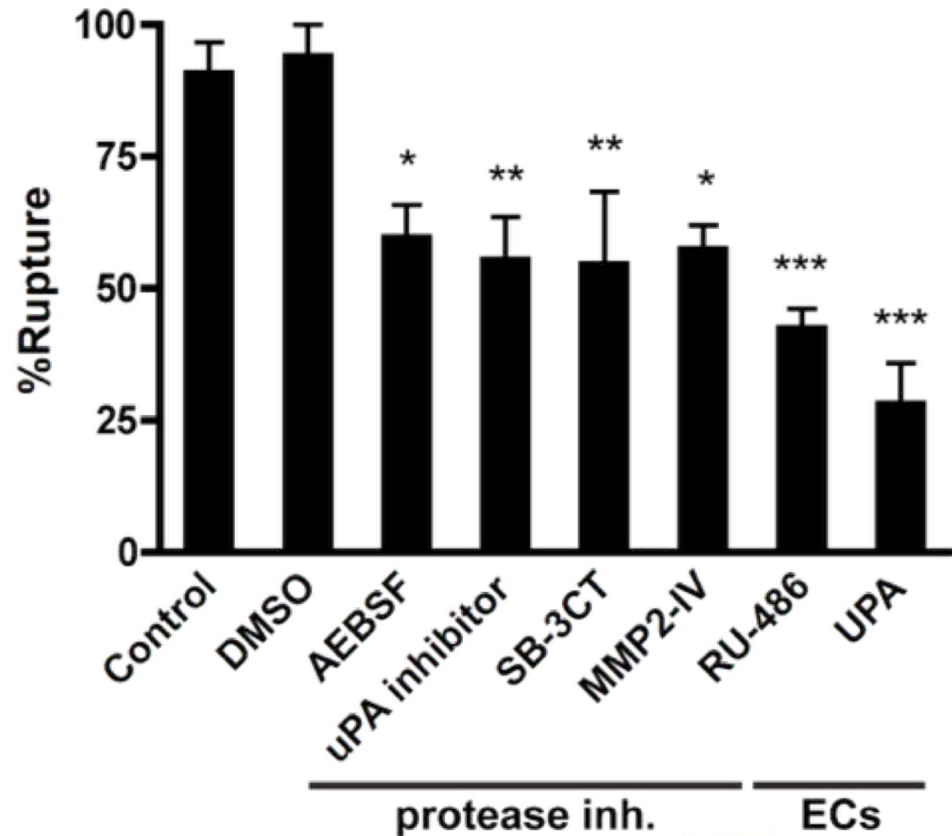
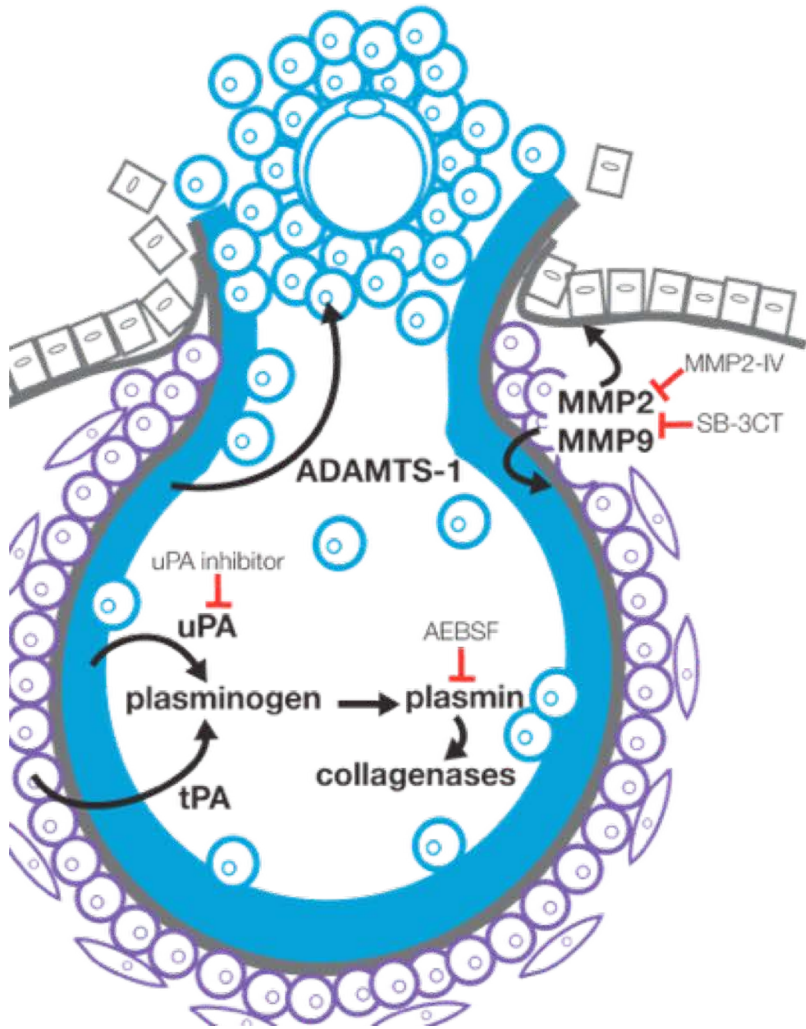


96% rupture (n=70)

In Vitro Ovulation and Luteinization

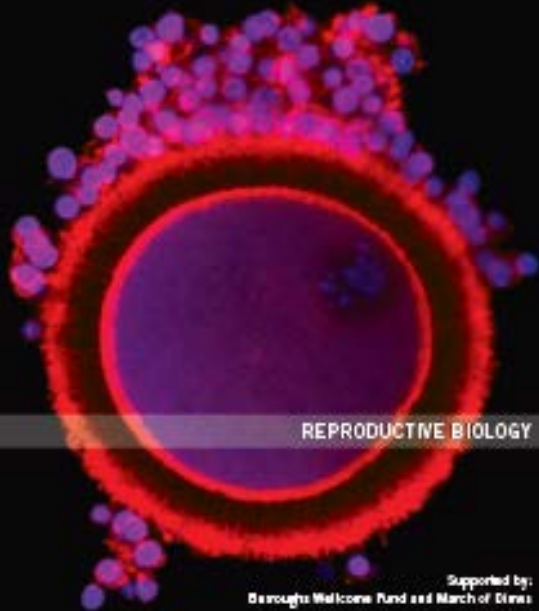


Mechanisms of Ovulation



eIVFG Produces MII Eggs and Supports Live Birth

nature medicine

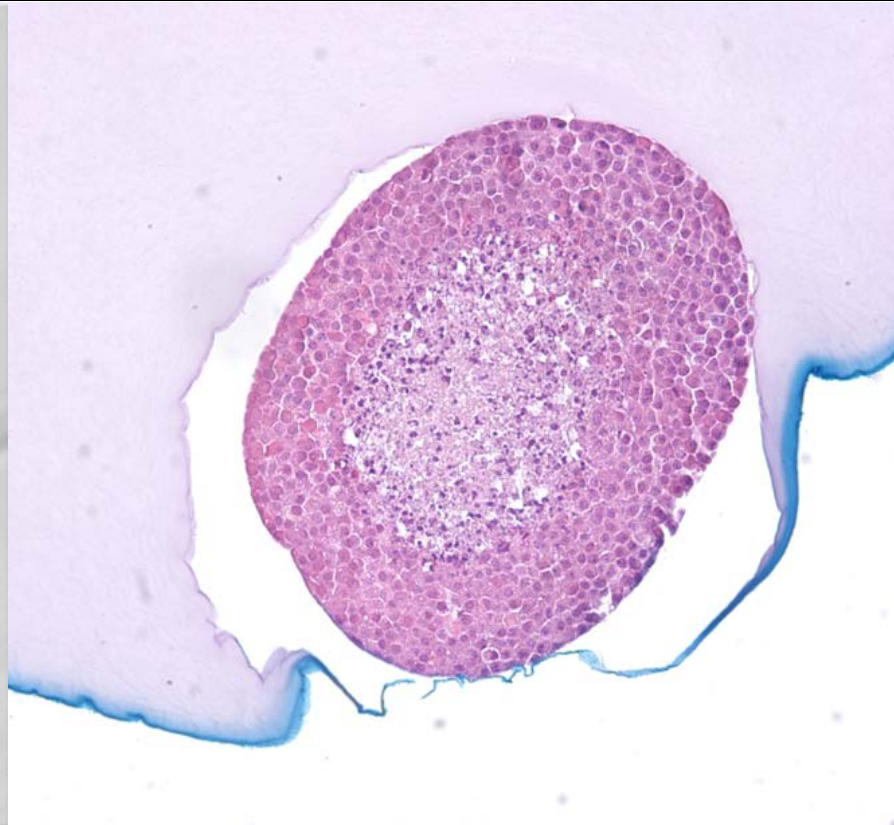
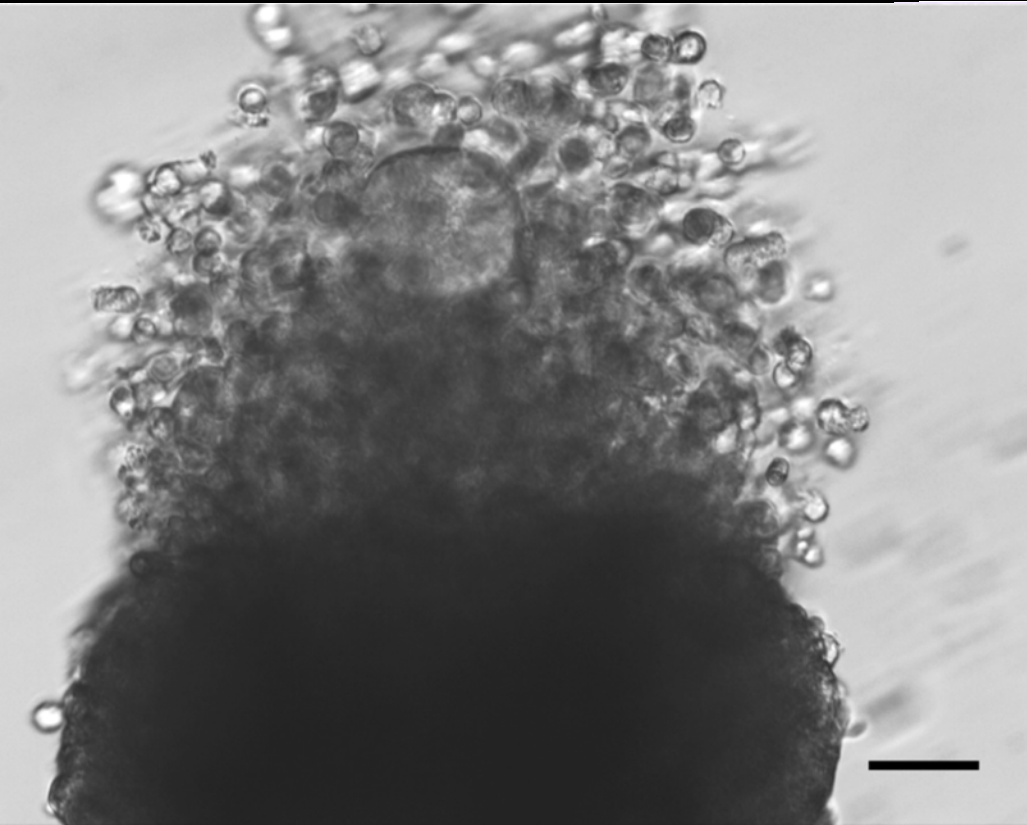
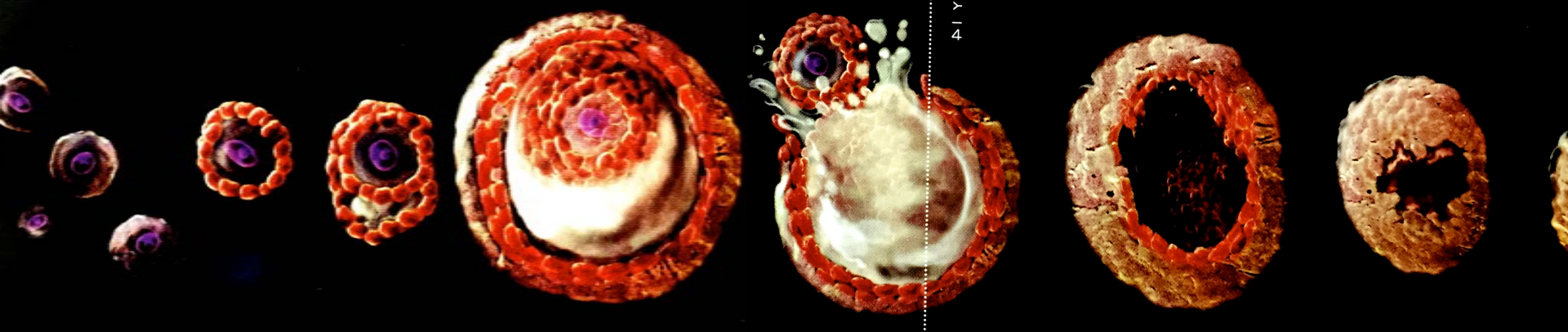


NICHD P50

Nature Medicine Breakthrough of the Decade (1998-2008)

Shea and Woodruff, Tiss Eng 2006



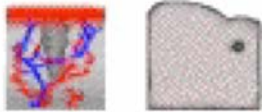
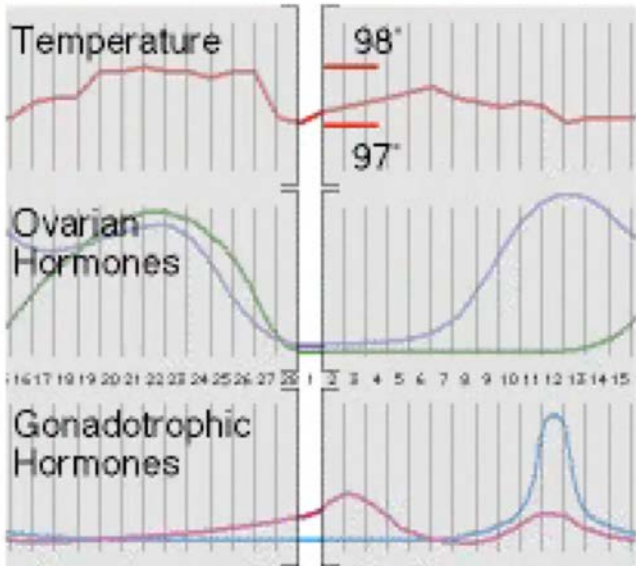


Premise: Reproductive Hormones Impact Every Tissue of the Body

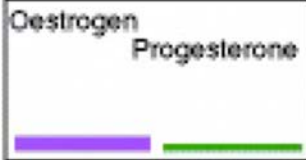
The Menstrual Cycle

© 1992 Roy Stringer, D. Brown, Dr. L. Turnbull, Dr. P. Smith, Liverpool J.M. University

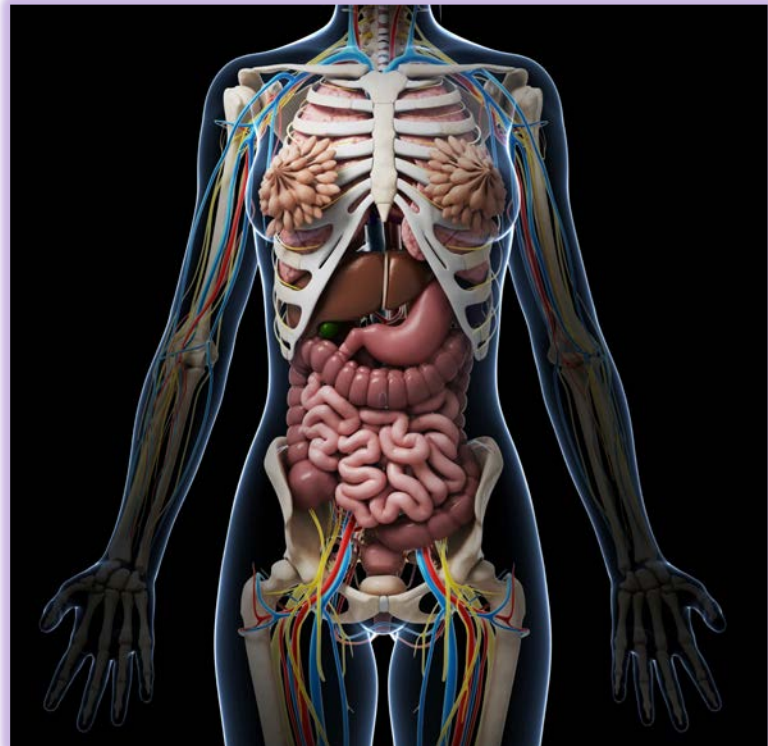
Day 1



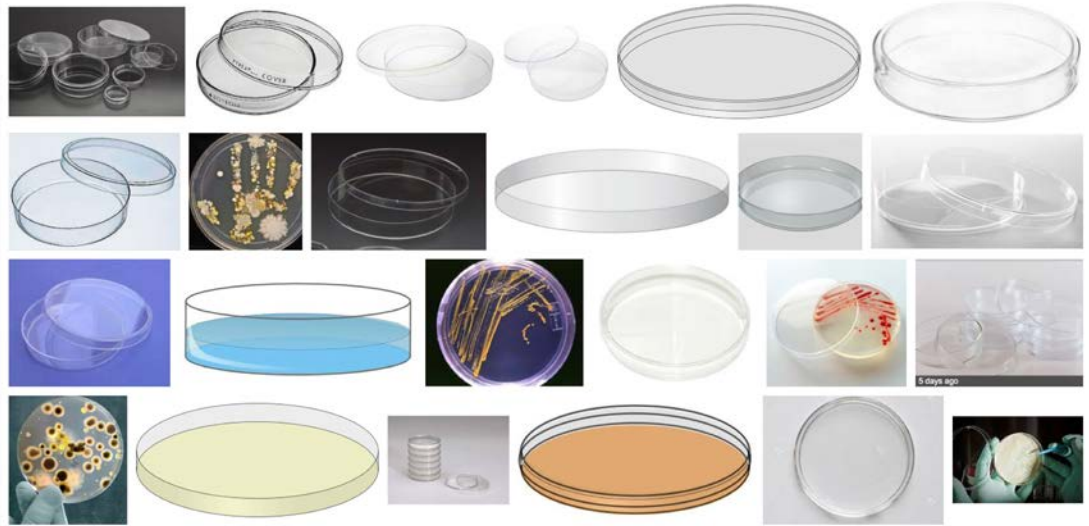
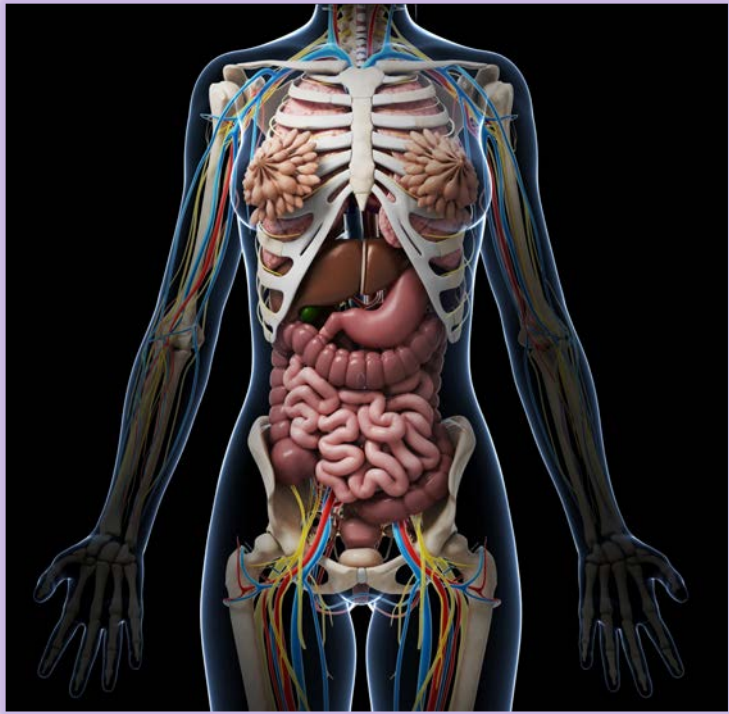
Endometrium Ovary



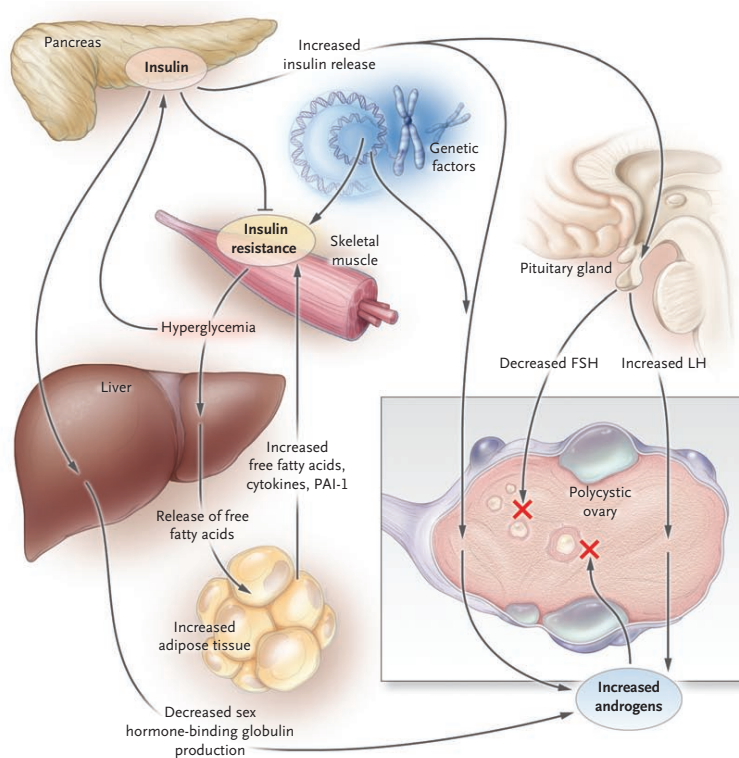
A Freeware sample from [Thecus Training for Cyto-Technologists](#) +44 51 645 8668



Problem: Petri Dishes Do Not



Potential: Health and Disease Models

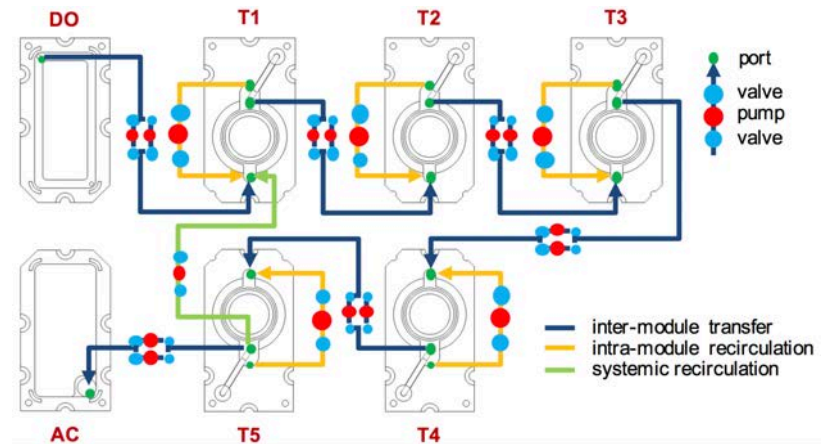
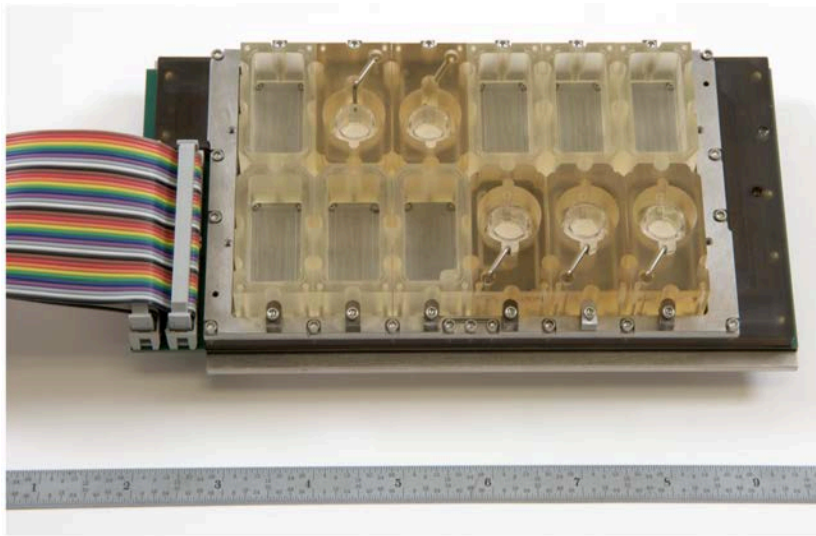


- Genes
- Epigenetics
- Environment
- Drug Interactions
- Obesity
- Age
- Sex

Multiorgan, Multi-etiology Disease;
Drug Metabolism



Promise: Endocrinology in a Dish



EVATAR Biology and Microfluidic Technology

Personalized: Your Genes, Your Environment



Each of Us!

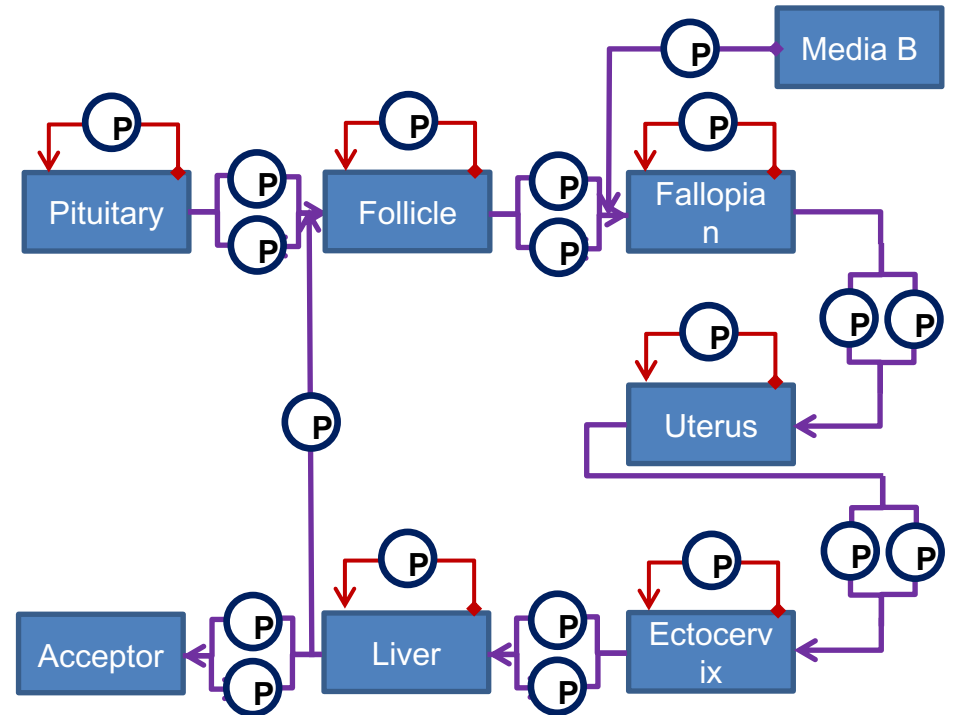


How Do We Change Basic Discovery Research?

Evatar Team



Coppeta, Bornstein, Xiao, Kim, Burdette, Rodgers, McKinnon and MANY OTHERS

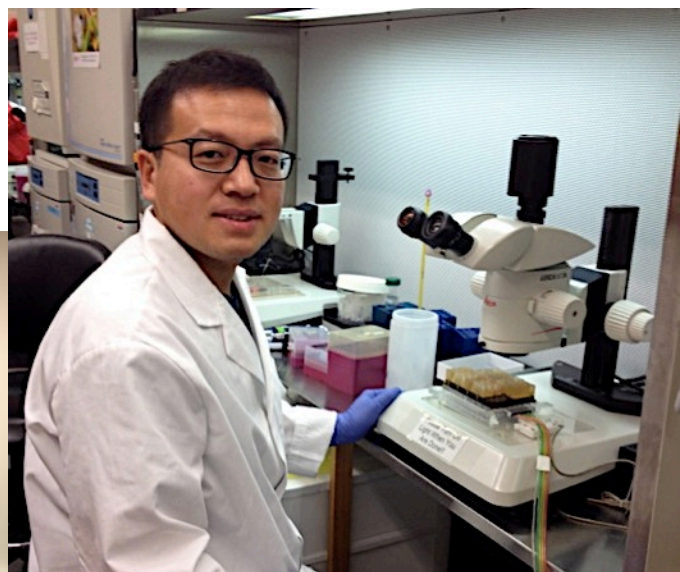


A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle. Xiao S, Coppeta JR, Rogers HB, Isenberg BC, Zhu J, Olalekan SA, McKinnon KE, Dokic D, Rashedi AS, Haisenleder DJ, Malpani SS, Arnold-Murray CA, Chen K, Jiang M, Bai L, Nguyen CT, Zhang J, Laronda MM, Hope TJ, Maniar KP, Pavone ME, Avram MJ, Sefton EC, Getsios S, Burdette JE, Kim JJ, Borenstein JT, Woodruff TK. **Nat Commun.** 2017 Mar 28;8:14584.

Discover Magazine, Top 100 Discoveries, 2017



Hunter Rogers, Graduate Student



Shuo Xiao, Ph.D. Postdoc 2013-2016
Assistant Prof USC



Jonathan Coppeta, Brett Isenberg, Jeff Borenstein
Draper Labs



Xiao et al, *Nature Commun*, 2017

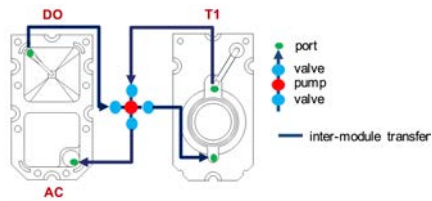
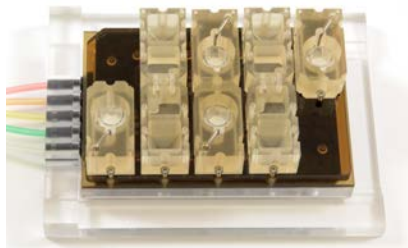
NIEHS/ORWH/NCATS UH3

Discover Magazine, Top 100 Discoveries, 2017



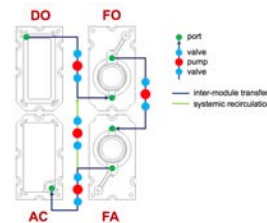
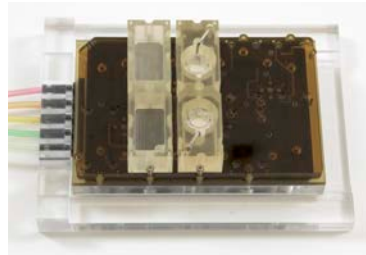
Microfluidic Systems

SOLO-MFP™



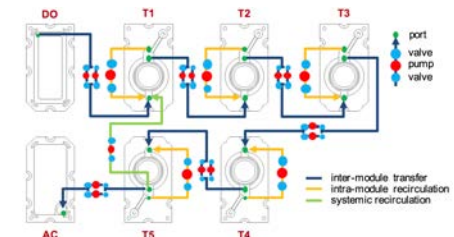
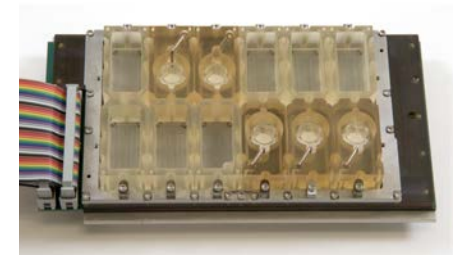
- Single tissue

DUET-MFP™



- Two tissue interactions
- Recirculation optional

QUINTET-MFP™



- Up to 5 tissues
- Recirculation

Xiao et al, *Nature Commun*, 2017

NIEHS/ORWH/NCATS UH3

Discover Magazine, Top 100 Discoveries, 2017



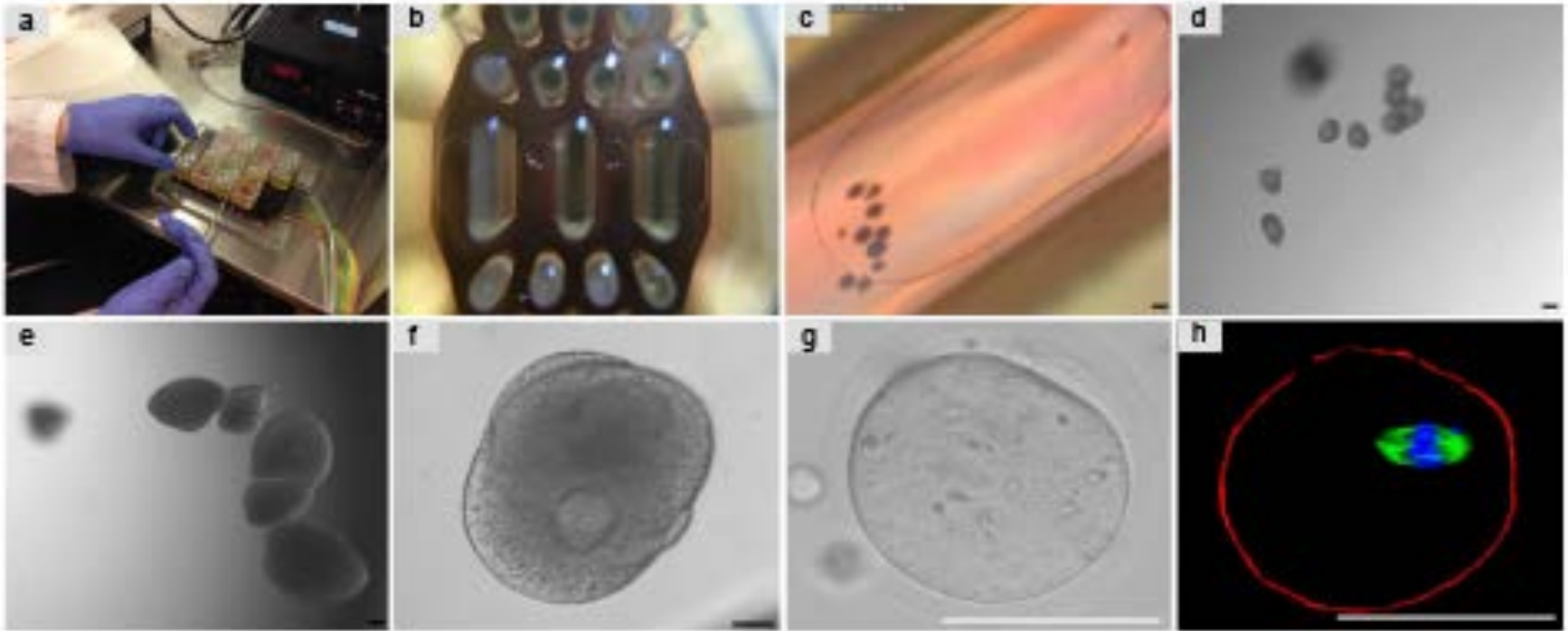
Microfluidic Follicle Culture



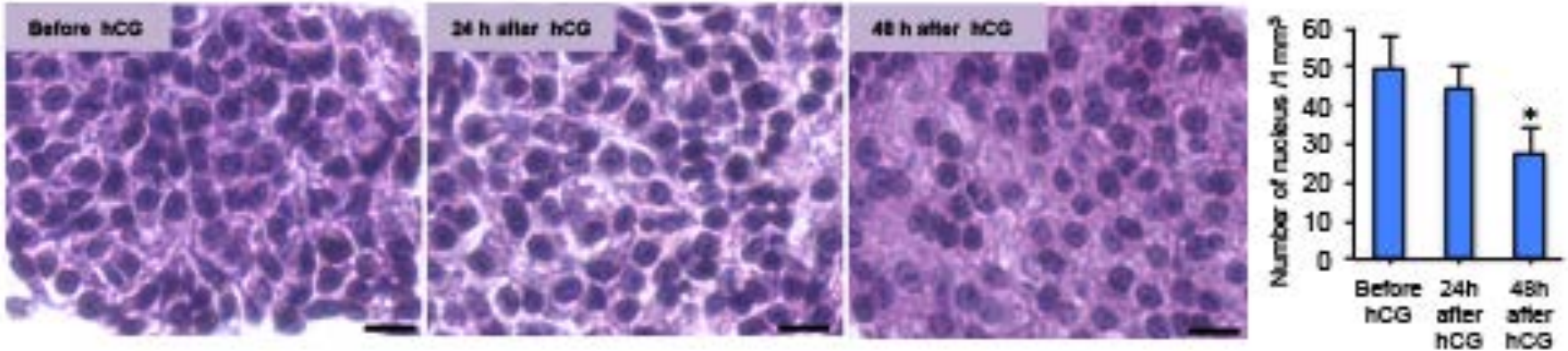
Imaging

Solo-MFP™ Supports 28 Day Follicle Function

A



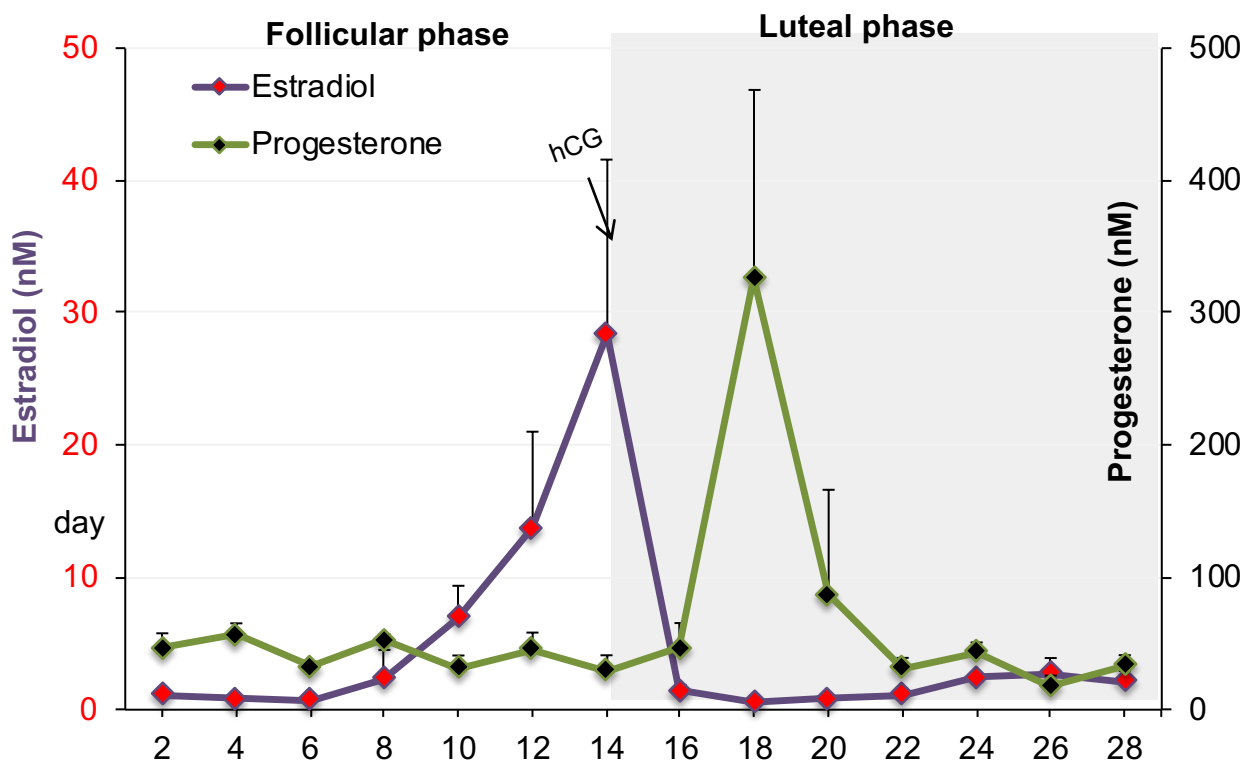
B



C

D

Microfluidic Follicle Culture



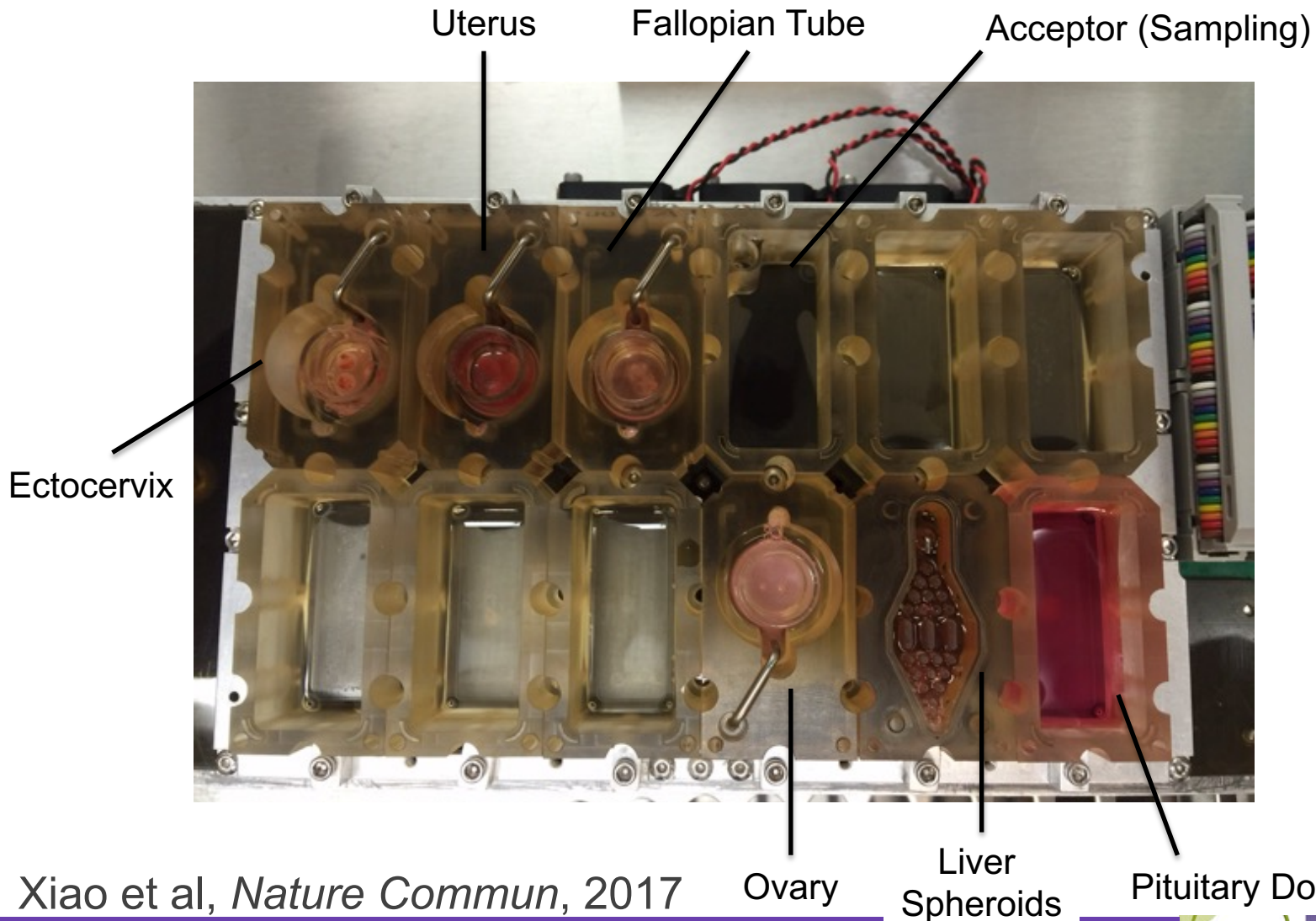
Xiao et al, *Nature Commun*, 2017

NIEHS/ORWH/NCATS UH3

Discover Magazine, Top 100 Discoveries, 2017



EVATAR – Ovarian Cycle in a Dish



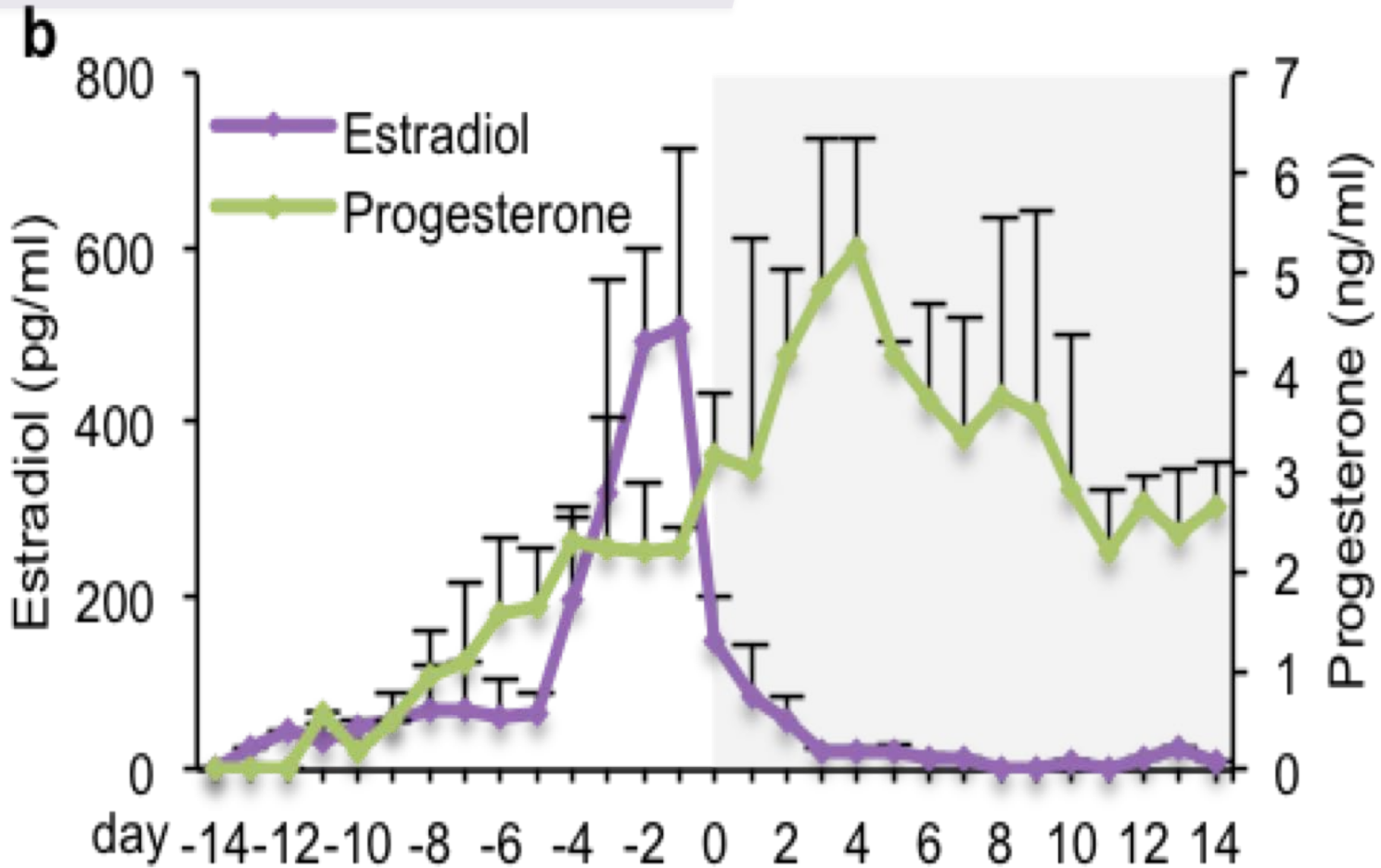
Xiao et al, *Nature Commun*, 2017

NIEHS/ORWH/NCATS UH3

Discover Magazine, Top 100 Discoveries, 2017



EVATAR™



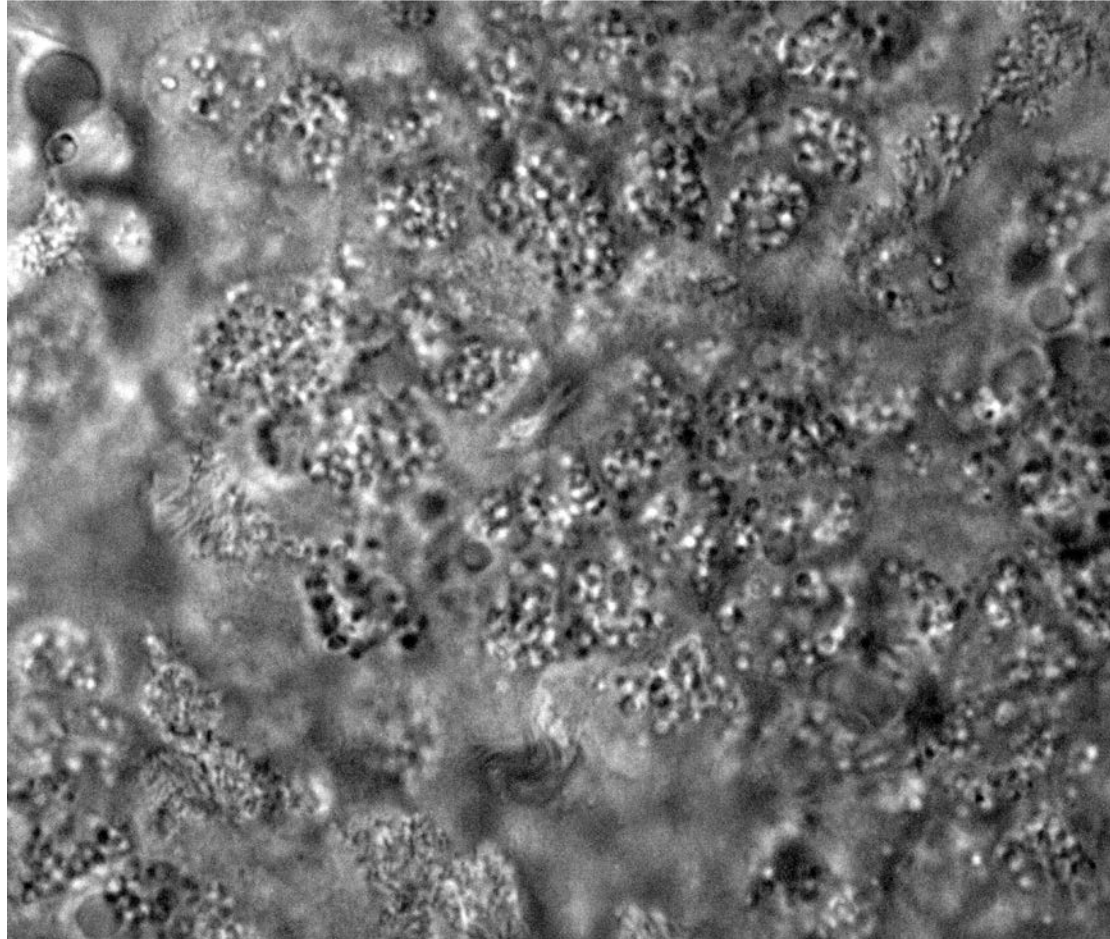
Xiao et al, *Nature Commun*, 2017

NIEHS/ORWH/NCATS UH3

Discover Magazine, Top 100 Discoveries, 2017



Evatar™ Quintet-MFP™ Fallopian Tube Functioning



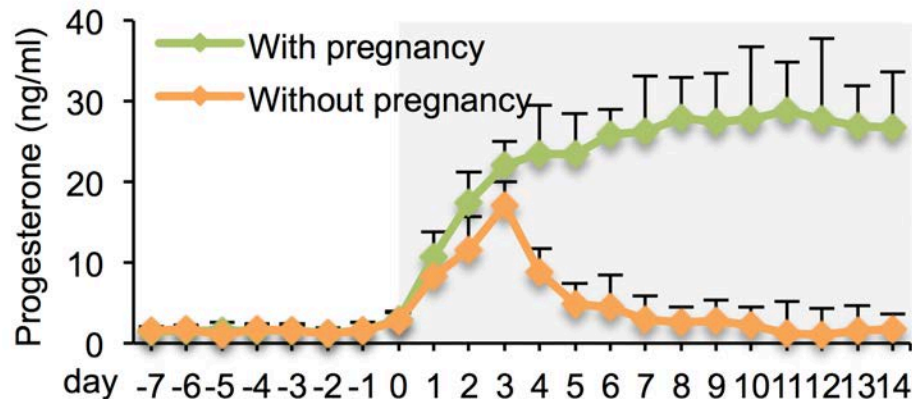
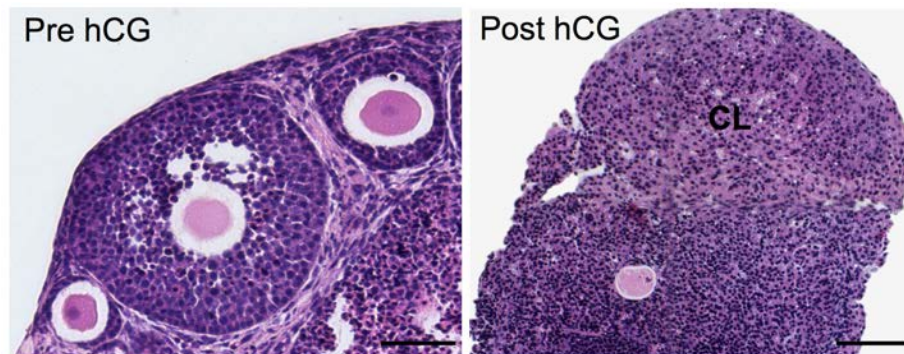
Xiao et al, *Nature Commun*, 2017

NIEHS/ORWH/NCATS UH3

Discover Magazine, Top 100 Discoveries, 2017



Quintet-MFP Supports Pregnancy-like Hormone Conditions



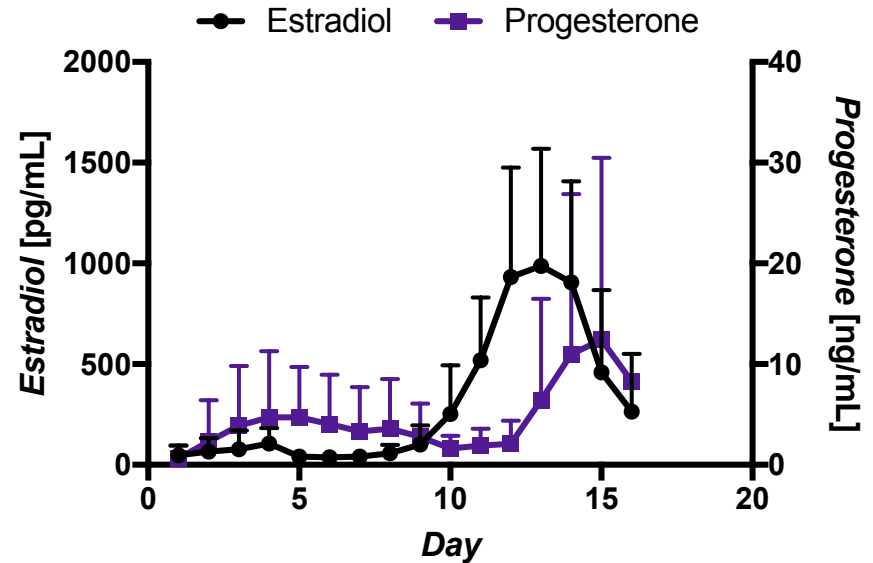
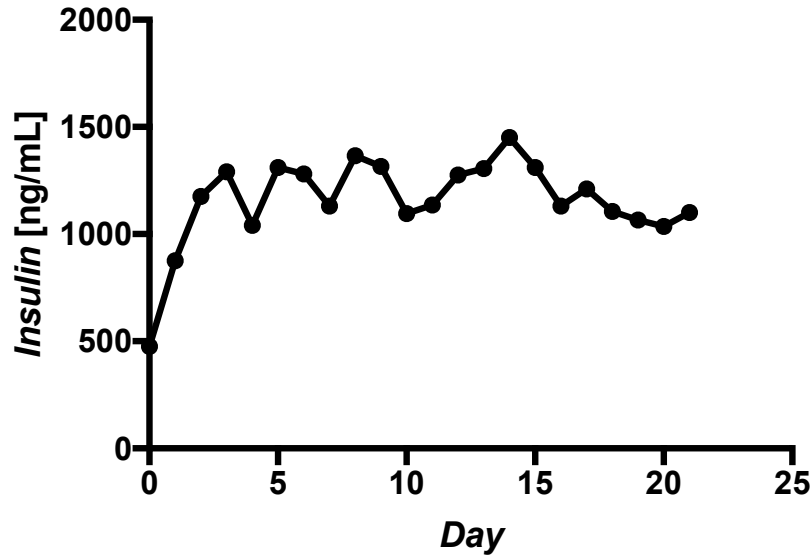
Xiao et al, *Nature Commun*, 2017

NIEHS/ORWH/NCATS UH3

Discover Magazine, Top 100 Discoveries, 2017

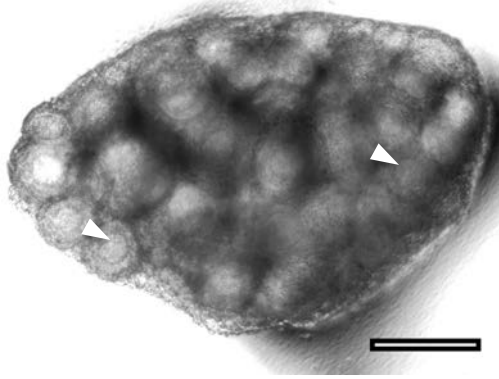


Microfluidic Culture of Islet and Ovary

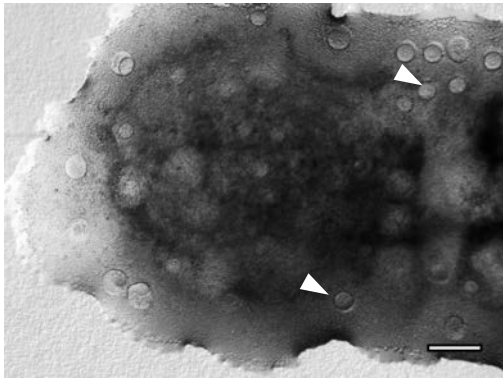


Encapsulated islets produce stable insulin levels across 21 days of culture and support normal ovarian endocrine patterning.

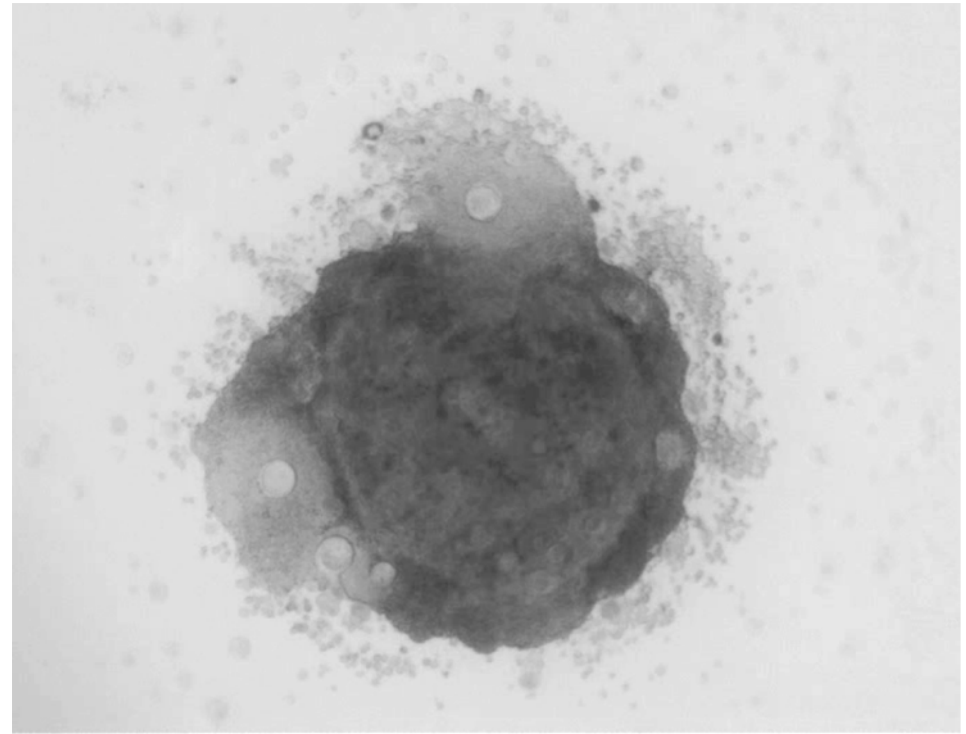
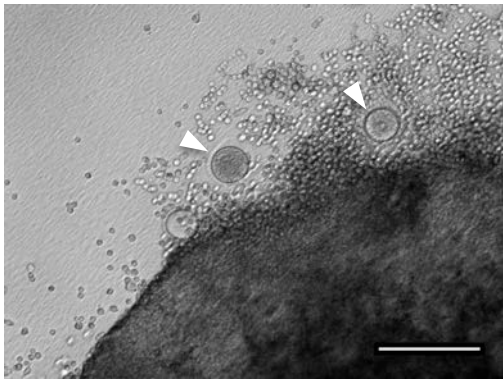
Day 0



Day 13



Day 14



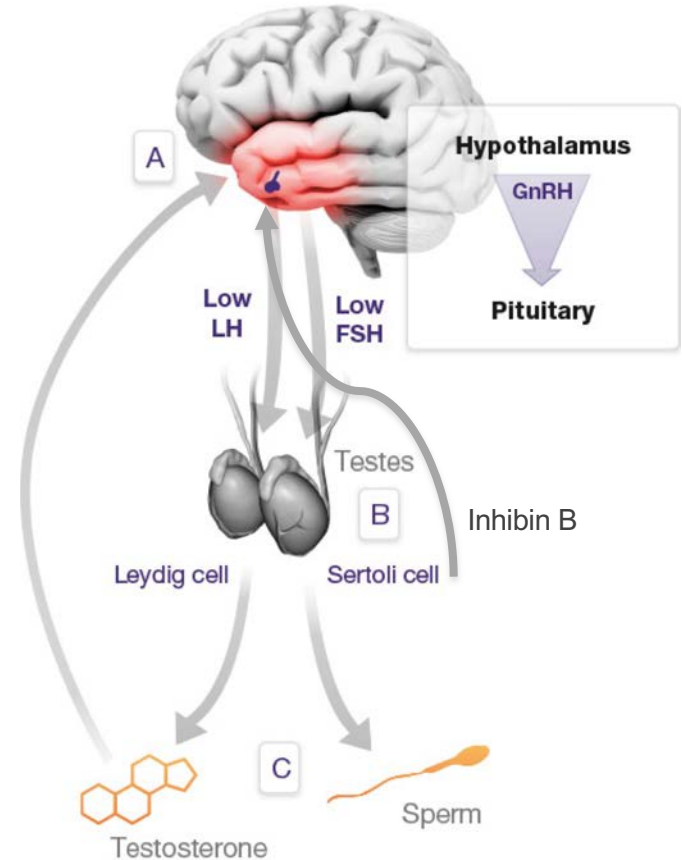
- Ovarian explants maintain structure throughout culture period without addition of exogenous insulin.
- Explants exhibit normal functional responses to endocrine patterning, including ovulation following hCG surge.

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

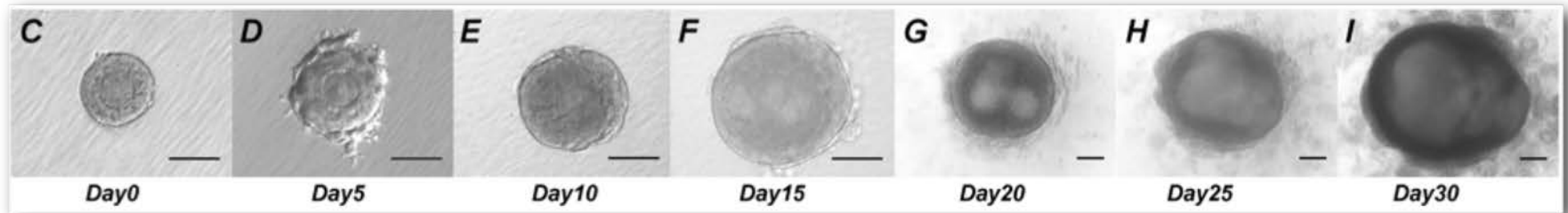
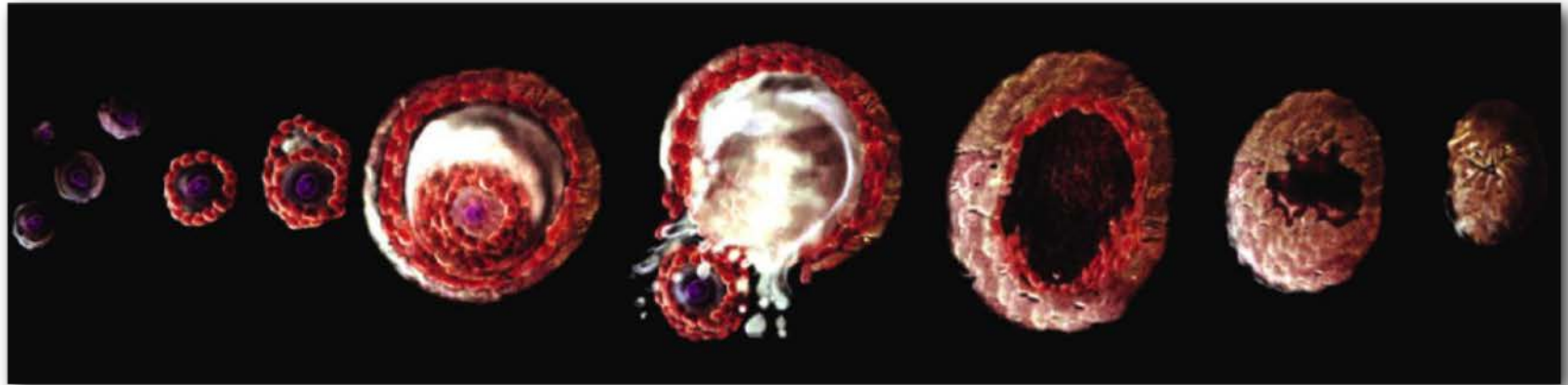


[Device Mimicking Female Reproductive Cycle Could Aid Research](#), *NPR*
[Meet Evatar: The Lab Model That Mimics the Female Reproductive System](#), *New York Times*
[How to build a female reproductive system that fits in the palm of your hand](#), *PBS News Hour*
[‘Organ On A Chip’ Re-creates the Female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

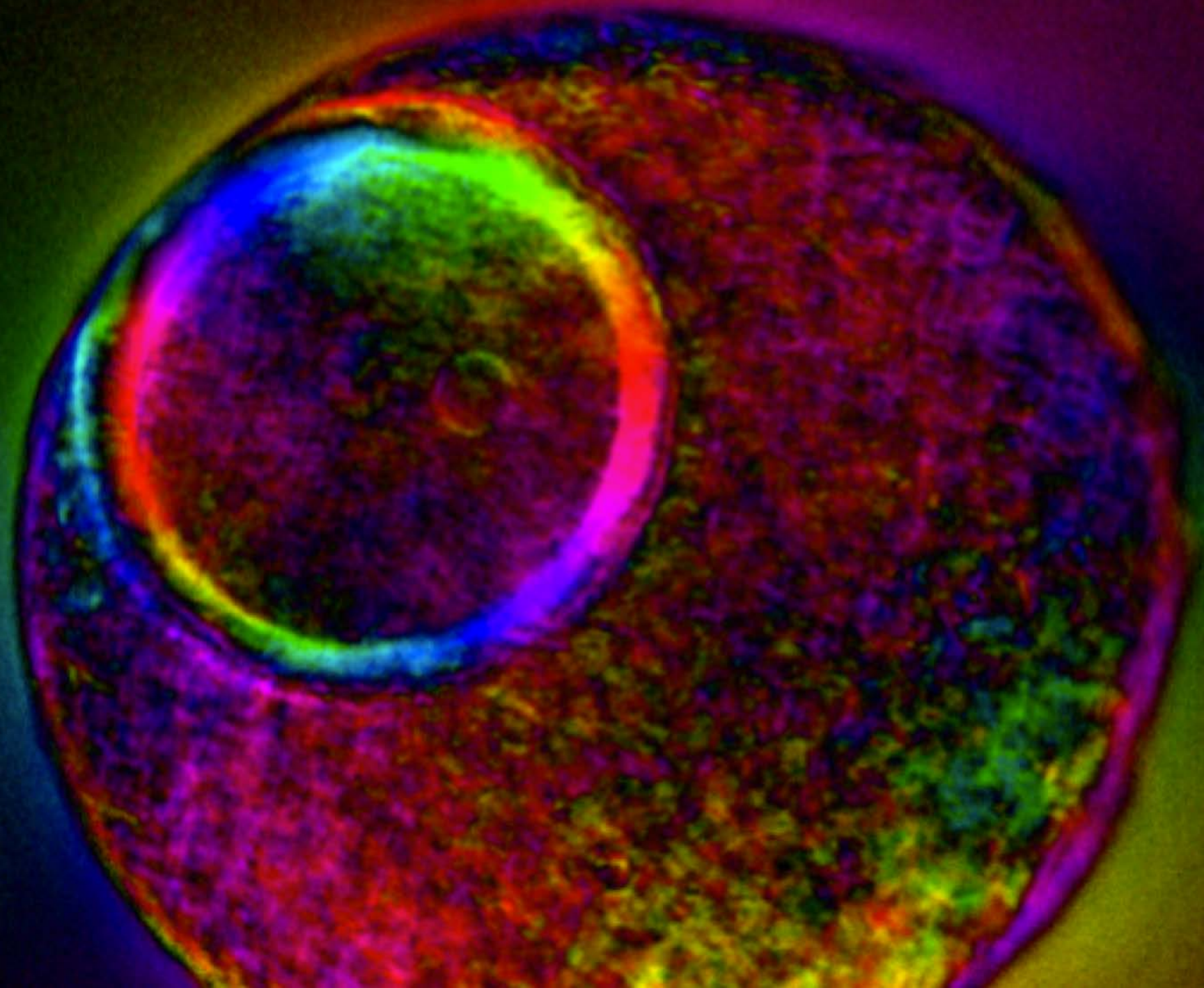
Funded by: NIEHS/ORWH/NCATS

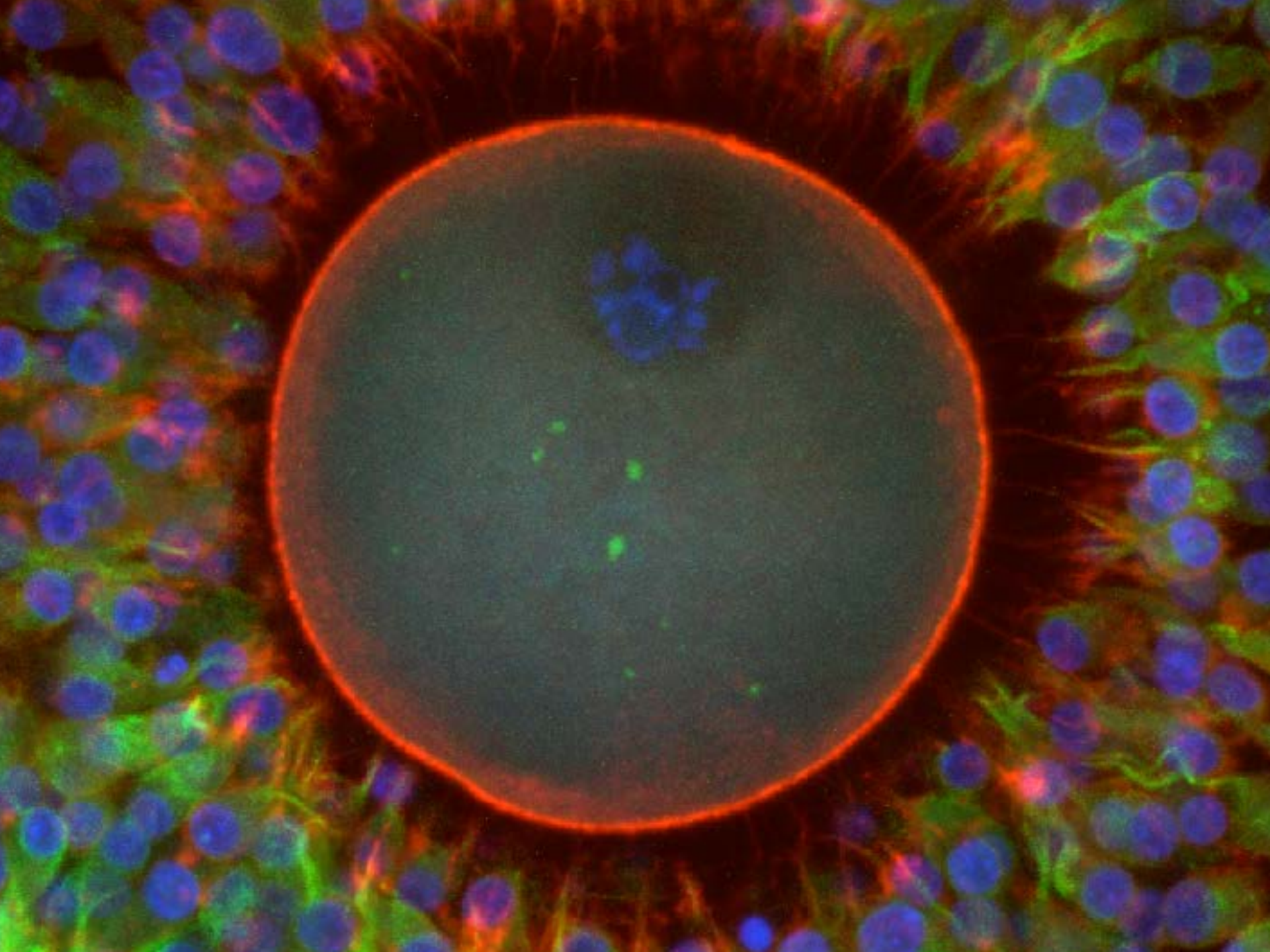


Follicle maturation *in vitro* phenocopies *in vivo* development

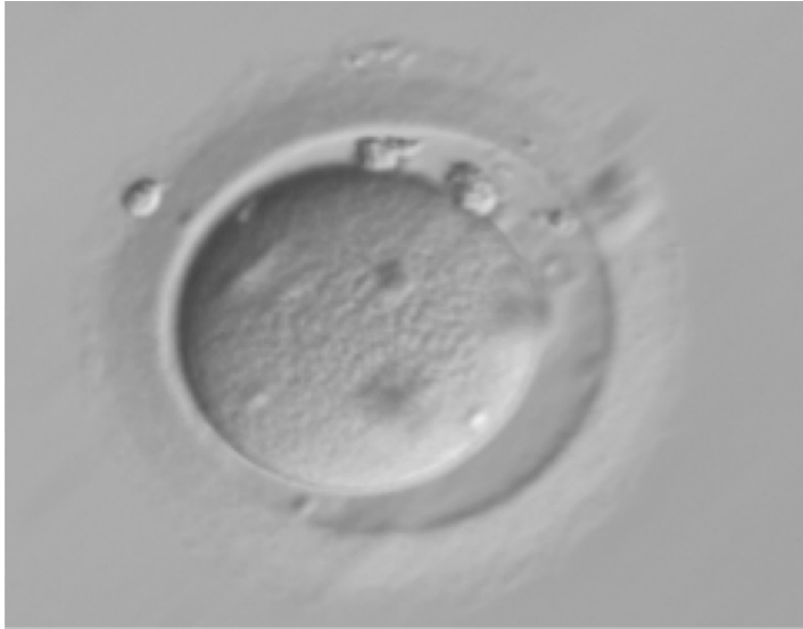


Grow - Secrete Hormones - Differentiate
Egg Matures - Ovulatory Mechanics - Connections
Live, Healthy Births in Mice
Architecture, Environment and Hormones

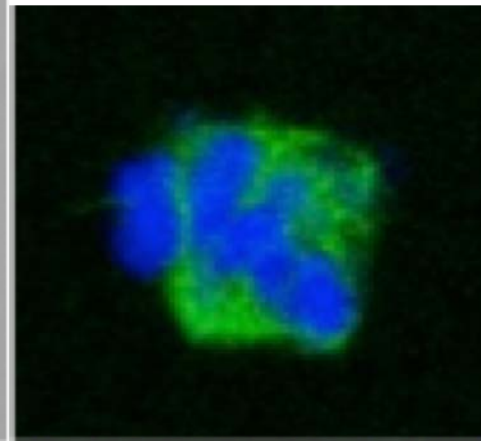




eIVFG Matured Human Oocytes



44 patients; 65 follicles; 4 MII

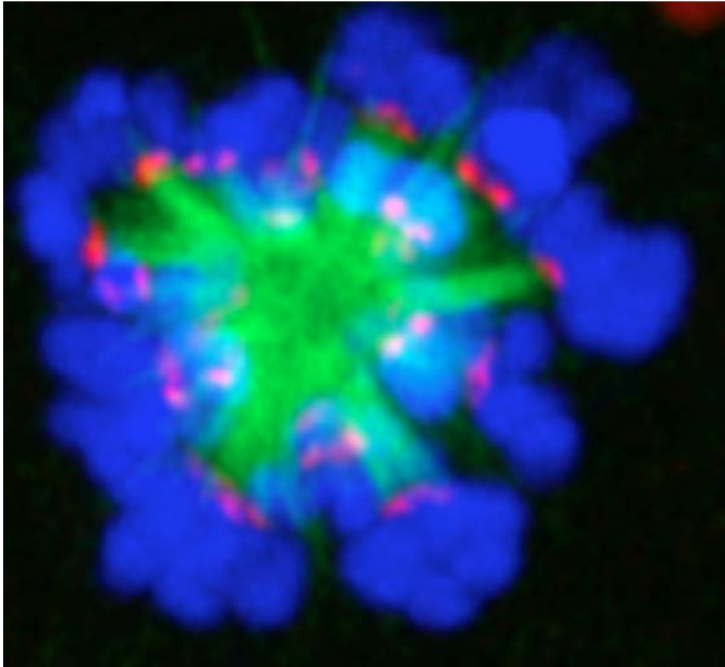


National Center for Translational Research in Infertility – NICHD P50

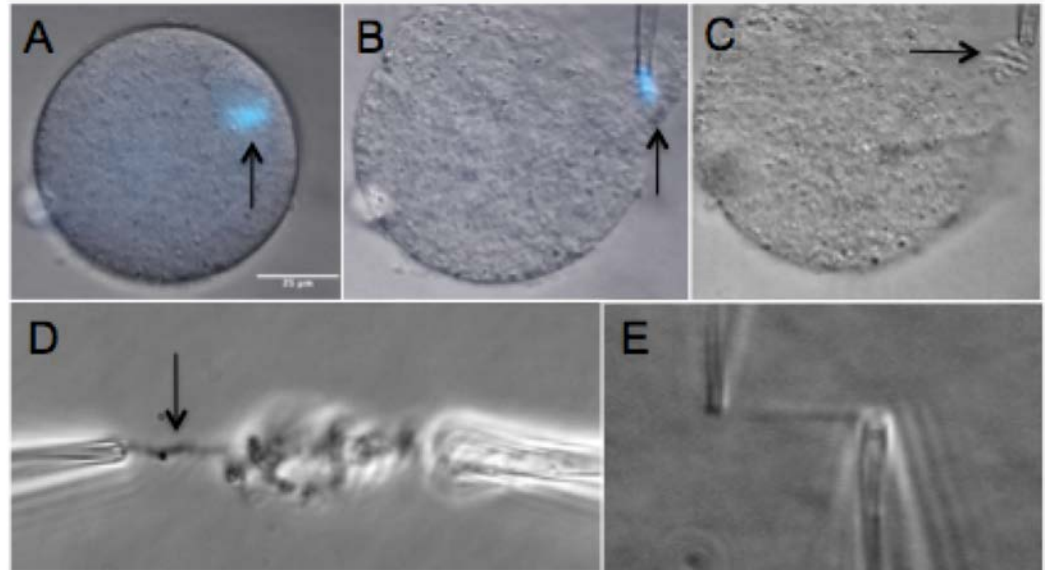


What Makes a Good Egg?

Structure Informs Function

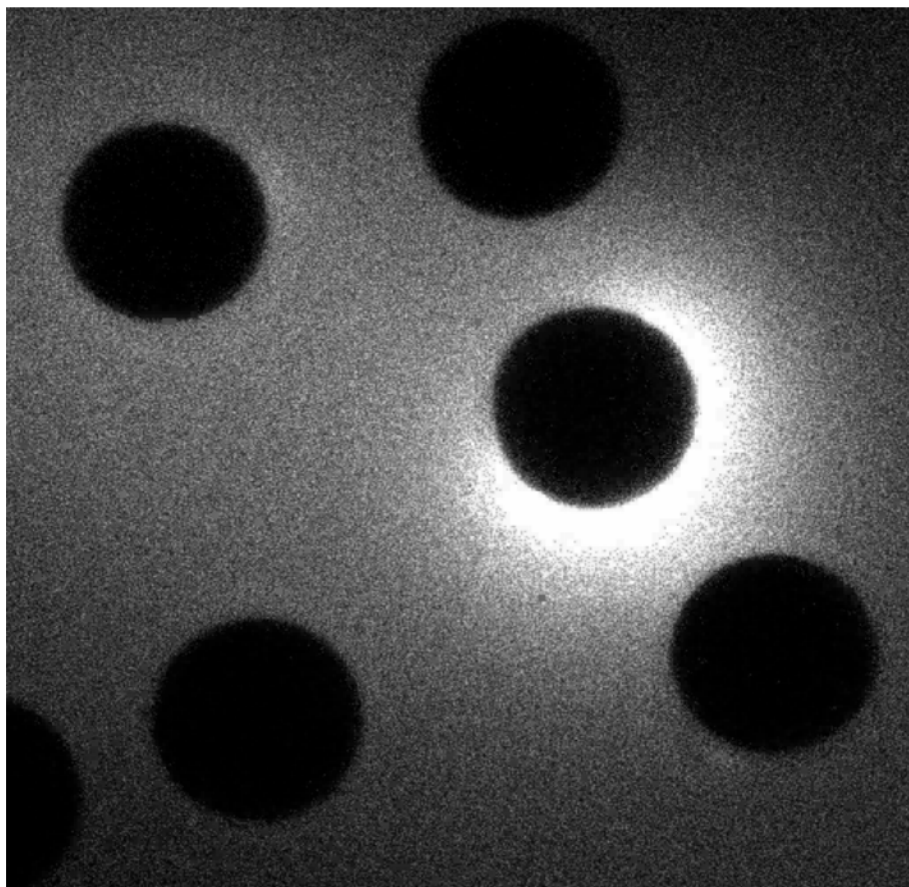


Duncan...Woodruff, **Aging Cell**, 2012



Hornick, Duncan, Marko, Woodruff, **JARG**, 2014

What Makes a Good Egg?



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



Francesca Duncan, Ph.D.
Assistant Professor
Northwestern University



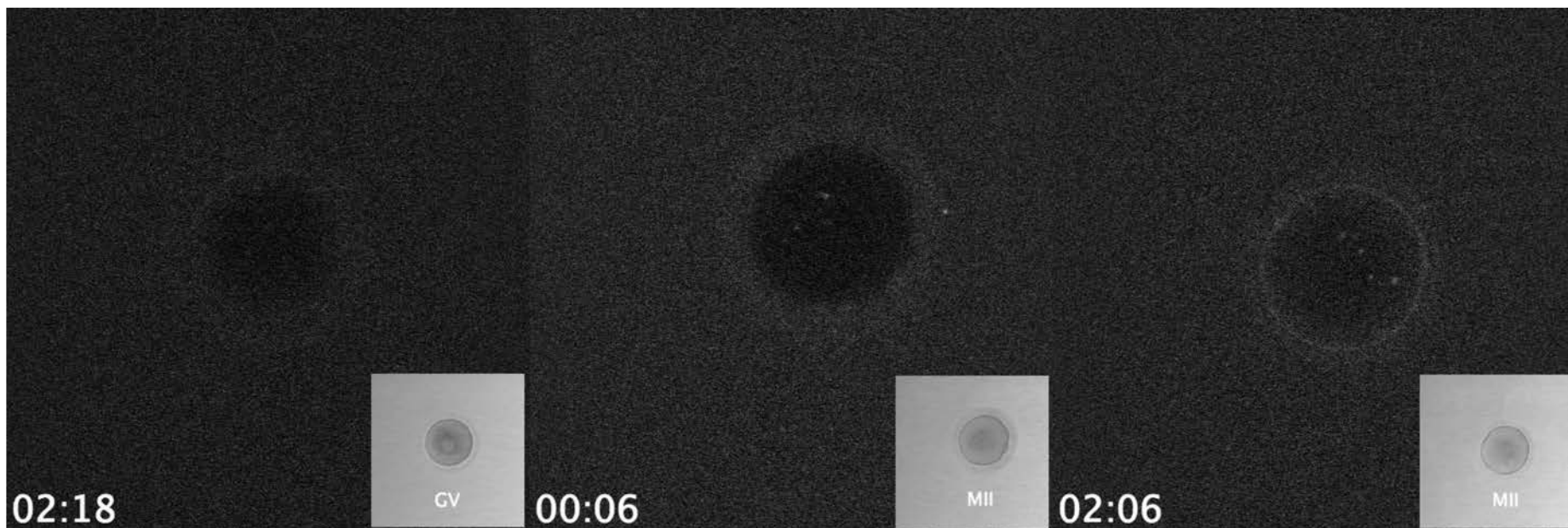
Hoi Chang Lee, Ph.D.
Postdoctoral Fellow
Woodruff Lab

Kim, Bernhardt, Kong, Duncan, Que, Zhang
***Nature Chemistry, Nature Chemical Biology,
Scientific Reports etc*** (2009-present)

NICHD, Ferring



Zinc is only released from mature, health human eggs



DISCOVER Magazine – Top 100 Discoveries of the Year, 2016

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

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 "assisted hatching," 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. 

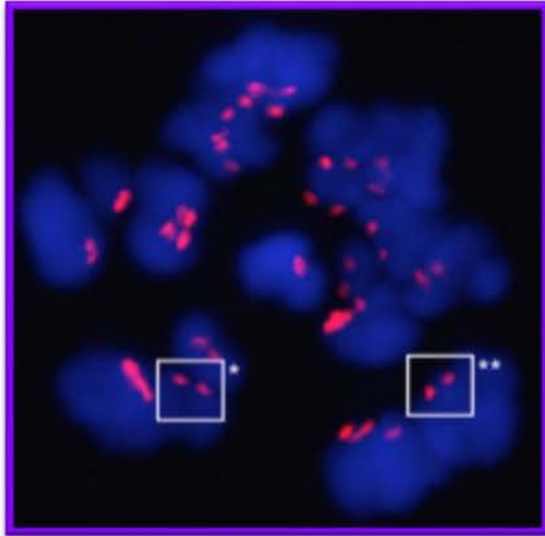
2016 Researchers find that when sperm enzymes fertilize the egg, there is a burst of naturally occurring zinc. These zinc "sparks" are brighter for healthier eggs, which could lead to more effective fertilization techniques.



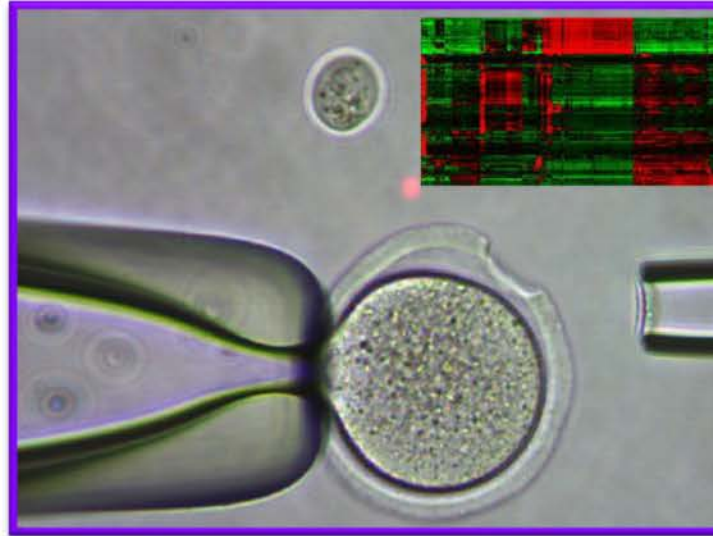
ATTILA VERECZKEY/CREATIVE COMMONS 3.0 (2). TIMELINE F



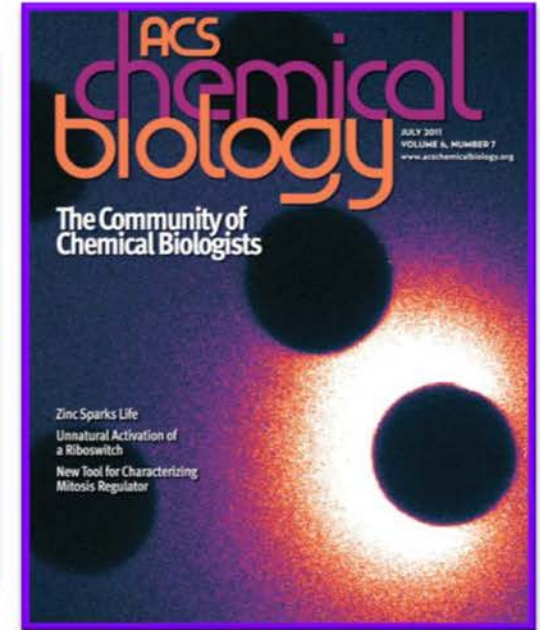
What makes a good egg?



Chromosome
number
and Structure

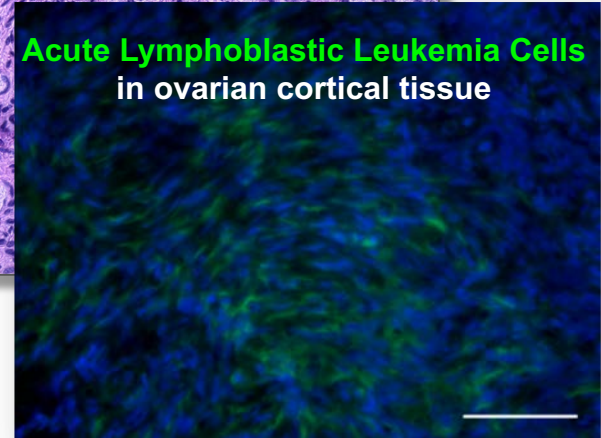
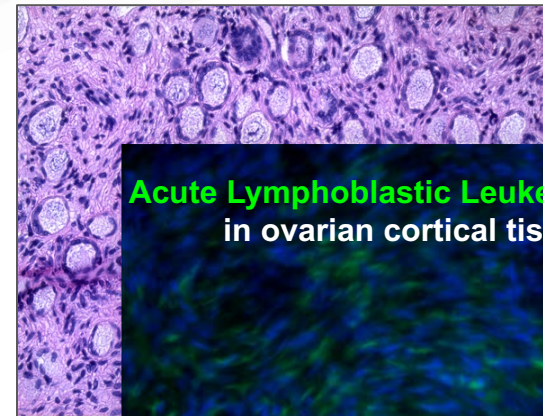
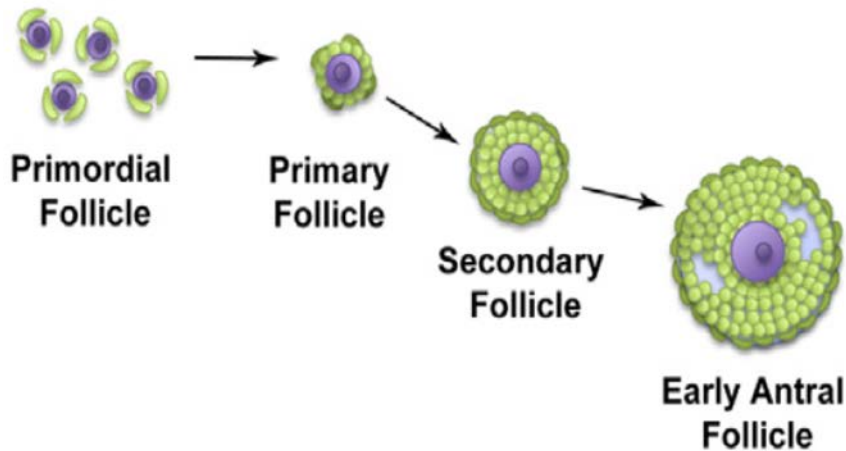


Oocyte-specific
gene expression



Zn exocytosis
at fertilization

Fertility Needs in Pediatric Cancer Patients



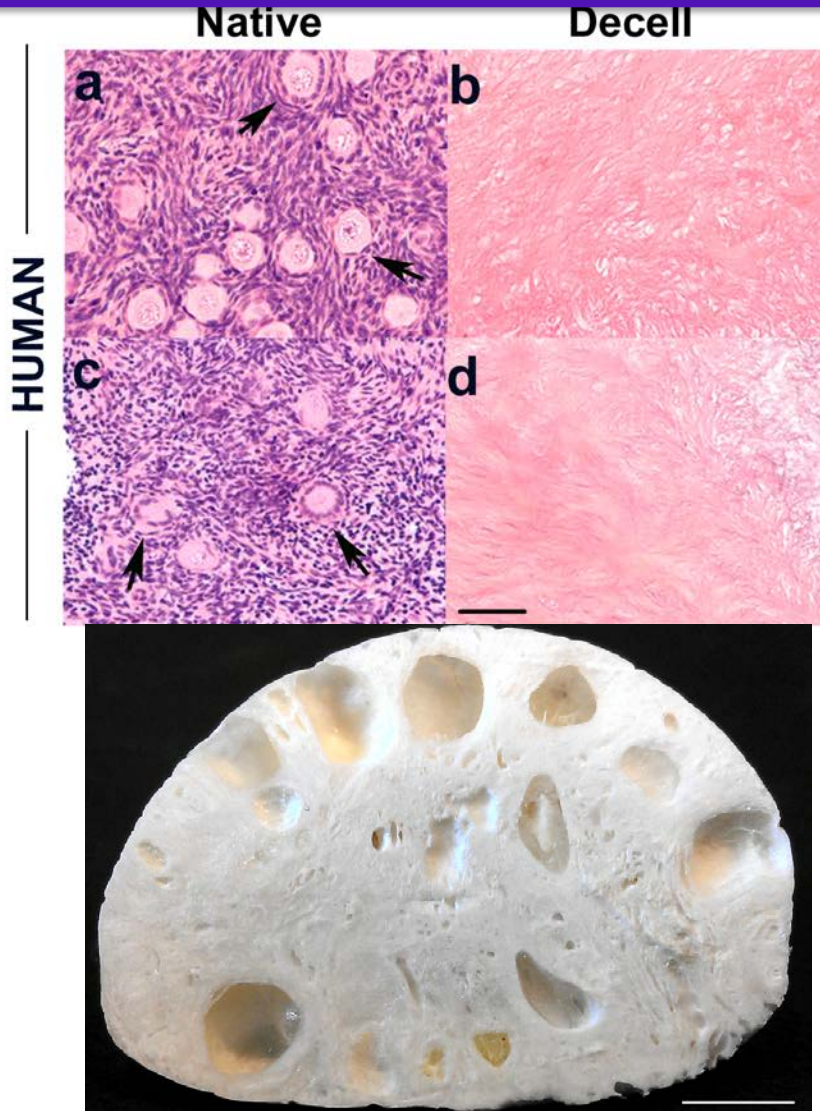
Ovarian cortical tissue from NPC participants; Scale bar = 50 um Laronda, et al. (2015) Biomaterials

Modified from: Cordeiro, Kim, Woodruff. *Cancer Treatment and the Ovary* (2015)

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)

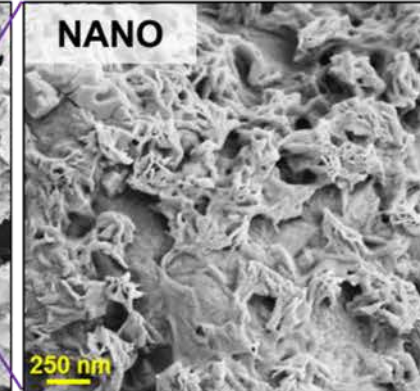
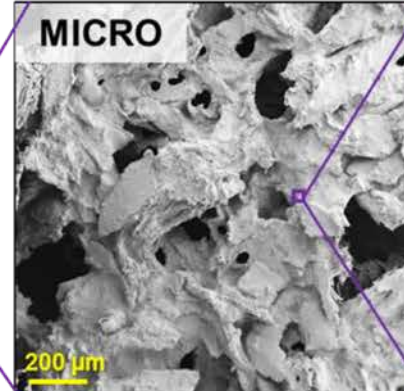


40 cm² TP
<20 min. Fabrication



65 vol.% Ovary dECM
35 vol.% PLGA

1 cm



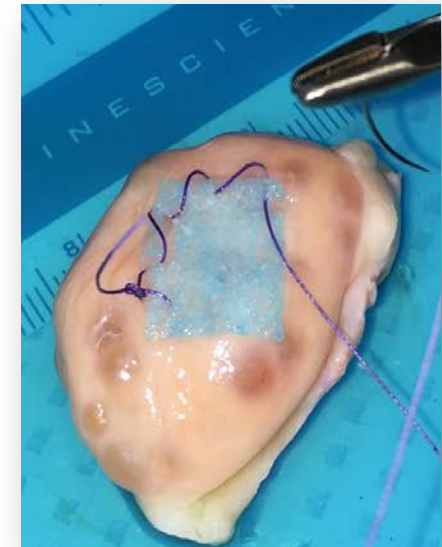
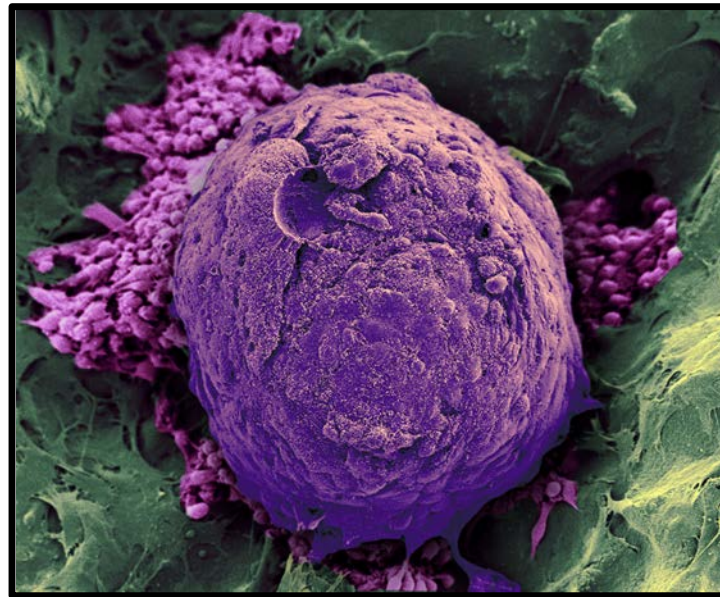
Decell'd Bovine
Ovary

Powdered
dECM

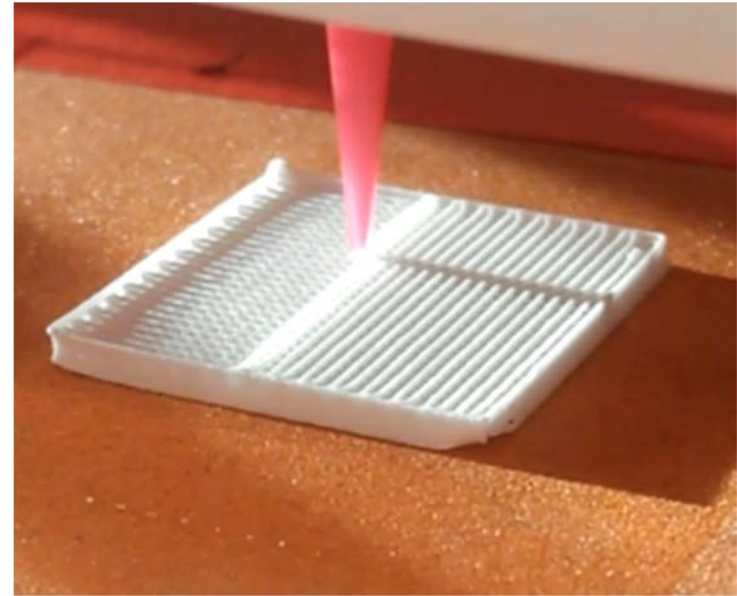
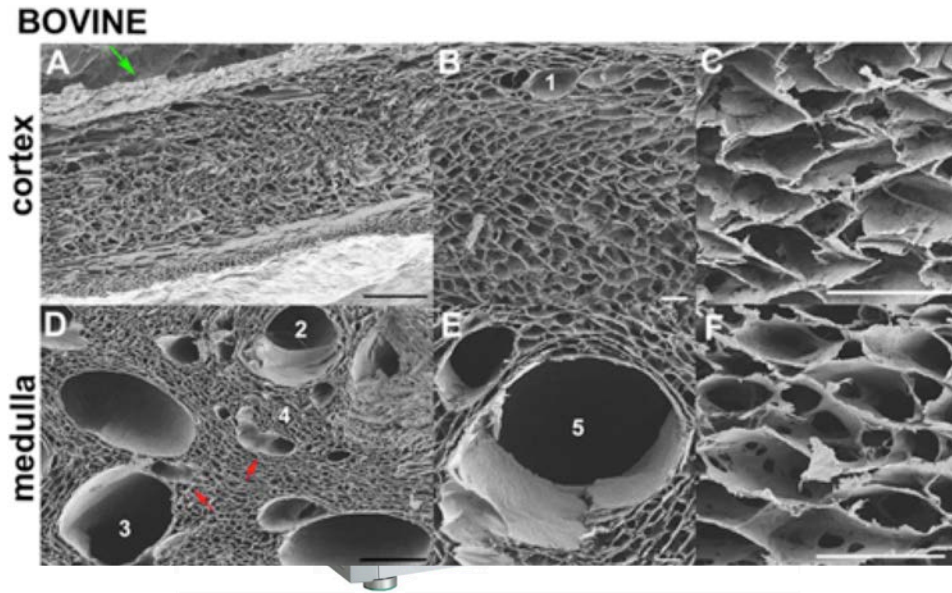
dECM
"Ink"

Ovarian dECM
"Tissue Paper"

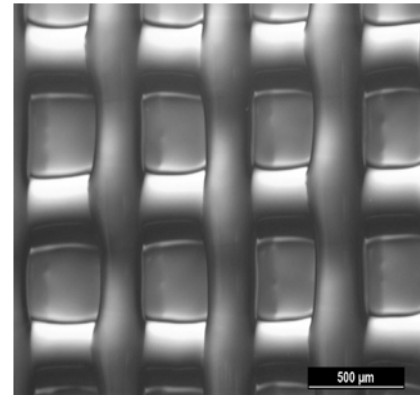
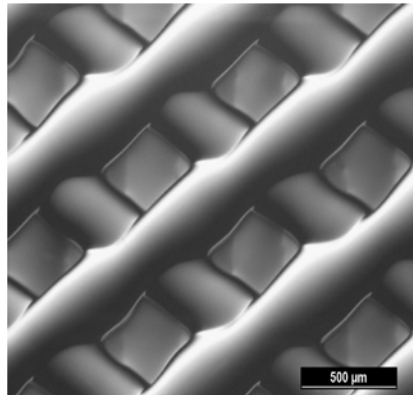
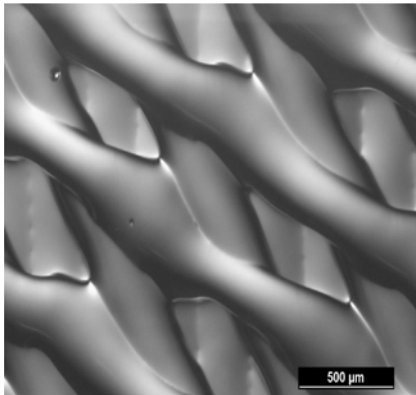
Retains substantial porosity and texture



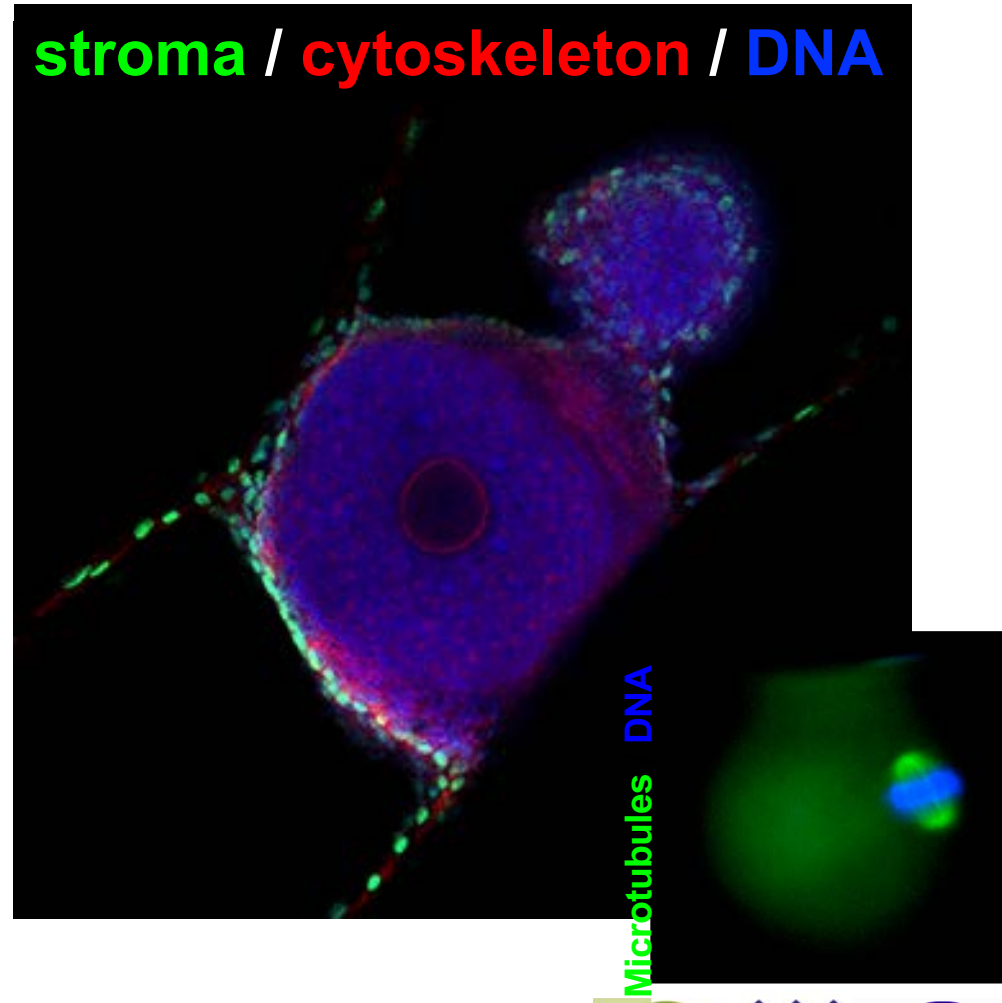
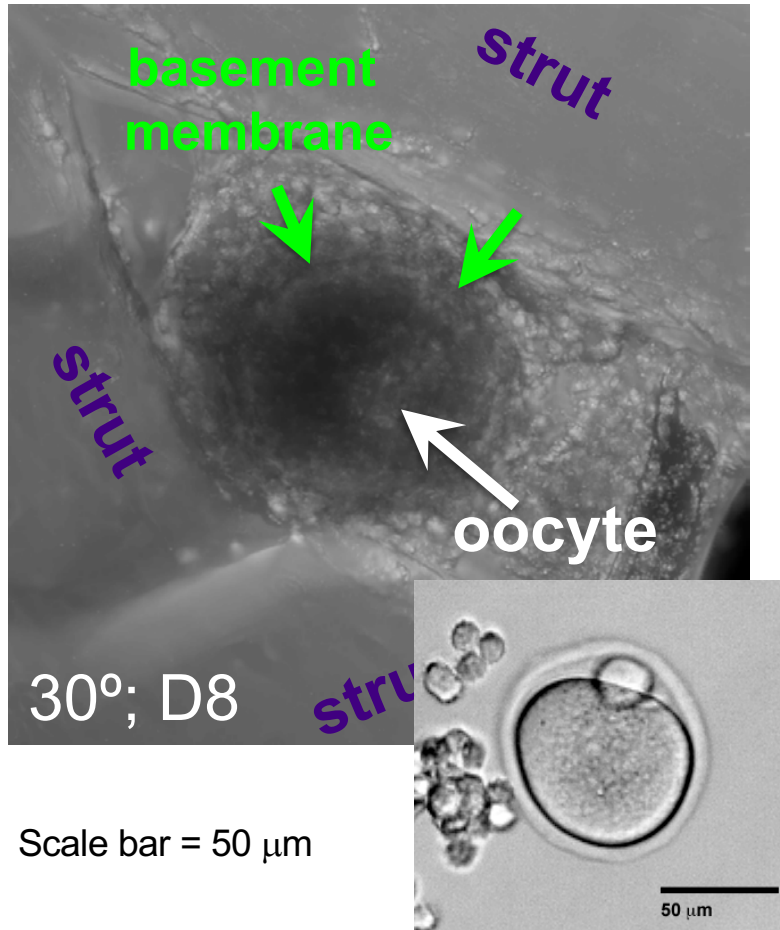
Bioinspired design of structural ECM as organ scaffolds



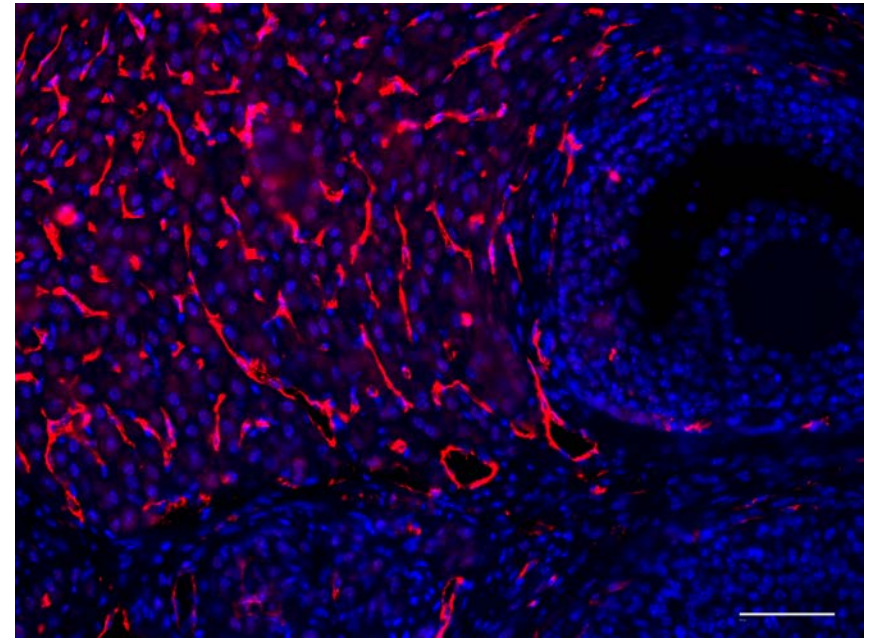
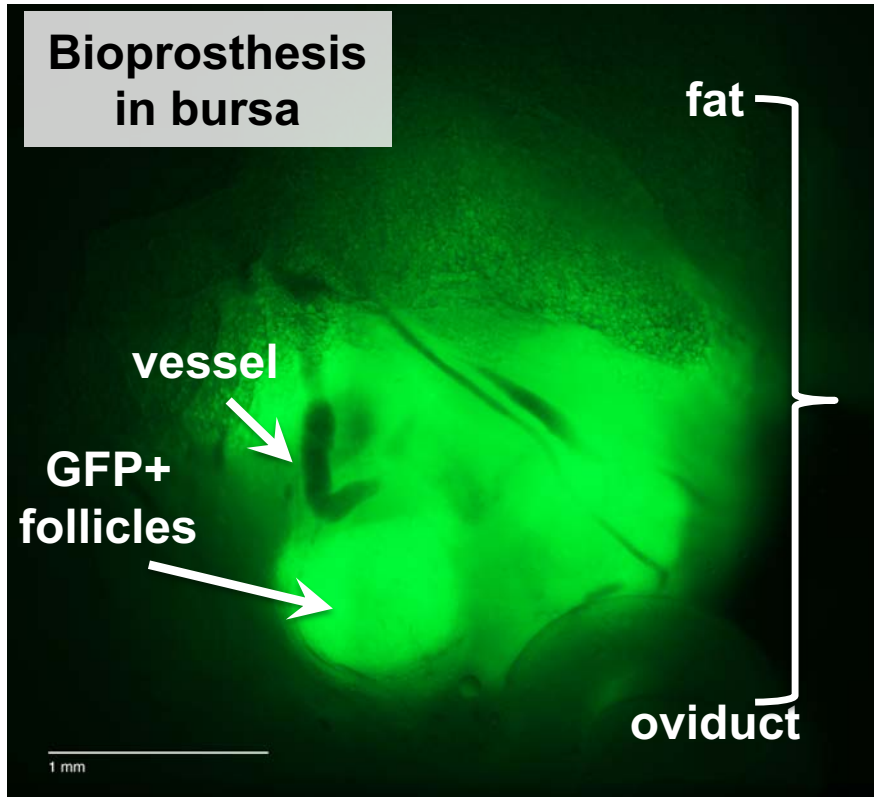
30° advancing angle 60° advancing angle 90° advancing angle



Bioinspired Scaffold Design –Follicle Development and Oocyte Maturation

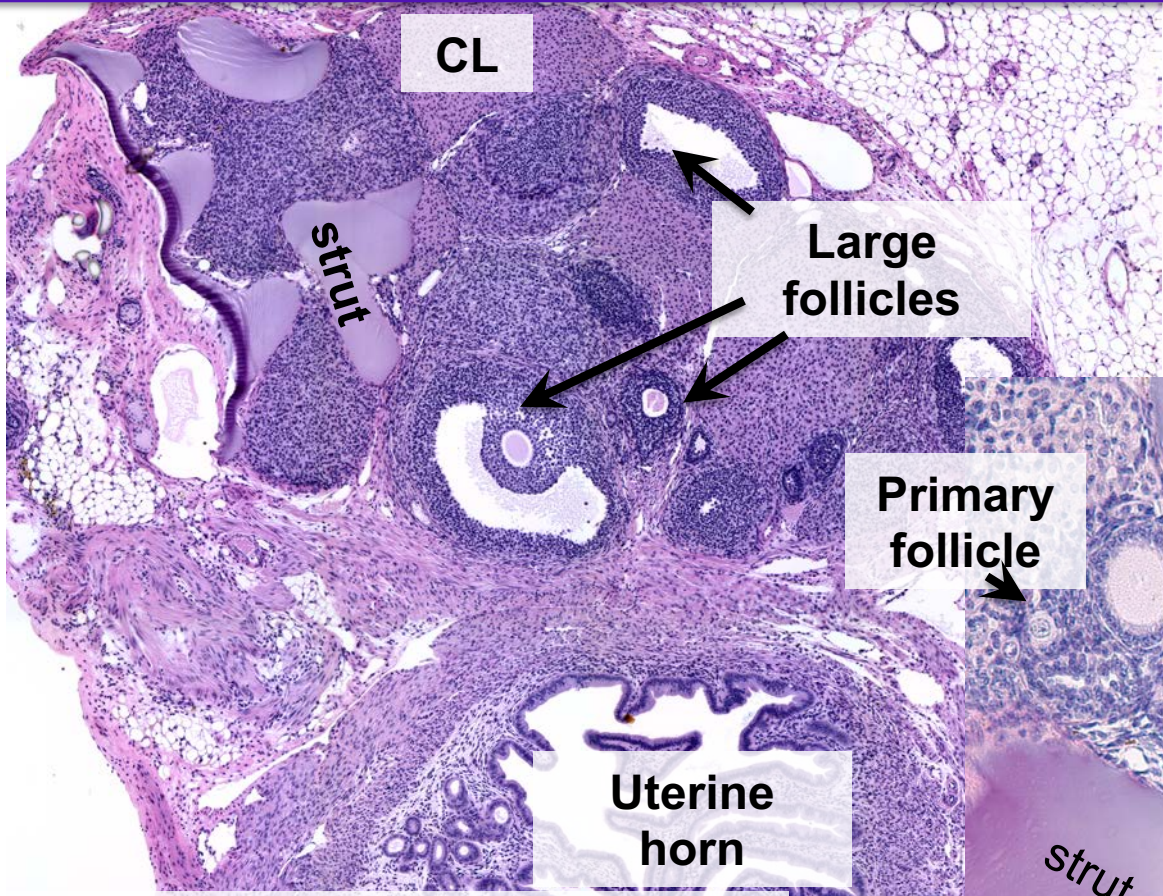


Bioinspired Scaffold Design – Soft Tissue Transplant

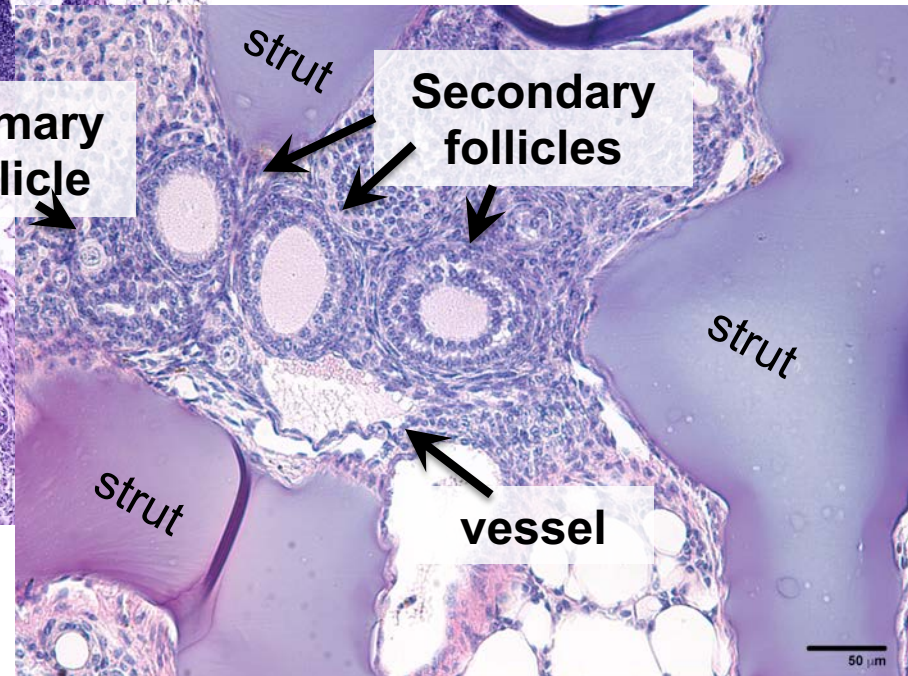


PECAM **DNA**

Bioinspired Scaffold Design – Soft Tissue Transplant



Bioprosthesis 8wks post-surgery

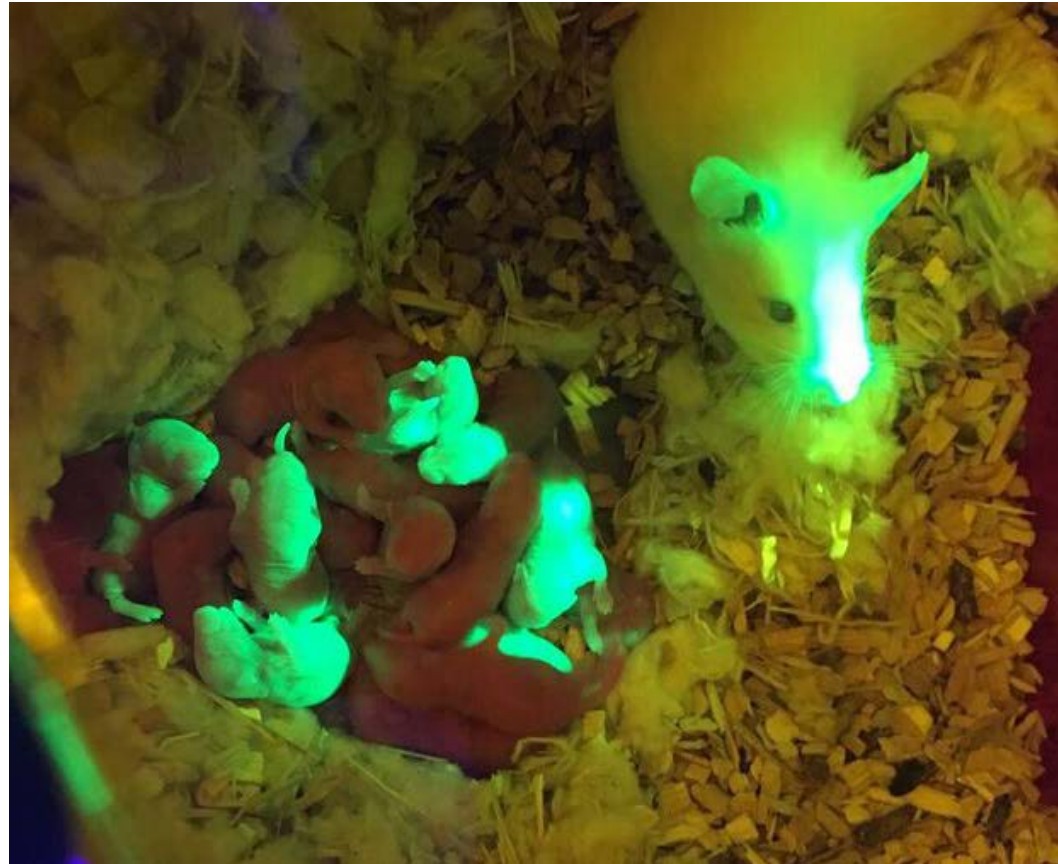
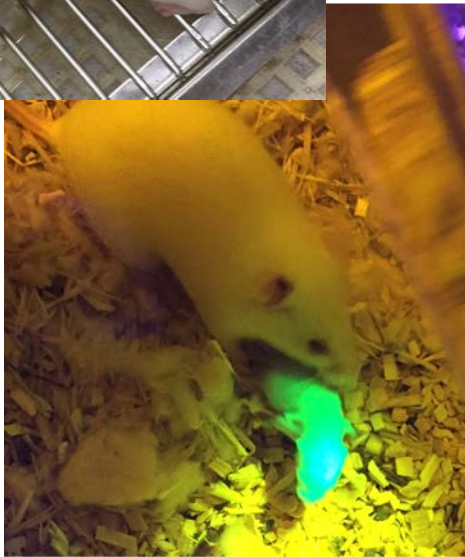


Bioprosthesis 3wks post-surgery

Scale bar = 200 and 50 μm



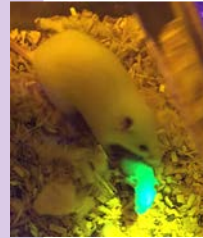
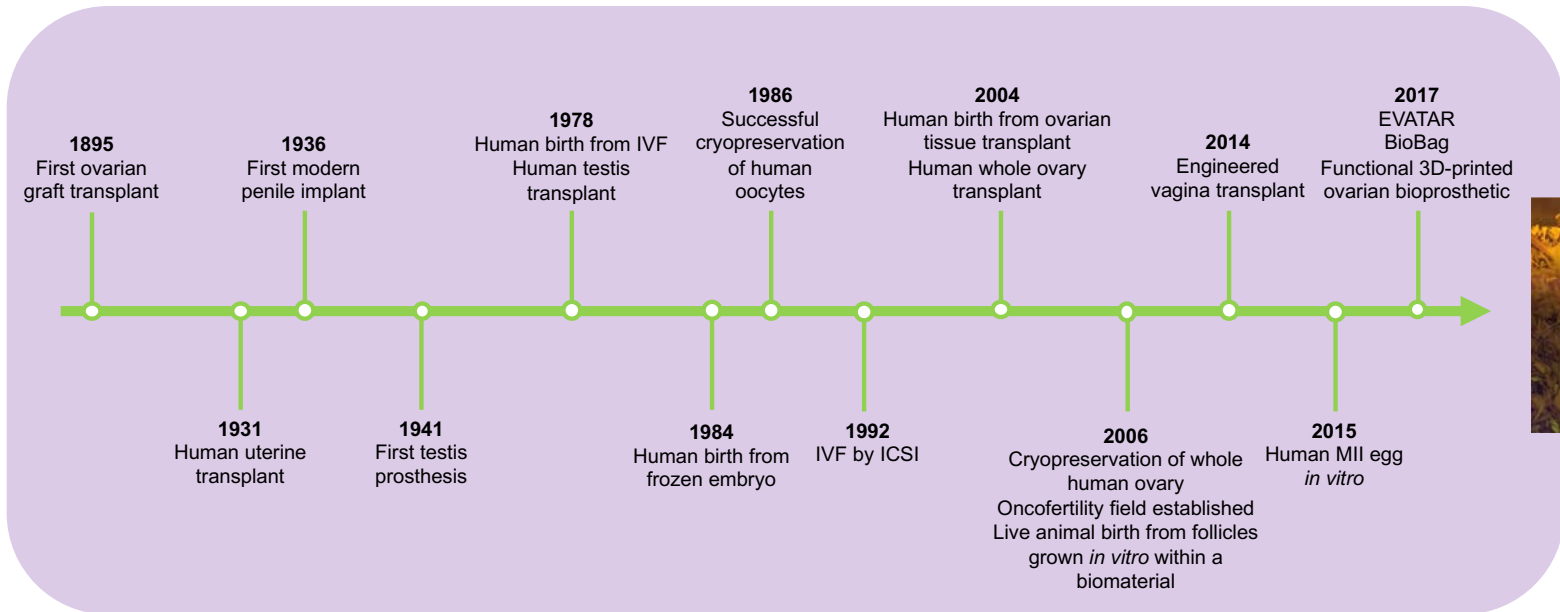
Live Birth from Ovarian Bioprothesetic Transplant



Transplant recipient (EGFP-)
with EGFP+ pup



Timeline of Discovery



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



Roger Gosden, Ph.D.
Ovarian Tissue Cryopreservation



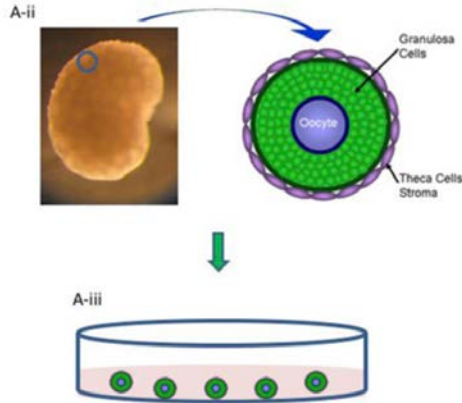
Kutluk Oktay, M.D.



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

Engineering the Reproductive Axis

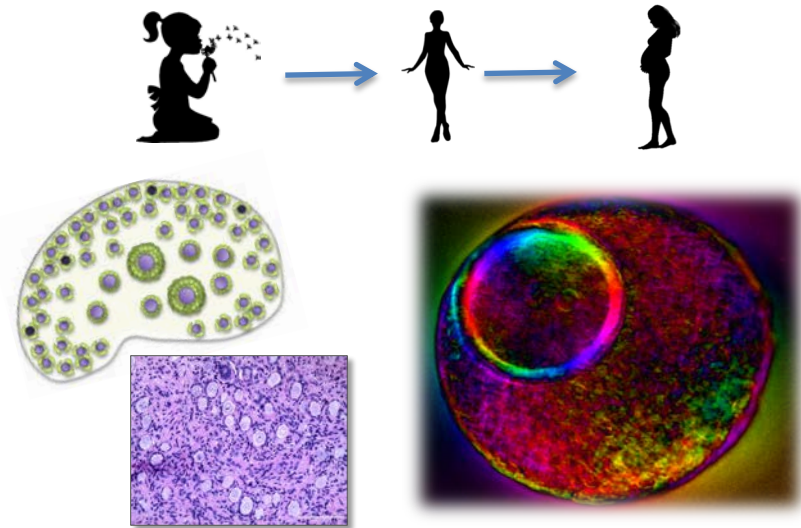
Follicle Maturation in a Dish



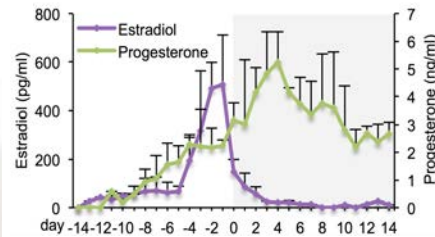
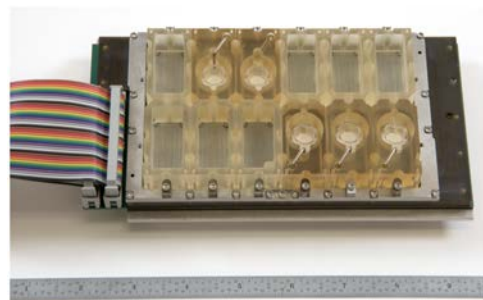
NUBorn & NUAge



Oncofertility Solutions



Engineered Reproductive Tract

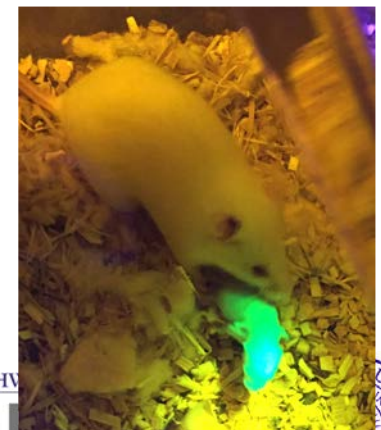


MFP-Ovulation



Artificial Ovary

Decellularized Ovary



Oncofertility 2018



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

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

Steven Chu, March, 2009

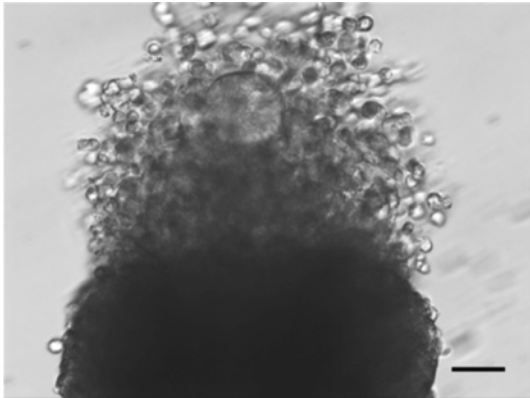
Oncofertility 2018-2058



- ✓ Better Cancer Control and Tx
- ✓ Higher Selectivity of Patients
- ✓ Neo-adjuvant Fertoprotectives
- ✓ In Vitro Follicle Maturation
- ✓ Designer Ovarian Bioprosthesis
- ✓ 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

NATIONAL
PHYSICIANS
COOPERATIVE



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Oncofertility[®]
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NIH/NICHD: P50HD076188

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

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

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Draper Labs
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