Purrfecting Microfluidic Chips for Ovarian Tissue Culture

By
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About Me

Chester, New Jersey

B.S. in Biology from University of Miami

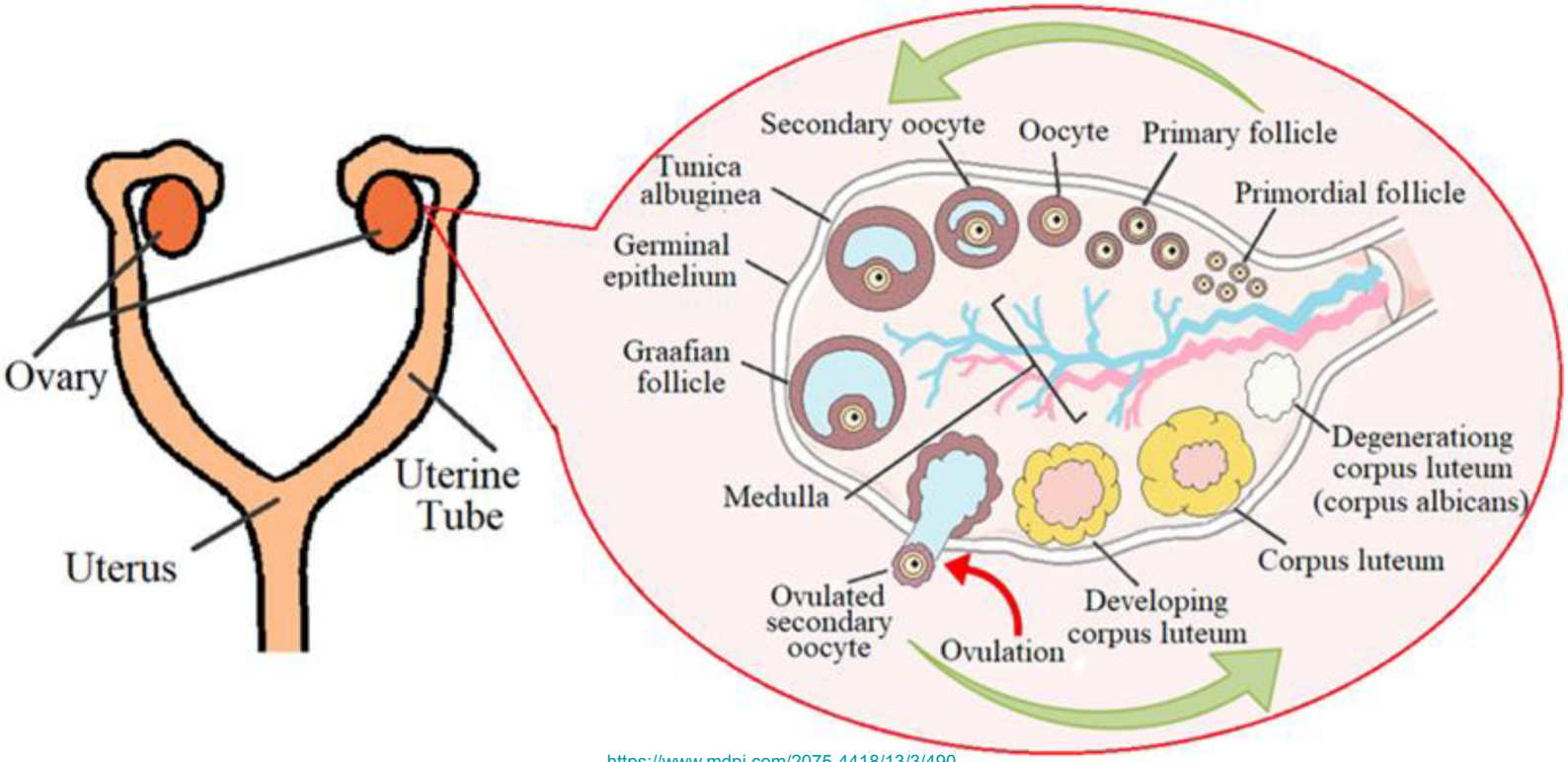
→ pre-veterinary track

Why SCBI?

- Exploration of research behind vet/conservation medicine
- Exposure to useful laboratory techniques
- Understanding of ethical conservation efforts

Introduction

Brief overview of ovarian structures & folliculogenesis:



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Introduction

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Evaluation of an ovary-on-a-chip in large mammalian models: Species specificity and influence of follicle isolation status

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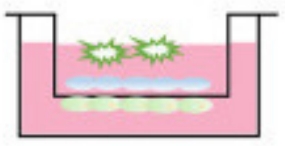


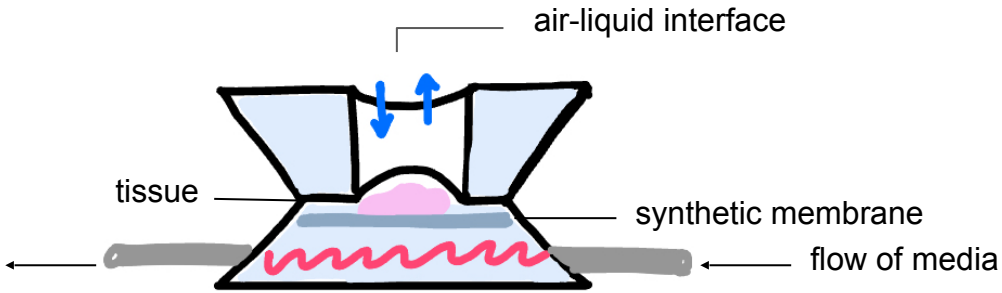
3D co-culture

VS.

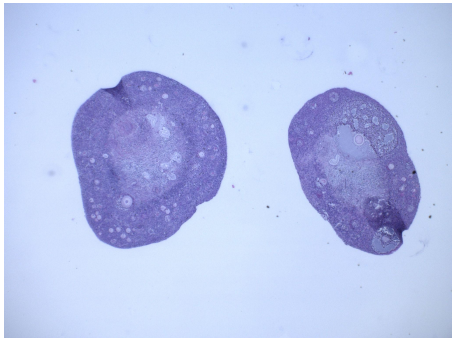
organ-on-a-chip

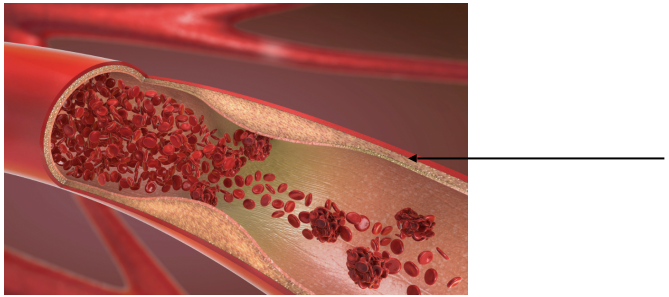
Introduction

How can we combine the advantages of both these culture systems?



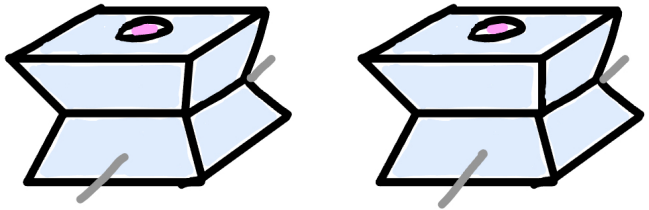
How can we promote tissue growth throughout the entire tissue?





Our Goal

Assess this microfluidic chip using an endothelial cell co-culture as an *in vitro* environment suitable for overall ovarian tissue growth/folliculogenesis in domestic dogs & cats



(-) cells (+) endothelial cells

Methods: Setup

1. **Microfluidic chip fabrication**

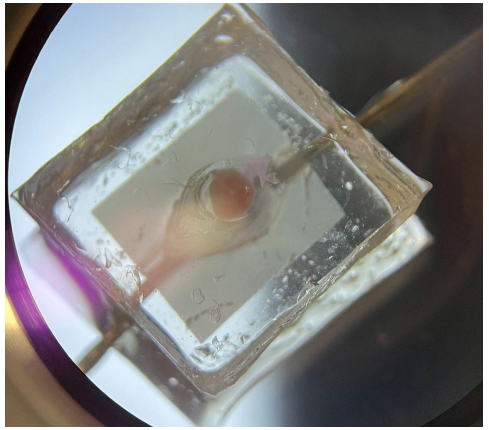
- Formlabs 3D printer for mold
- PDMS

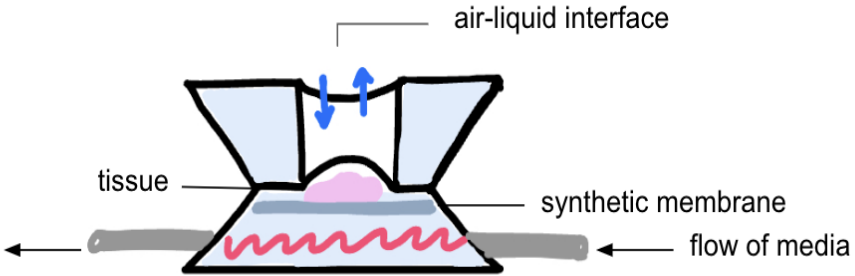
1. **Prep for culture**

- Wash chips
- Prep cell culture media
- Thaw presumed endothelial cells

1. **Isolate ovarian tissue**

- Cats 1 yr. or younger in age (~ 1-2 mm depth)



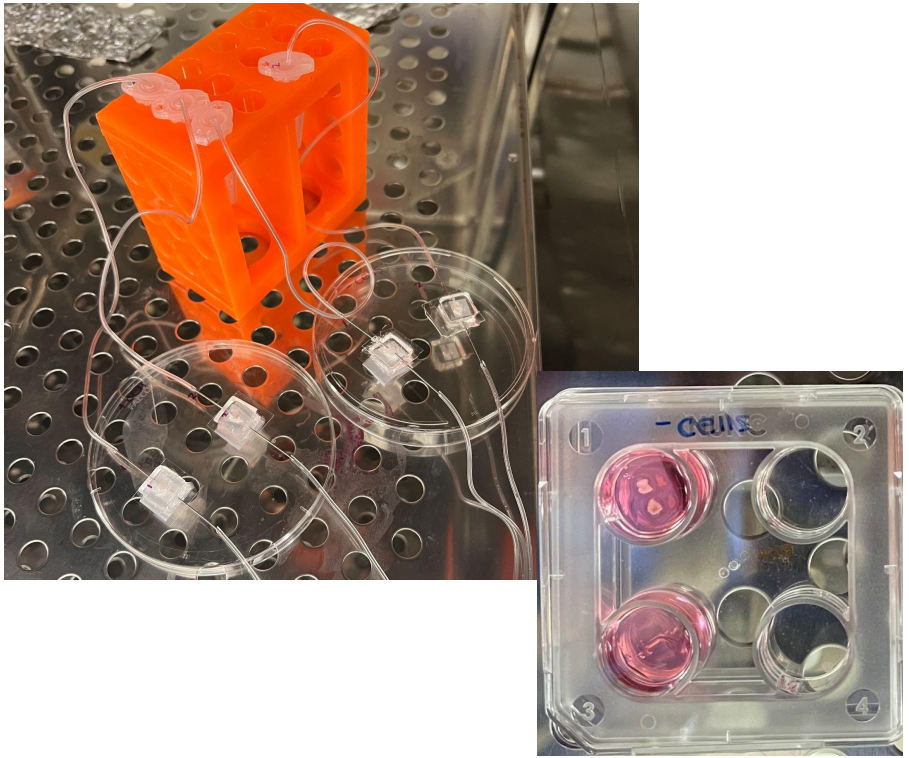


Methods: The Culture

7 Cats (A,B,C,D,E,F,G)

Five treatment groups:

1. Agarose gel block (-) endothelial cells
2. Agarose gel block (+) endothelial cells
3. Chip (-) endothelial cells
4. Chip (+) endothelial cells
5. Fresh control

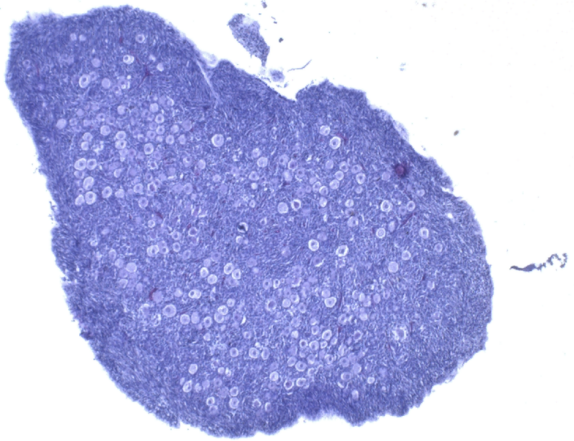


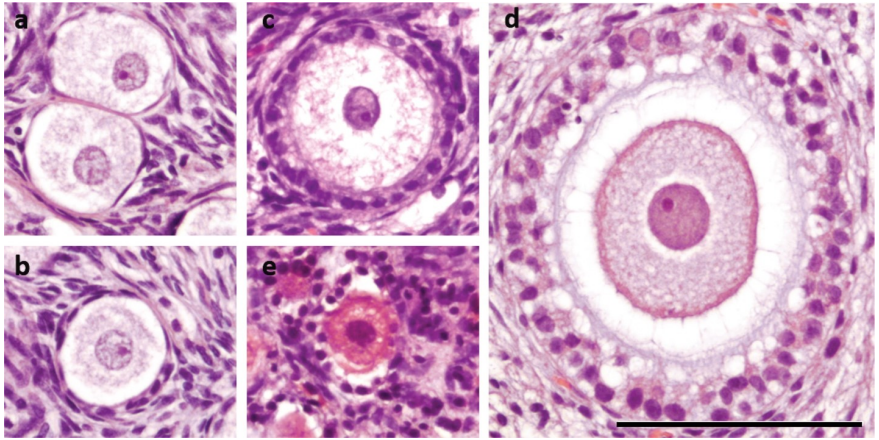
Cultures ran for 6 days
e/o day refreshing
culture media

→

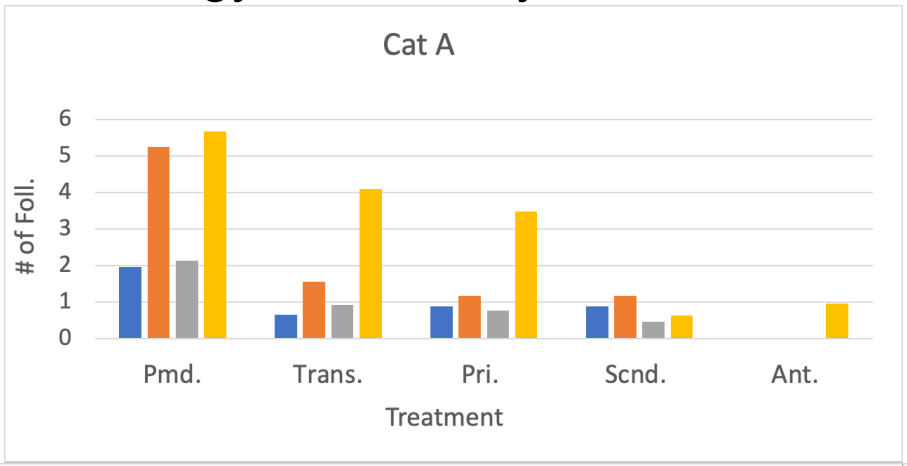
Gene Expression + Histological
Analyses

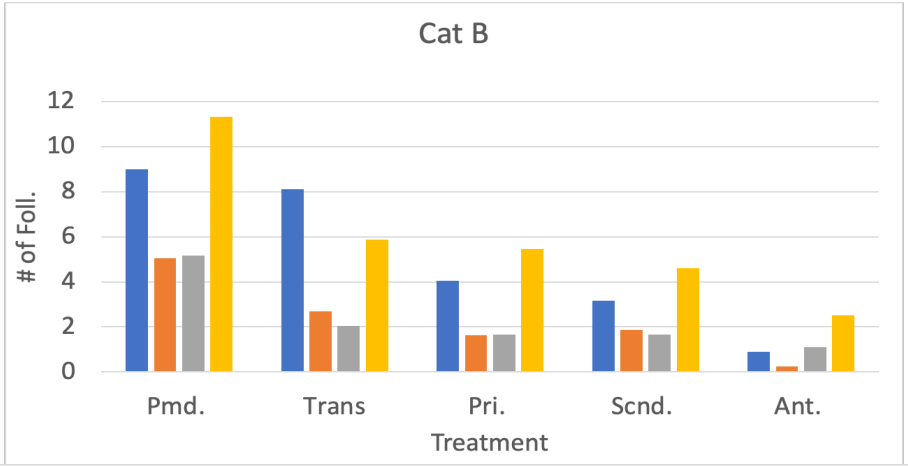
Histology Analyses

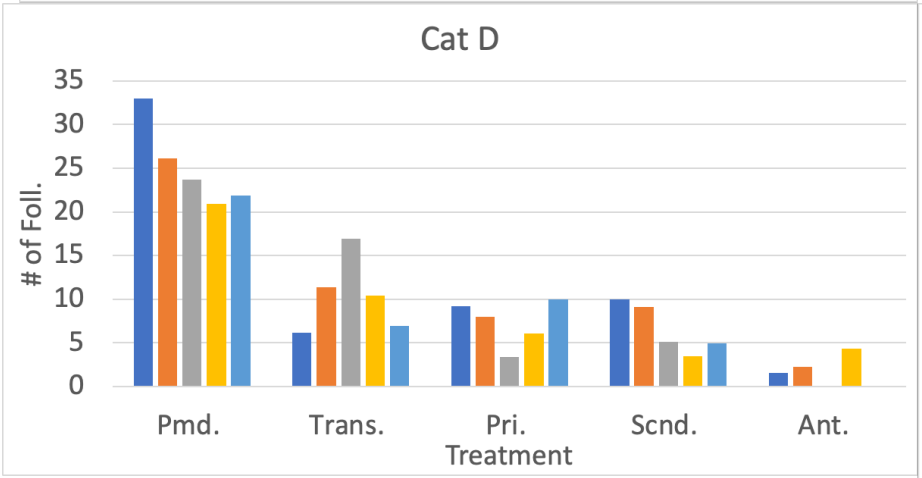


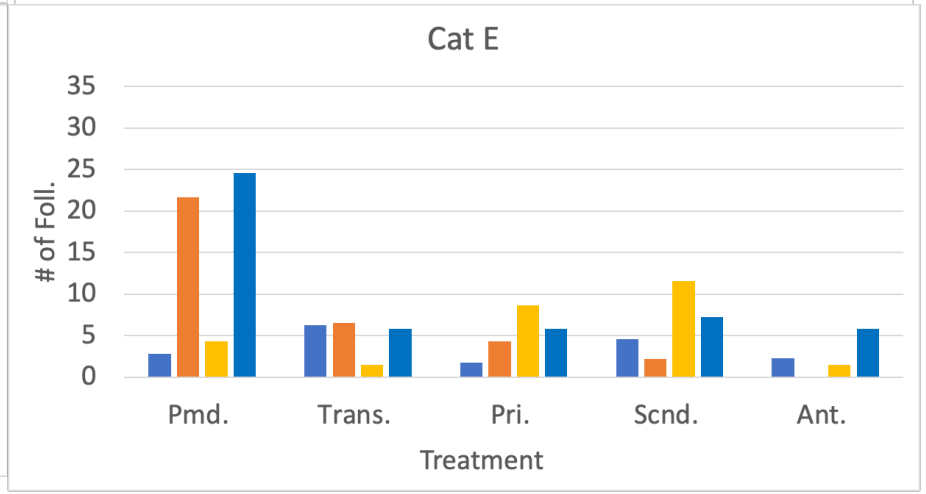


Histology Results: By Cat







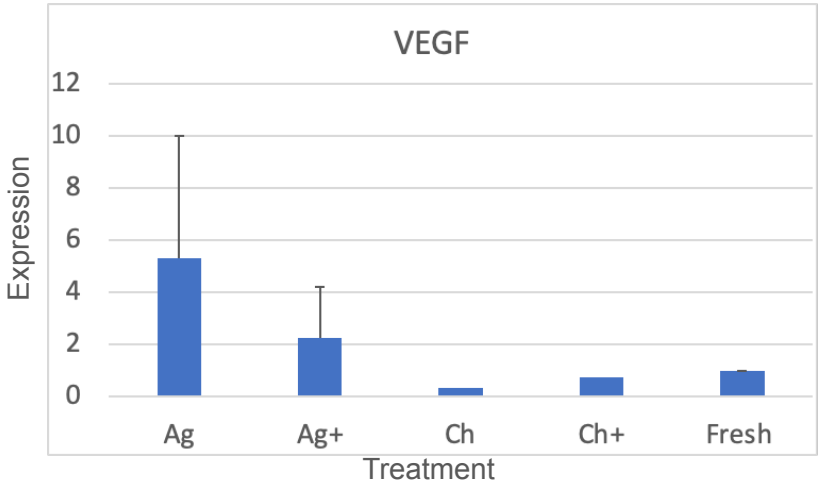


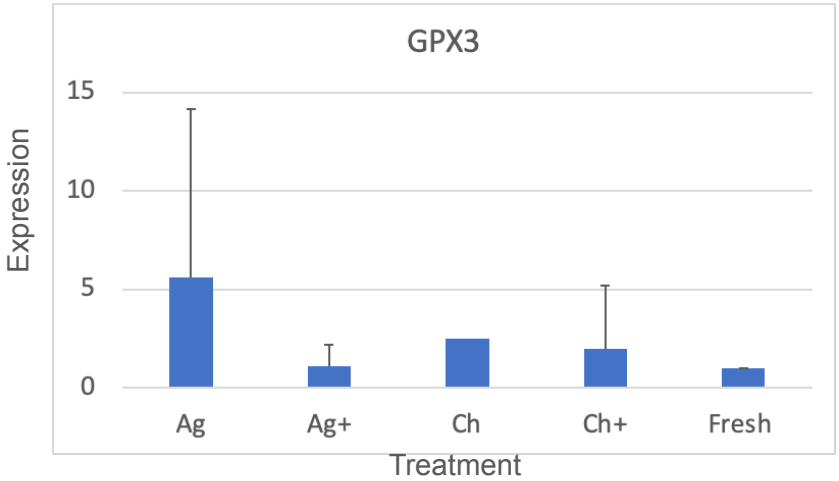
RT-PCR Analyses

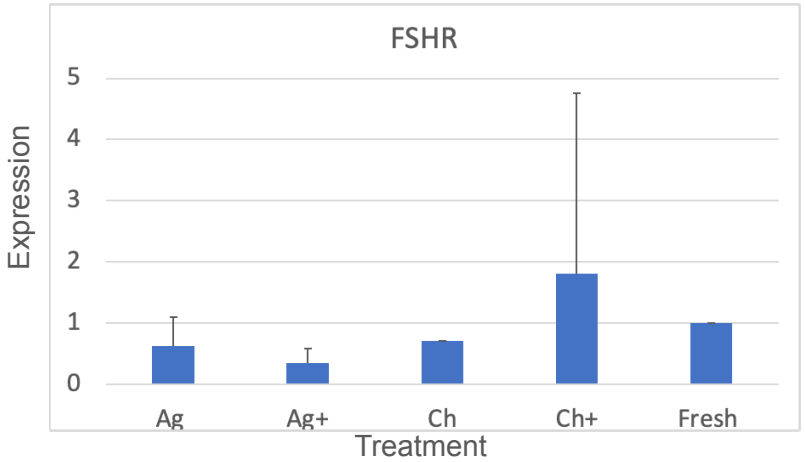
Genes:

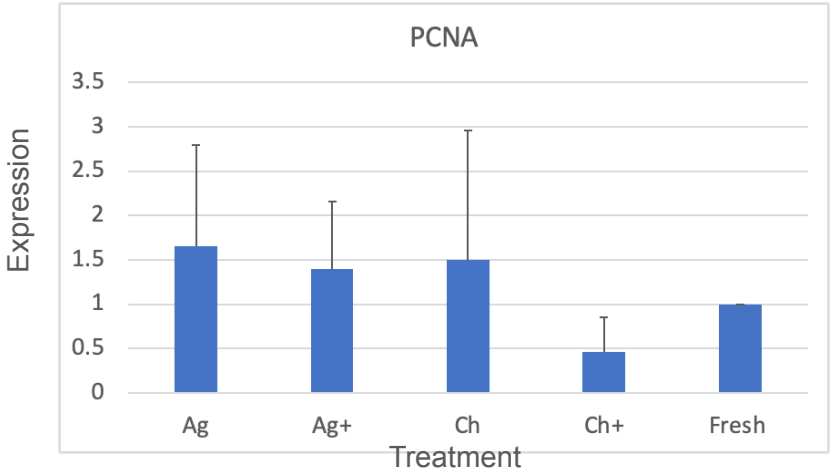
- **Beta-actin** = housekeeping control gene
- Glutathione Peroxidase 3 **(GPX3)** = oxidative stress
- Proliferating Cell Nuclear Antigen **(PCNA)** = cell proliferation
- Follicle Stimulating Hormone Receptor **(FSHR)** = folliculogenesis
- Vascular Endothelial Growth Factor **(VEGF)** = angiogenesis

RT-PCR Results: Gene Expression vs. Treatment Group









Overall Results

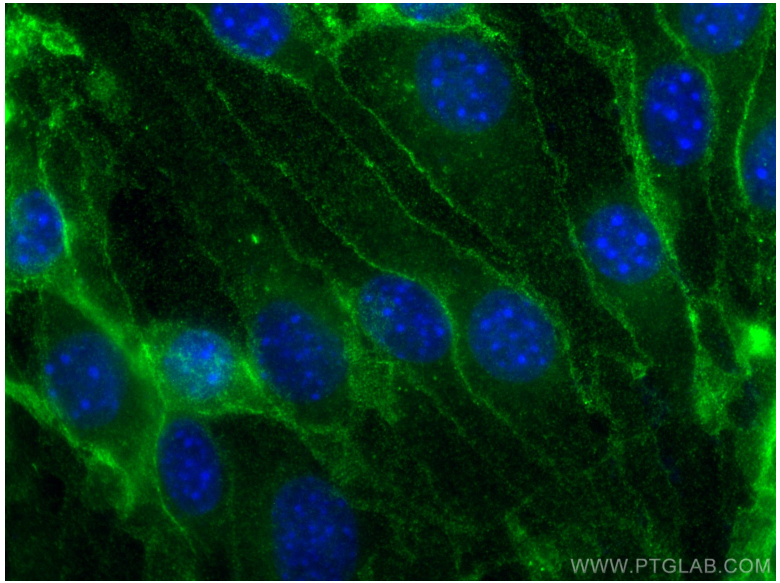
Results not statistically significant due to small sample size

However:

- Ch+ showed support in maintenance & development of later stage follicles
- Gene expression trends among treatment groups holds hope for larger sample sizes

What’s Next?

- Increase sample size
- Complete IHC analysis on membranes
- Bigger chips
- Focus on species specificity



My Overall Experience

What I learned:

- Importance of collaboration
- Variety of conservation efforts

What I loved:

- Meet the Scientist
- Small events with interns in other departments

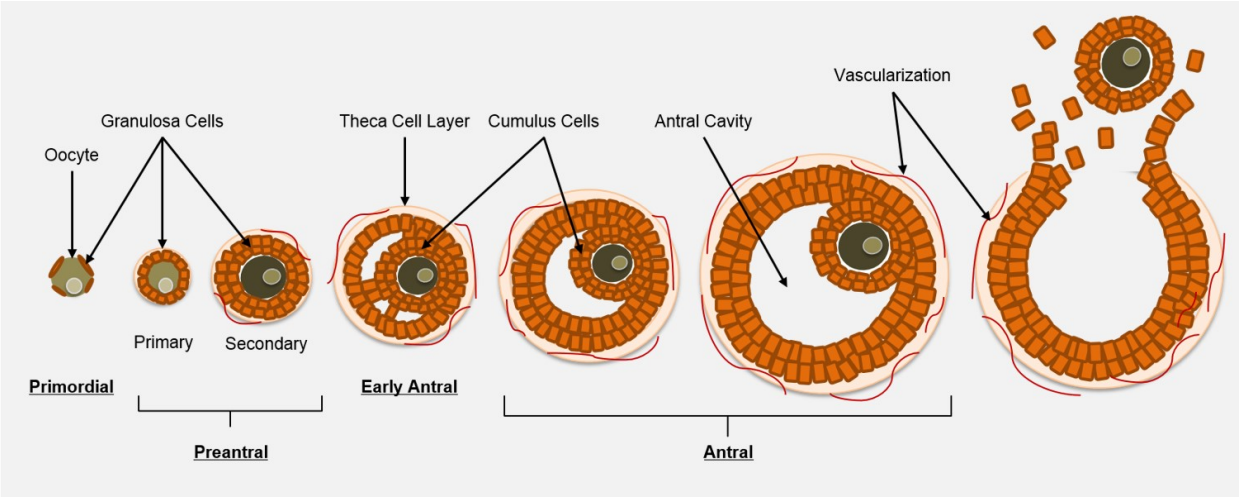
Thank You!

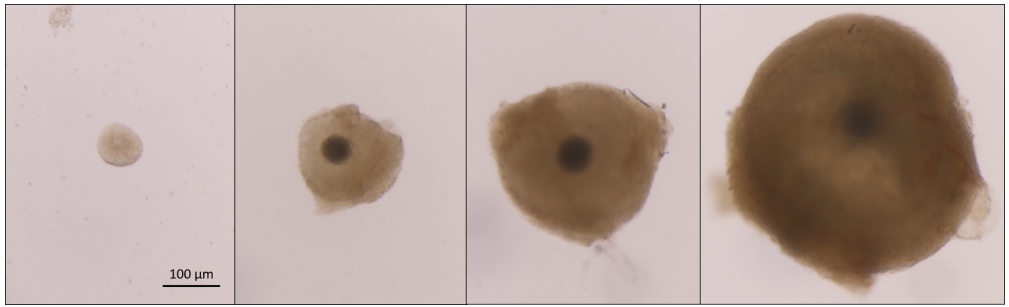
A special thank you to Dr. Jennifer Nagashima, all of those apart of SCBI & other students in the gamete lab!

Smithsonian Conservation Biology Institute

Questions?

Additional Images





Additional images