Microphysiological Modeling of the Female Reproductive System: Implications for Toxicity Testing and Understanding Disease Pathophysiology

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Overview

1. Why the Female Reproductive Tract?
2. Building and testing the Repro *in vitro* systems
3. Integration - FemKUBE
4. Beyond the FemKUBE
The Female Reproductive Tract

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Research in the Female Reproductive Tract is limited

- Ethical issues associated with research in women of reproductive age/pregnancy
- Vast species differences in reproductive processes
  - The human model is the best model for human reproduction

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Female reproductive hormones influence the whole body

- Ovary, Fallopian tubes, uterus, cervix
- Breast
- Heart
- Bone
- Liver
- Brain
- Gastrointestinal system

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Preclinical and clinical research are done in males

"We don't study females... Their menstrual cycles would mess up our results."

The female is very complex
- menstrual phases
- pre- and post-menopausal states,
- pregnant women

In the past decade, 8 of the 10 drugs that were pulled from the market for being unsafe were found to have more serious side effects in women than in men

Women - higher rates of anxiety and dementia
Men - greater likelihood of autism and Parkinson's disease

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Ex-Vivo Female Reproductive Tract: Reproductive Toxicology and Drug Testing

- Early Testing of Drugs
  - Reduce sex bias in pipeline
  - Increase safety of drugs
- Sex Specific Drug Metabolism
- Gestating Uterus Drug Development
- Contraceptive Design and Targets
- Environmental Health Toxin ID
- Vaccine Development
- Mechanisms of Cancer Prevention and Therapy

Laronda et al. *Stem Cell Research and Therapy*, 2014
The Reproductive Tract Is An Integrated System With Functional Hallmarks That Can be Modeled *In Vitro*

Primate Reproductive Tract

FemKUBE Integration:
NU/MIT/Draper Gen 0.3W

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The Hormonal Changes of the Menstrual Cycle
Growing Reproductive Tract Tissues In Vitro

Teresa Woodruff

Joanna Burdette

Julie Kim

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Recapitulating Ovarian and Endocrine Changes of the Human Menstrual Cycle *In Vitro*

**Characterize Hormonal Responses in Vitro**

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OvaryKUBE: Human Follicle 3D Culture in Alginate

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OvaryKUBE Validation and Functional Assay: In vitro Human Follicle Hormone Profile (N=5)

A

B

C

D

E

F

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Yuanming Xu, Teresa Woodruff
OvaryKUBE: Using 3D Mice Multiple Follicle Culture to Phenocopy the Hormones of the Human Menstrual Cycle
OvaryKUBE: Applications of *In Vitro* Follicle Growth (IVFG) system

Source of ovarian hormones

Testing of compounds that affect follicles

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Yuanming Xu
OvaryKUBE Validation of Compounds: In Vitro Follicle Growth (IVFG) Provides a Method of Predicting Reproductive Outcomes in Mammals in Response to Environmental Exposures

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Effect of chemotherapeutics known to have adverse reproductive effects on Follicles *In Vitro*

- Cyclophosphamide (CTX)
- Cisplatin (CDDP)

![Graph A](image1)

![Graph B](image2)
Effect of Corexit 9500 on Follicles *In Vitro*

- Corexit 9500

**Graph:**
- Y-axis: Percent survival
- X-axis: Culture time (days)
- Graph showing survival rates for different concentrations over time.

**Images:**
- Comparative images for different days (Day 0, Day 4, Day 6) showing follicles at various concentrations (Control, 25 ppm, 50 ppm, 75 ppm).

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Effect of Nalbuphine on Follicles *In Vitro*

- Nalbuphine (analgesic)
TubeKUBE: Human Fallopian Culture
TubeKUBE: Cilia Beating
TubeKUBE: Hormonal Regulation of Cilia Beating

Ctrl

E2 1nM

E2 1nM + P4 10nM

E2 0.1nM + P4 50nM

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TubeKUBE:
Hormonal Response to Secreted Factors

OVGP1

IGF1

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Jie Zhu
 TubeKUBE: Testosterone Alters Hormonal Responses

E2 1nM

E2 1nM + T4 100nM

OVGP1

hIGF1

α-tubulin

Jie Zhu
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UteroKUBE: Human Myometrium and Endometrium

Proliferative stage endometrium

Myometrium

Endometrial epithelial and stromal cells

Myometrial cells

UteroKube

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CervixKUBE: 3D Human Endocervical Cultures

Control

E₂

E₂+P

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Sevrim Yildiz Arslan
CervixKUBE: Hormone Stimulation of Mucin Production

MUC16 (CA125)

Control

E2+P4

PAS (Red: neutral mucins)

Alcian Blue (Blue: acidic mucins)
CervixKUBE: Validation of Secreted Factors

- HGF
- IL-6
- LIF
- MCP-1
- MIP-1β
- TNF-α
- GRO-α
- IL-1α
- IL-1β
- IL-1RA
- PDGF-BB

Hormone levels

- Estrogen (E2)
- Progesterone (P4)

Time (days)

Proliferative phase

Secretory phase
Moving Static to Microphysiologic

Teresa Woodruff, Department of Obstetrics and Gynecology, Northwestern University

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Jeff Borenstein, Ph.D., Biomedical Engineering Center, Draper Laboratory

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Microphysiologic (MPS) Modules
- Plug-and-play module interface
- Media/collection MPS module
- Follicle MPS module
- Modules have 4 ports to support 2 separate flows

Fluidic Interface
- Connects modules to each other
- Can direct flow within modules
- Interfaces to actuator layer to pump fluid

Actuator Interface
- Pneumatic layer
- Air pressure drives the pumps by actuating the membrane located between the fluidic and pneumatic layers

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Jonathan Coppeta, Brett Isenberg Draper Laboratory
Jackie Shepard Northwestern University
In Vitro Hormone Delivery Mimics of the Fluctuating Estrous Cycle

Microfluidic Culture
- Real time delivery of hormones to downstream tissue
- Integration of tissues

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Jackie Shepard
Flexible Microfluidic designs

Diagram: Two syringes connected to a network of blocks labeled 'Foll', 'Tube', 'Ut', and 'Cerv'.
FemKUBE: Beyond the Female Reproductive Tract

• Disease Modeling

– Cancer - Endometrial, Ovarian, Breast
FemKUBE: Beyond the Female Reproductive Tract

• Disease Modeling
  – Leiomyoma

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FemKUBE: Beyond the Female Reproductive Tract

• Disease Modeling
  – HIV transmission/Vaccines
FemKUBE: Beyond the Female Reproductive Tract

- Pregnancy Modeling: Placenta
- Liver
- Bone
- DudeKube
FemKUBE: Challenges and solutions

• Human tissue availability
  – iPS cell differentiation into reproductive tract tissues

• Optimizing 3D cultures
  – Decell ECM and bioplotted scaffolds
FemKUBE Team

- PI: Teresa K. Woodruff (OvaryKube)
  - Jie Zhu
  - Yuanming Xu
- Julie Kim (UteroKube and CervixKube)
  - Yanni Yu
  - Sevim Yildiz Arslan
- Joanna E. Burdette (TubeKube)
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