JULY 26 2022 - UC DAVIS SVM STAR PROGRAM

The Effect of Preservation Protocols on Mitochondrial Respiration in Ovarian Cortex of the Domestic Cat Model

STAR Student: Julianne Nussbaum

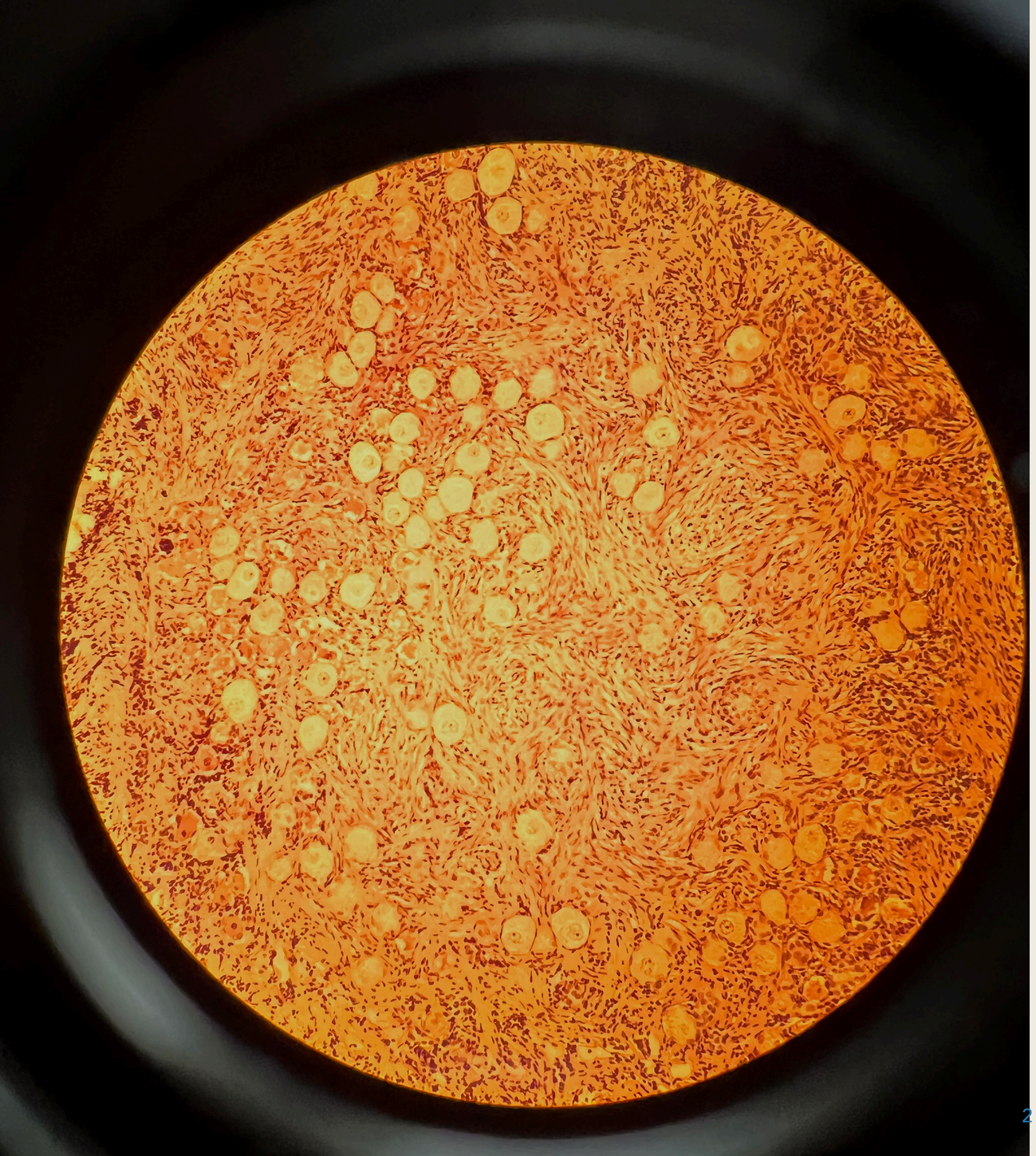
UC Davis SVM Mentor: Dr. Stuart Meyers

Smithsonian National Zoological Park Mentors:

Dr. Pierre Comizzoli and Dr. Olga Amelkina

Smithsonian
National Zoological Park
Conservation Biology Institute

UCDAVIS
VETERINARY MEDICINE



Road Map

- The Need
- Founding concept
- Methods
- Results
- The Future
- Acknowledgements

The Importance of Reproductive Science to the Zoological Conservation Mission

There are currently 41 extant species in the Felidae family

3 near threatened
14 vulnerable
8 endangered[1]

Therefore zoo's play an important role in advancing conservation reproductive methods to help **ensure survival** and **optimize genetics.**

The Push to Advance Preservation Technology

- The current gold standard for reproductive preservation **depends on liquid nitrogen** preservation - *slow freezing and vitrification*
  - This method is expensive and requires proper storage/infrastructure
- Optimizing alternate methods that **avoid dependency on low temperature** preservation is our study goal

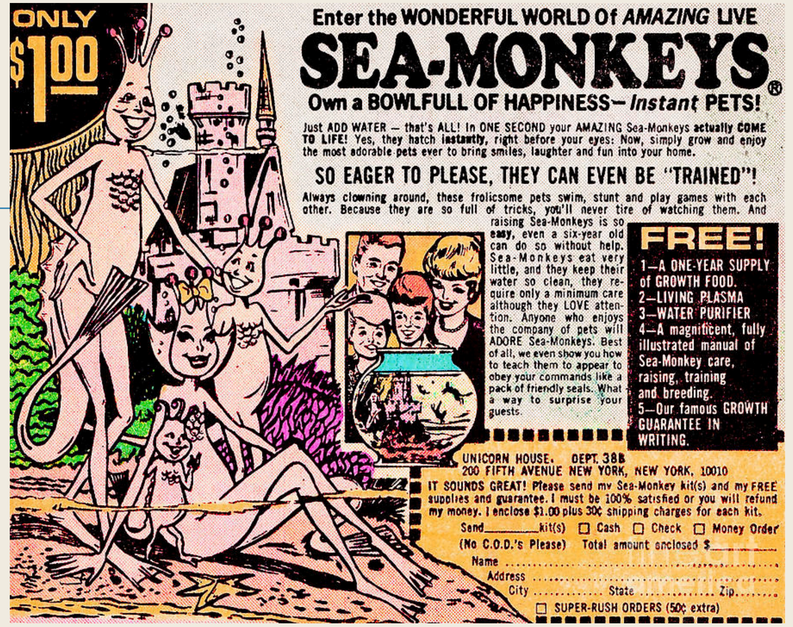
Storage = -196 degree C

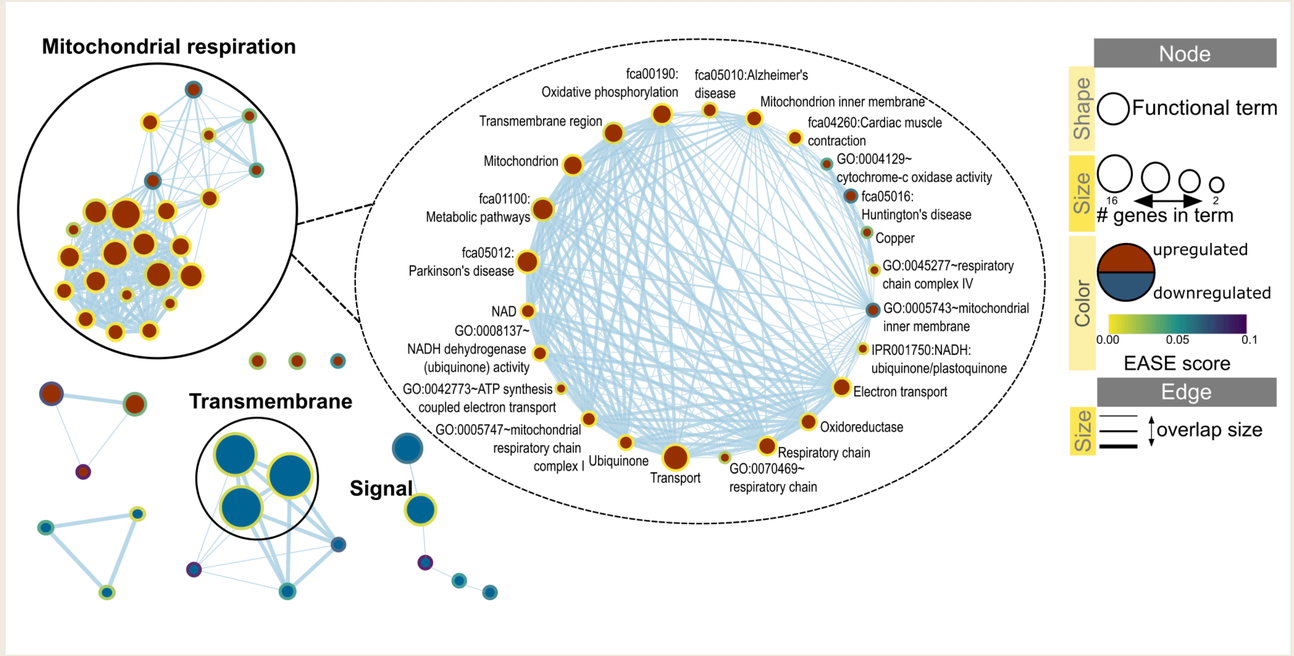
Storage = room temperature or 4 degree C

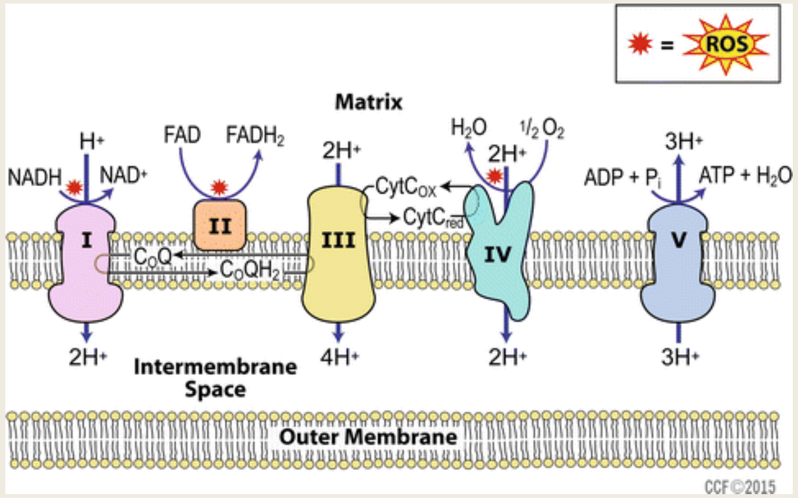
Founding Concept

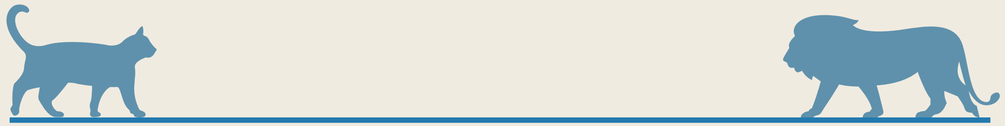
Anhydrobiosis: life suspended in a dry state

- The process depends on sugar production for tissue preservation -> trehalose and sucrose[2]
- Purpose?
  - **Water replacement theory:** preventing drying induced denaturation of proteins
  - **Glass formation:** inhibit chemical reactions
- Upon rehydration metabolic function returns









Guiding study:

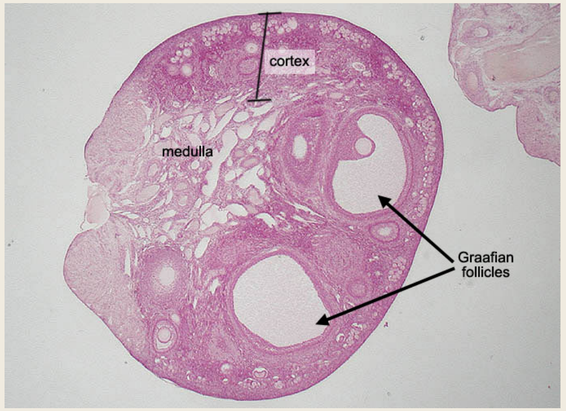
Initial response of ovarian tissue transcriptome to vitrification or microwave- assisted dehydration in the domestic cat model[3]

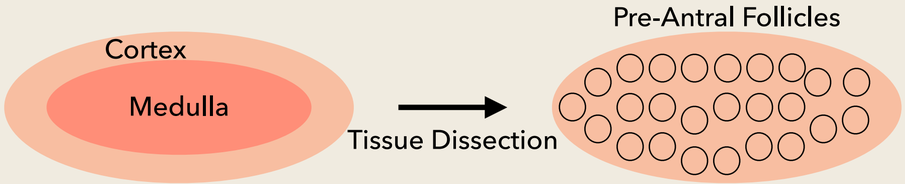
- The Smithsonian NZP has been working to optimize their dehydration and vitrification protocols
- Genetic analysis in the 2020 study showed **genes pertaining to mitochondrial respiration are up regulated following vitrification and warming**
- There is evidence that in the short term, increased mitochondrial activity promotes survival from endoplasmic reticulum stress[4]
- Prolonged up-regulation of mitochondrial respiration is cause for concern due to the **increased production of ROS**[5]

Are antioxidant or other ROS treatments needed for ovarian tissue during the preservation process?[5]

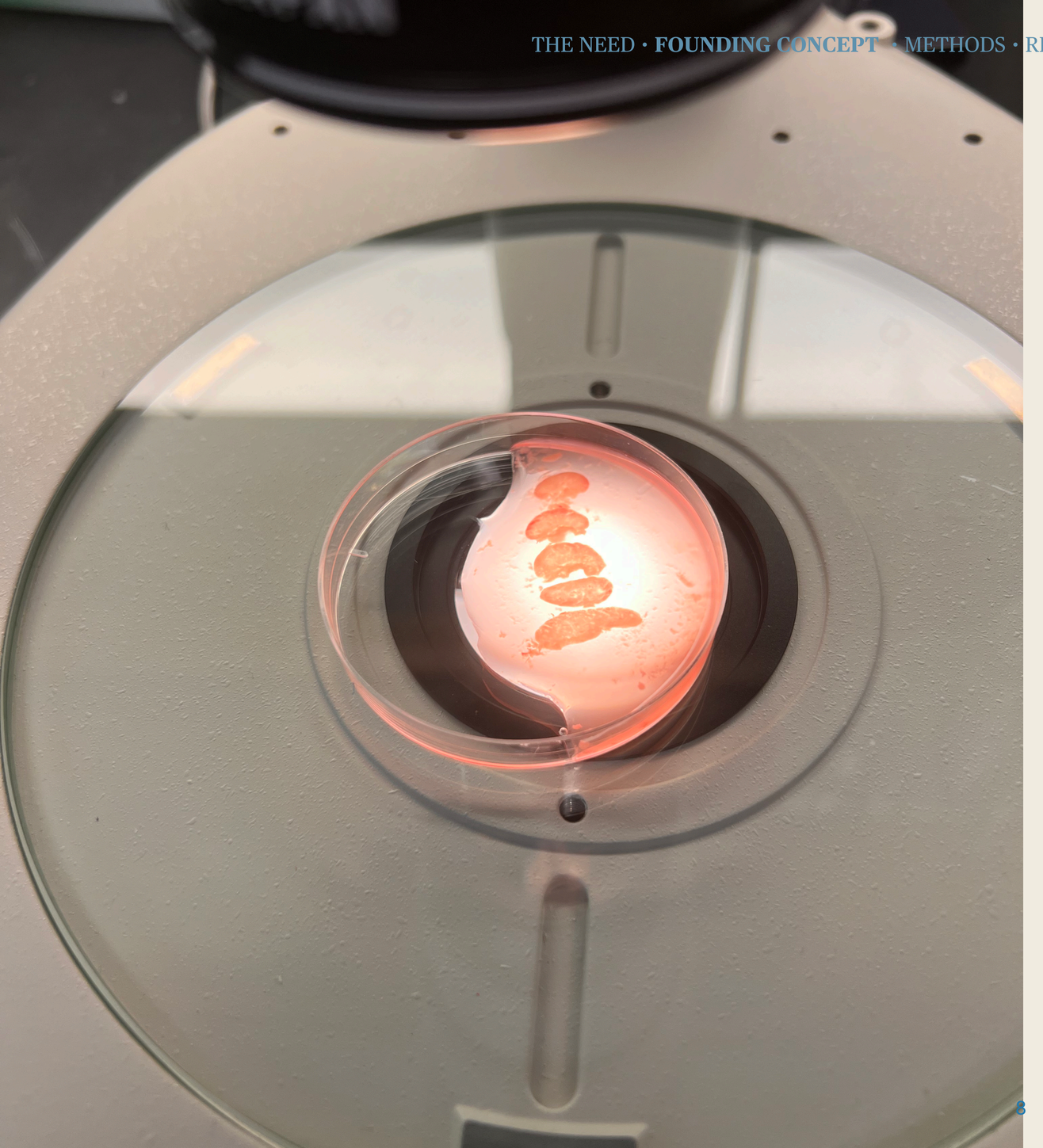
The Cortex and the Cat

- Two options for reproductive preservation: gametes or whole tissue
- Preserving whole ovarian cortex tissue gives us access to an untapped supply of pre-antral follicles -> these can later be cultured and used for conservation breeding programs (genome rescue banking)[6]





- We are using the domestic cat as our model species for human and for rare and endangered felids due to comparable traits in anatomy and physiology.[7]
  - Ovaries are provided by spays at a local animal clinic.

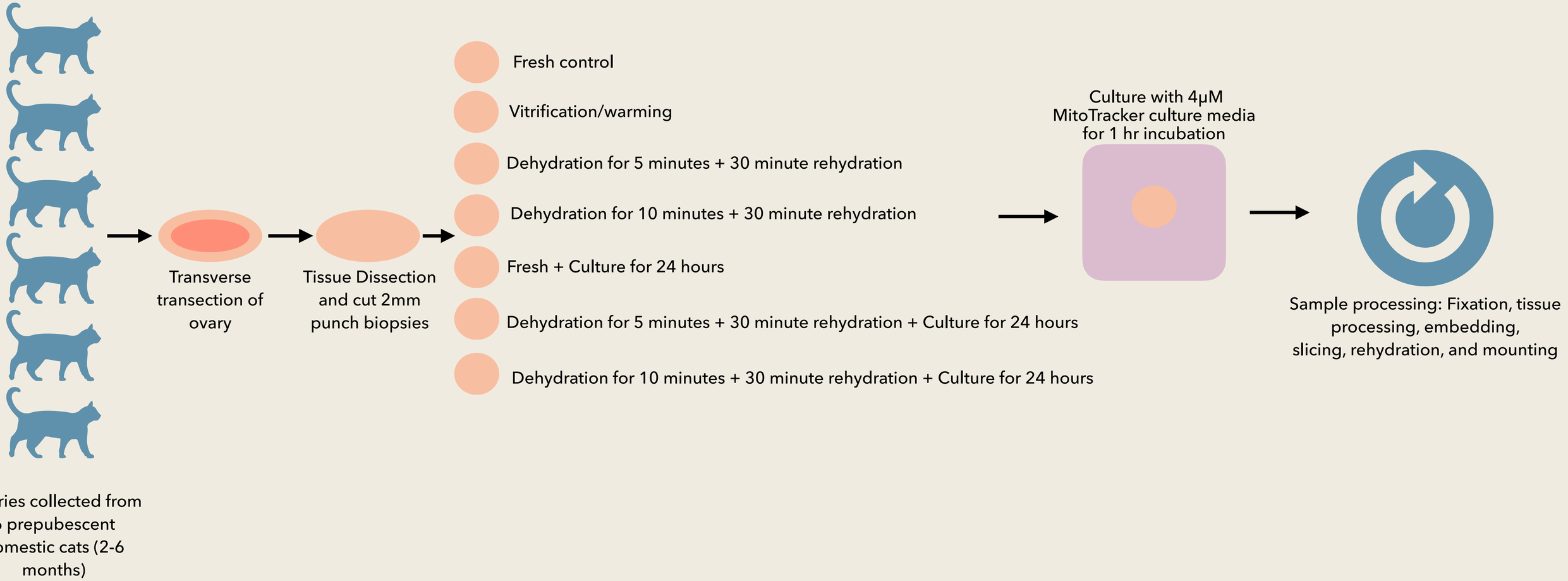


Hypothesis

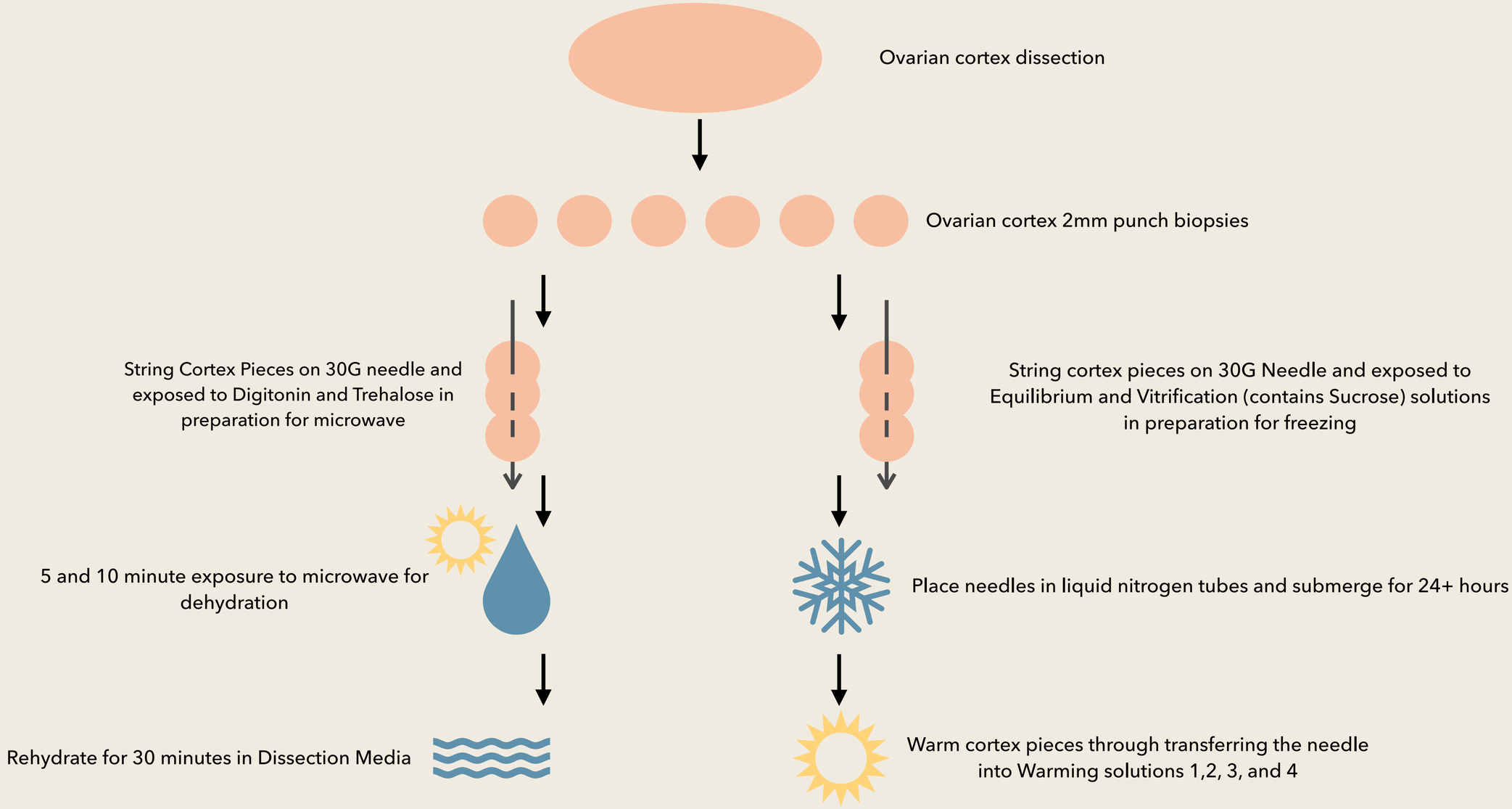
Elevation in mitochondrial activity is an initial adaptive stress response to mild stress caused by vitrification. Ovarian tissue responds differently to vitrification and dehydration protocols.

Methods

3-4 days for completion

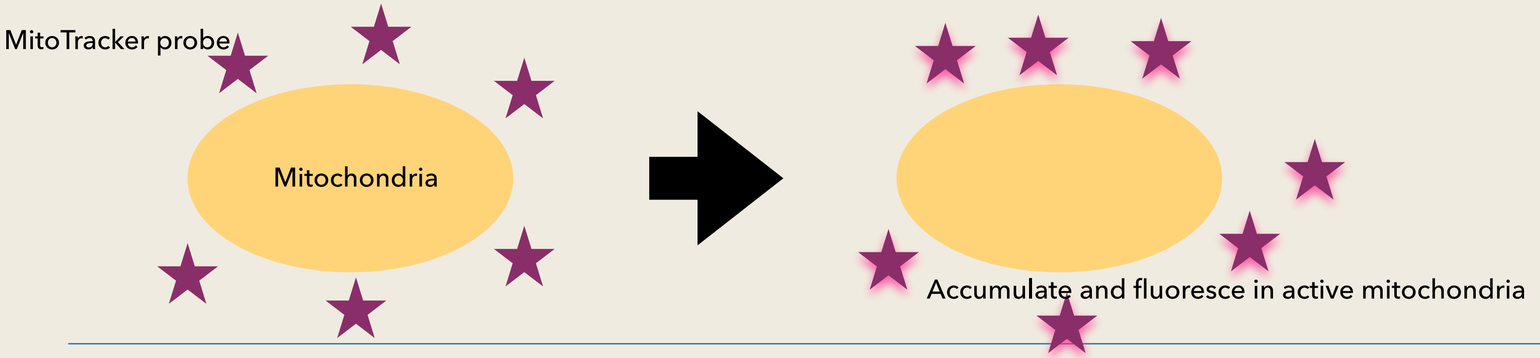


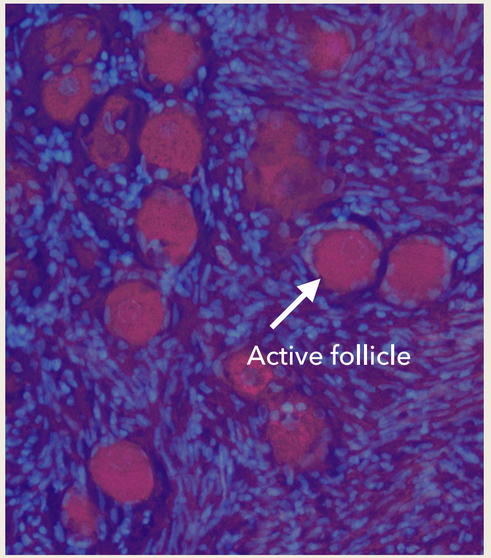
Methods: Dehydration & Vitrification Protocol

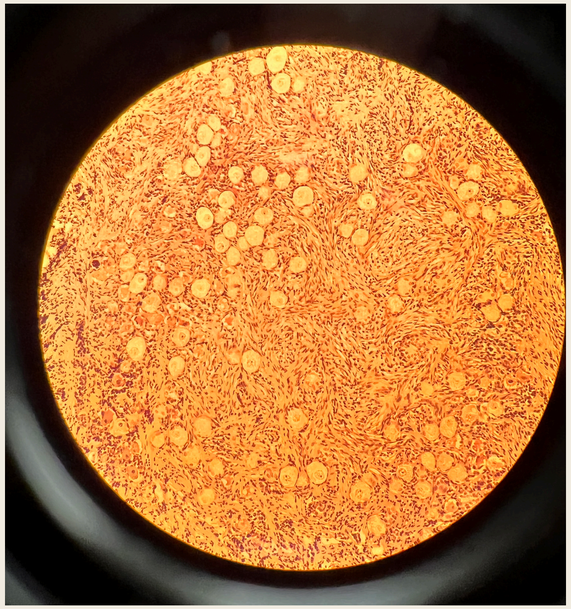


Methods

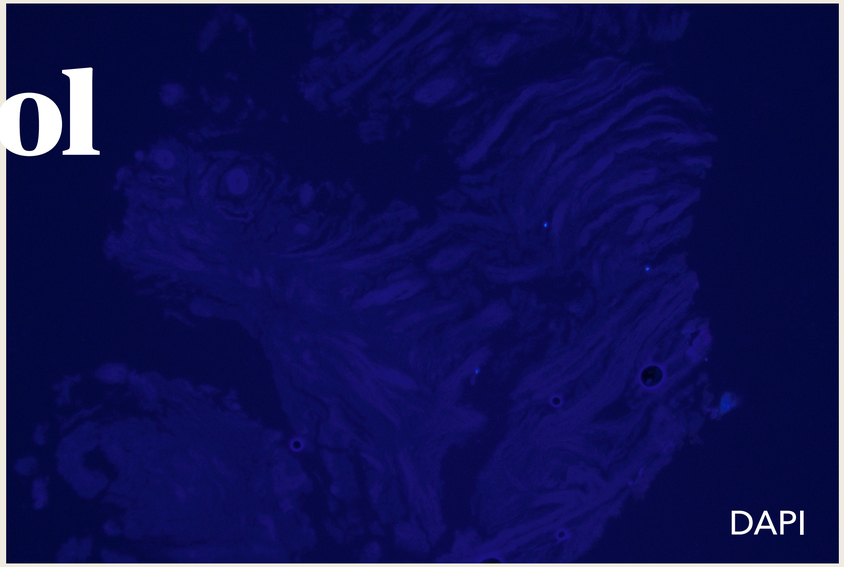
- MitoTracker probe culturing allowed for monitoring of mitochondrial activity levels
- Probes **passively diffuse** across plasma membranes and accumulate in active mitochondria -> we added DMSO for penetration assistance
- We mounted with Vectashield Hardset with DAPI to allow visualization of total follicles present
- Under TXRed channel (579/599 nm) we were able to view number active follicles and total follicles under DAPI channel (350/470 nm)

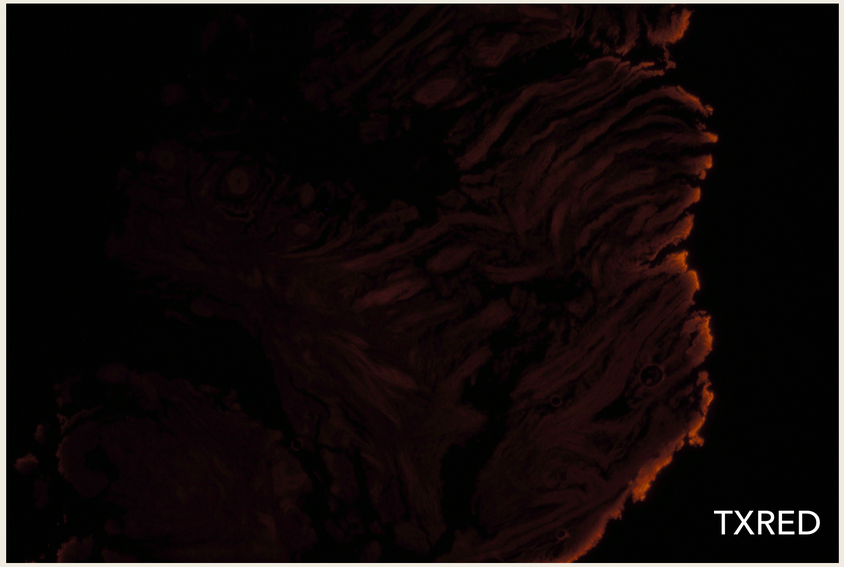






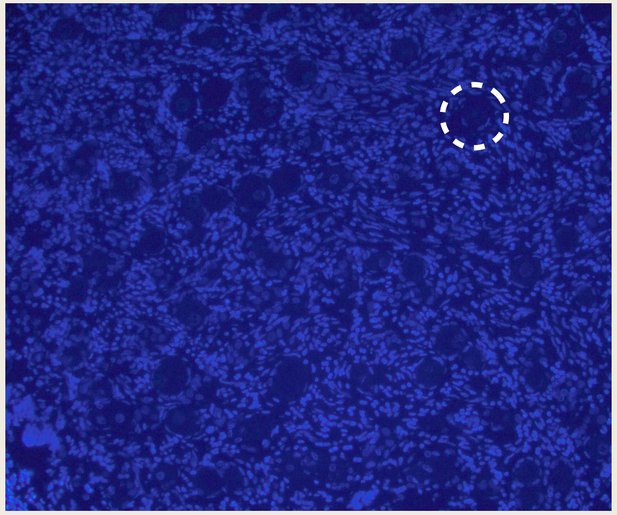
Dead Control



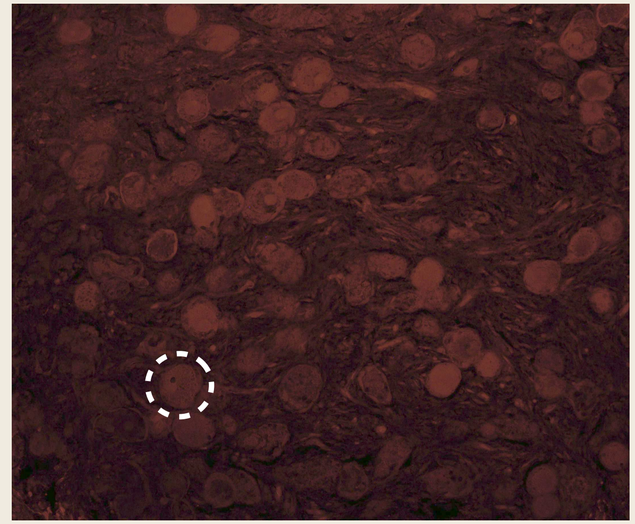


= follicle

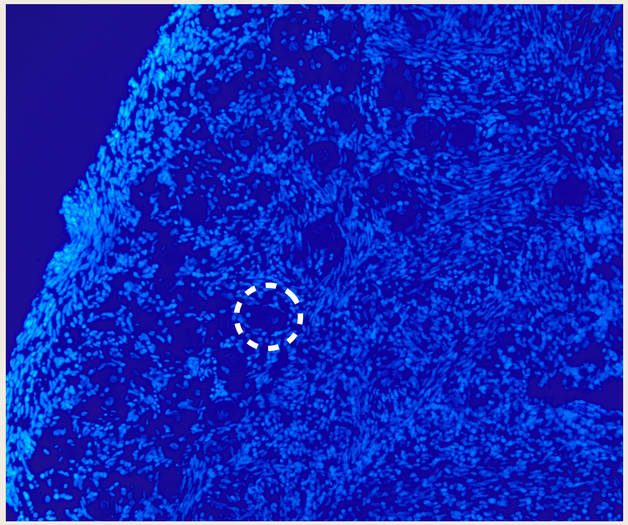
Results



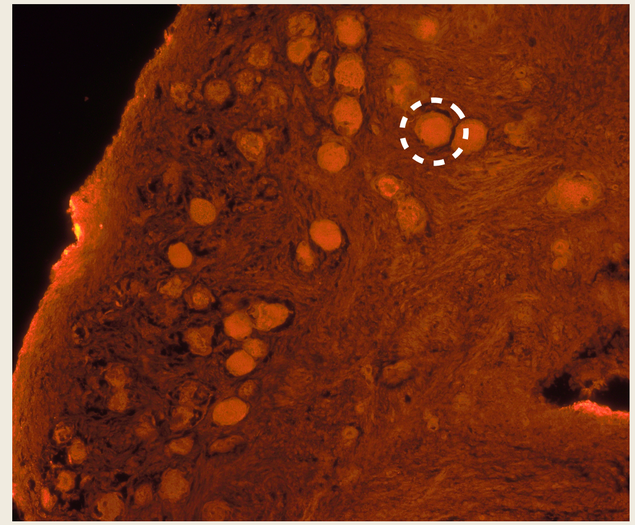
Typical intensity of fresh tissue



+



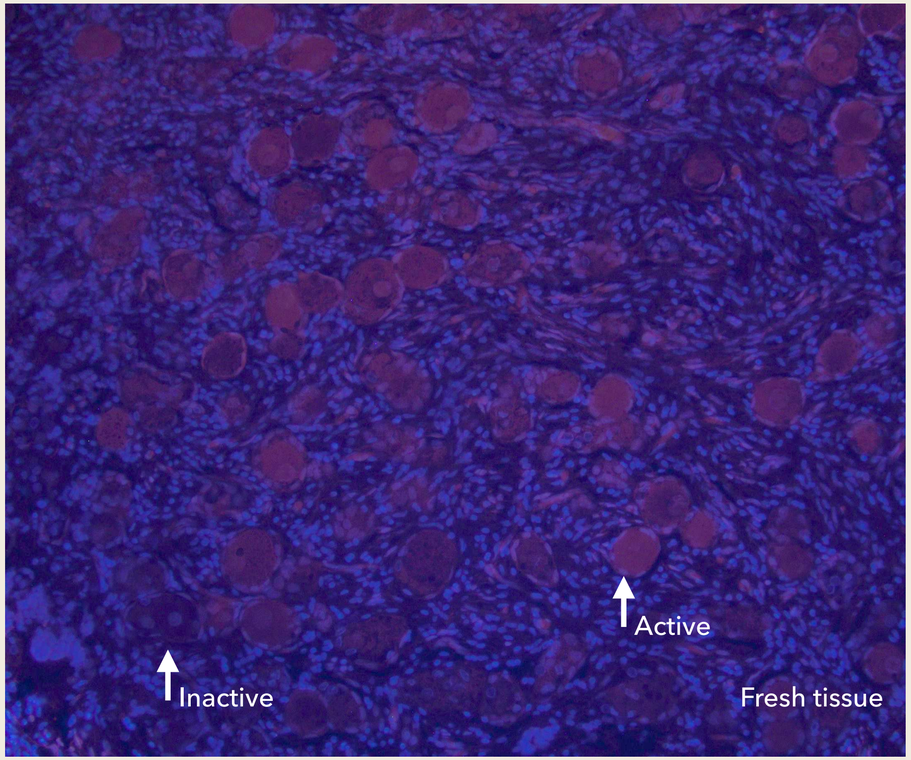
Typical intensity of treated tissue (D10)

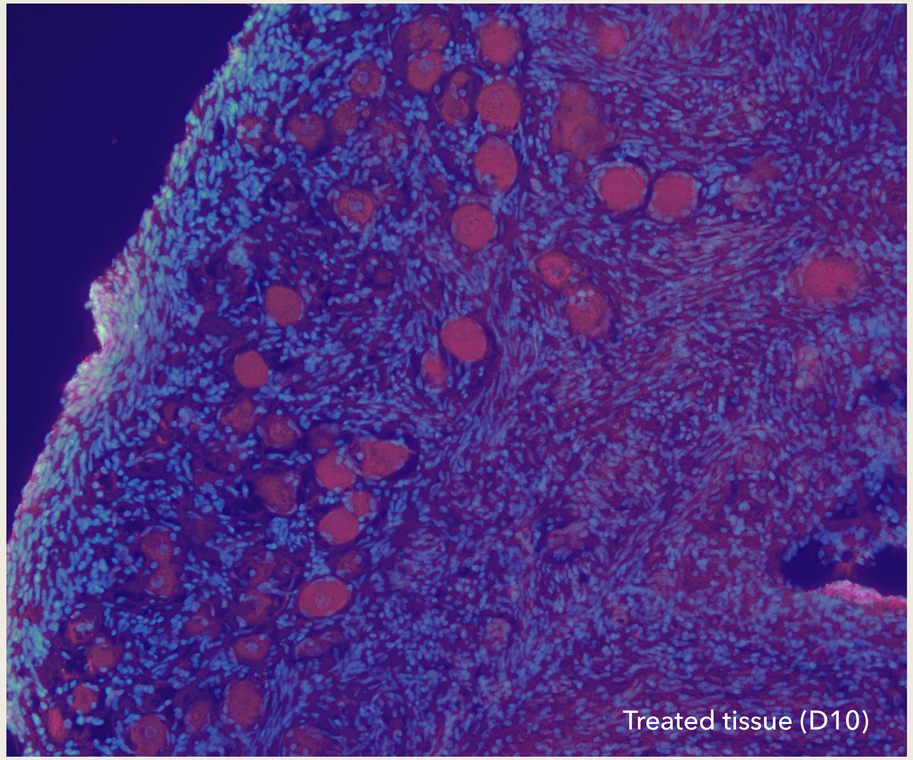


DAPI Image

TXRED Image

Results



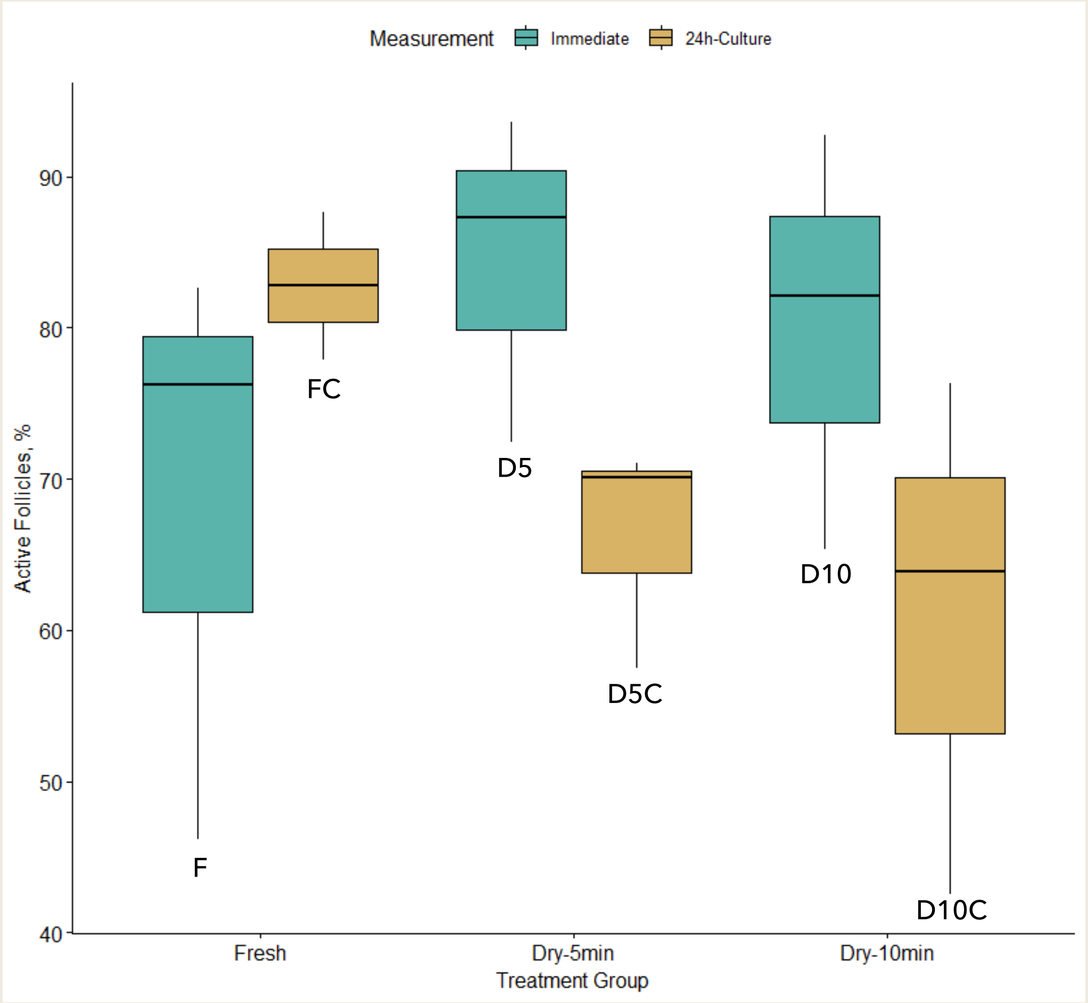


Merged images

Higher fluorescence = Increased mitochondrial respiration

Results

High mitochondrial activity



Low mitochondrial activity

F - Fresh

FC - Fresh cultured

D5 - Dry 5 minutes

D5C - Dry 5 minutes + 24 hrs culture

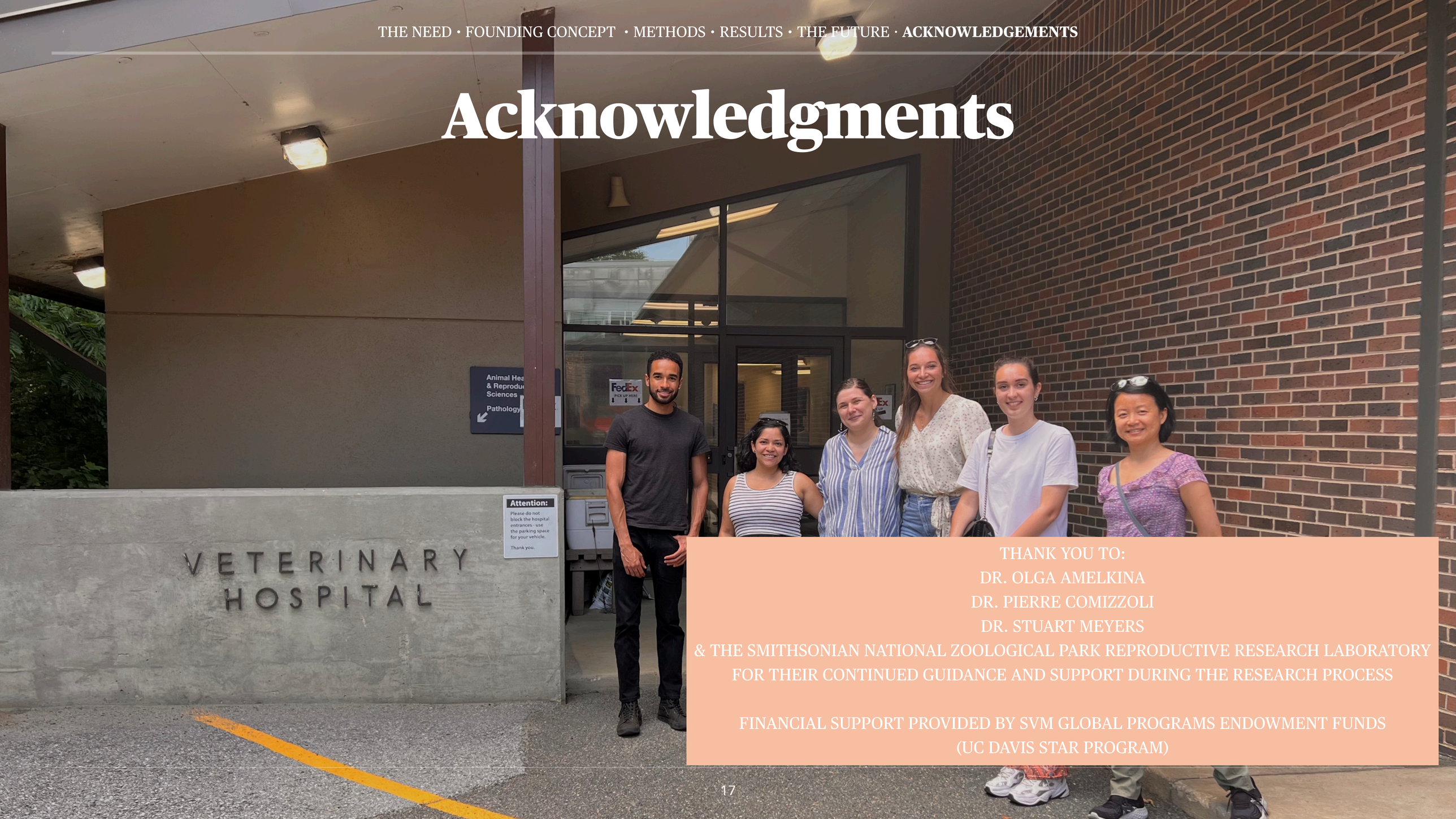
D10 - Dry 10 minutes

D10C - Dry 10 minutes + 24 hrs culture

What does this mean? Where to go from here?

- Our preliminary data shows an **adaptive stress response** (increase in mitochondrial activity) **immediately after cellular stress**
- **Mitochondrial activity decreases after culturing**
- We need to analyze our remaining cats and all vitrified treatment groups
- We will then compare dehydration and vitrification results and run statistical analysis on them to determine significance

Acknowledgments



THANK YOU TO:
DR. OLGA AMELKINA
DR. PIERRE COMIZZOLI
DR. STUART MEYERS
& THE SMITHSONIAN NATIONAL ZOOLOGICAL PARK REPRODUCTIVE RESEARCH LABORATORY
FOR THEIR CONTINUED GUIDANCE AND SUPPORT DURING THE RESEARCH PROCESS

FINANCIAL SUPPORT PROVIDED BY SVM GLOBAL PROGRAMS ENDOWMENT FUNDS
(UC DAVIS STAR PROGRAM)

Works Cited

1. “Big Cats.” *WCS.org*, https://www.wcs.org/our-work/wildlife/big-cats.

2. Crowe, John H., John F. Carpenter, and Lois M. Crowe. "The role of vitrification in anhydrobiosis." *Annual review of physiology* 60.1 (1998): 73-103.

3. Amelkina, O. A. and Pierre Comizzoli. “Initial response of ovarian tissue transcriptome to vitrification or microwave-assisted dehydration in the domestic cat model.” BMC Genomics 21 (2020): n. pag.

4. Knupp J, Arvan P, Chang A. Increased mitochondrial respiration promotes survival from endoplasmic reticulum stress. Cell Death Differ. 2019 Mar;26(3):487-501. doi: 10.1038/s41418-018-0133-4. Epub 2018 May 23. PMID: 29795335; PMCID: PMC6370866.

5. Devine, Patrick J et al. “Roles of reactive oxygen species and antioxidants in ovarian toxicity.” *Biology of reproduction* vol. 86,2 27. 9 Feb. 2012, doi:10.1095/biolreprod.111.095224

6. Bus A, Langbeen A, Martin B, Leroy JLMR, Bols PEJ. Is the pre-antral ovarian follicle the 'holy grail'for female fertility preservation? Anim Reprod Sci. 2019 Aug;207:119-130. doi: 10.1016/j.anireprosci.2019.05.017. Epub 2019 Jun 1. PMID: 31208845.

7. Comizzoli P, Songsasen N, Wildt DE. Protecting and extending fertility for females of wild and endangered mammals. Cancer Treat Res. 2010;156:87–100.

Questions?