THE EFFECT OF PRESERVATION PROTOCOLS ON MITOCHONDRIAL ACTIVITY IN OVARIAN CORTEX OF THE DOMESTIC CAT MODEL

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INTRODUCTION

Current reproductive technology depends on liquid nitrogen preservation which requires proper storage/infrastructure and is high in cost. Therefore, we are working to optimize alternate methods that **avoid dependency on low temperature preservation**. Tissue dehydration protocols are based on the concept of **anhydrobiosis**, where life can be suspended in a dry state using trehalose.[1] The decision to **preserve whole ovarian cortex tissue** gives access to an untapped supply of pre-antral follicles. This large supply of follicles will aid in conservation breeding efforts and genome rescue banking.

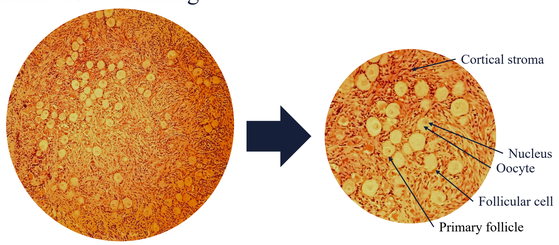


Figure 1. Ovarian cortex histology sample showing high levels of pre-antral follicles.

OBJECTIVE

The process of dehydration and vitrification have harsh effects on the ovarian tissue and follicles. If the follicle survives, large amounts of energy are required for cellular repair. Previous gene assays of treated ovarian cortices show an upregulation in mitochondrial activity following vitrification.[1] Mitochondria produce ATP necessary for the repair process; however, if increased activity persists beyond the initial recovery period, the high levels of ROS will cause damage to follicle lipids, proteins, and nucleic acids.[3]

Our study objective is to ***understand whether mitochondrial activity is a momentary response or a prolonged activity and if tissue responds differently between vitrification and dehydration.*** We hypothesize that elevation in mitochondrial activity is an initial adaptive stress response to vitrification and rehydration and that ovarian tissue responds differently to vitrification and dehydration protocols.

RESULTS

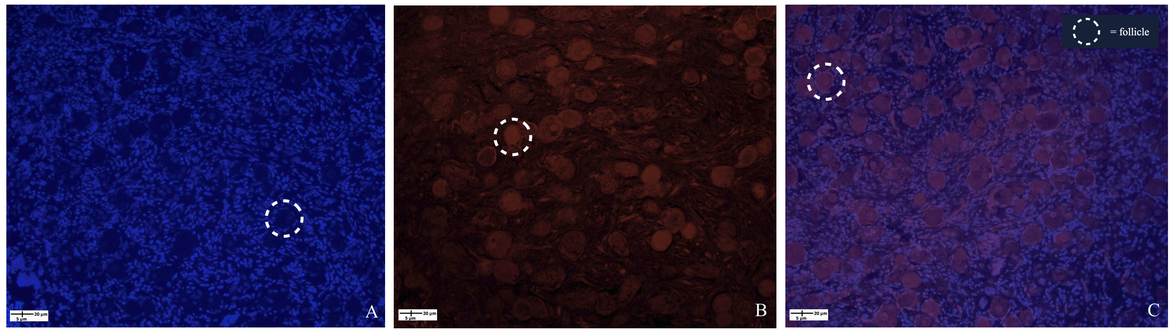


Figure 2. Fluorescent images taken from fresh ovarian cortex tissue treated with MitoTracker. A. DNA staining with DAPI viewed under DAPI channel (350/470 nm). B. Active mitochondria staining with MitoTracker probe viewed under TXRED channel (579/599 nm). C. Merged image for follicle counting

CORTEX TREATMENT TYPE AND PERCENT ACTIVE FOLLICLES

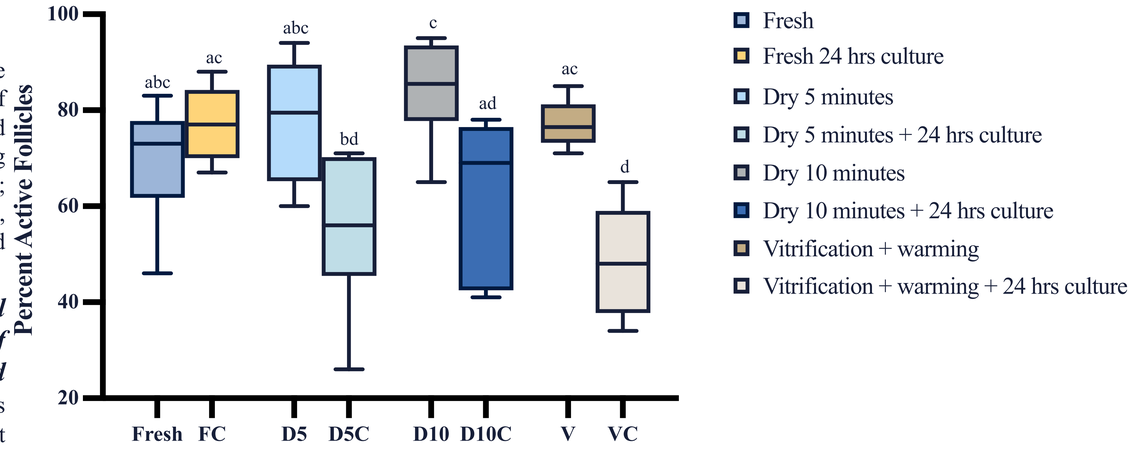
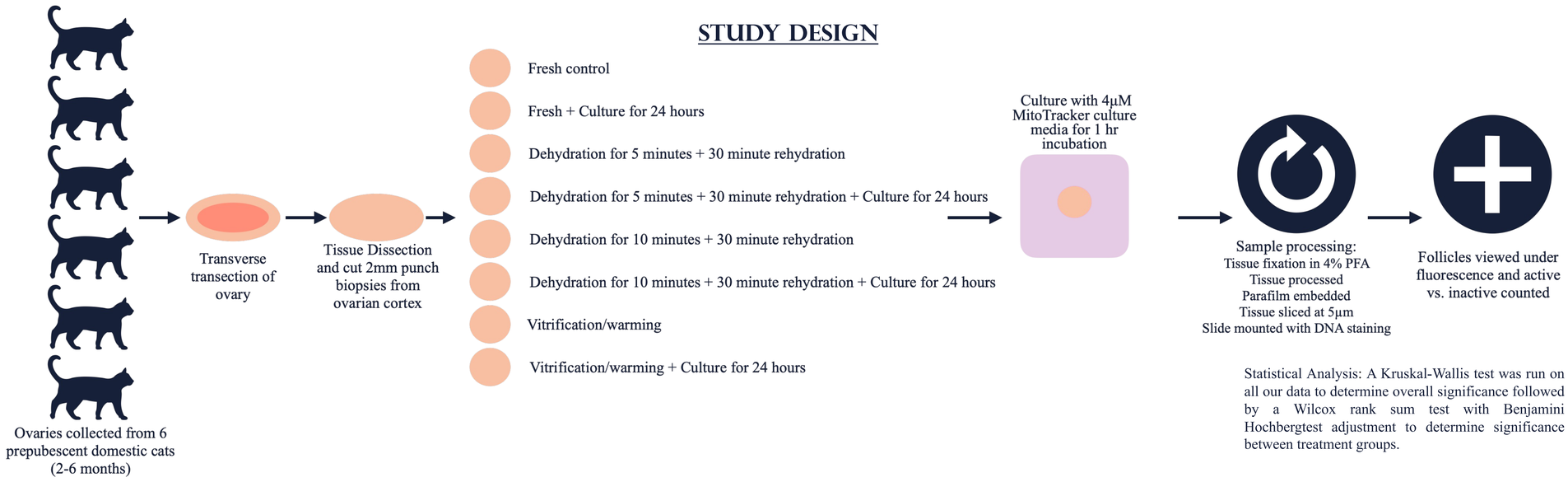


Figure 3. Box plot representation of active follicle percentages in cortex treatment groups. Values with different letters differ (P<0.05).

STUDY DESIGN



Statistical Analysis: A Kruskal-Wallis test was run on all our data to determine overall significance followed by a Wilcox rank sum test with Benjamini Hochbergtest adjustment to determine significance between treatment groups.

CONCLUSIONS

- Overall, we did not see an increase in follicular mitochondrial activity in response to vitrification and dehydration.
- There is a decrease in mitochondrial activity following 24-hour culturing in 10-minute dehydration and vitrification.
- In response to 24-hour culturing, the mitochondrial activity for 5-minute dehydration does not significantly decrease, making it more similar to fresh tissue than 10-minute dehydration and vitrification.

NEW PROPOSED QUESTION

Does mitochondrial activity decrease due to cellular death during culturing?

If yes: Alterations to culture media may be needed; however, fresh culture tissue had increased mitochondrial levels so this is not highly indicated. Otherwise, tissue treatments might be too harsh and protocols need to be evaluated.

If no: Follicular mitochondrial activity may need to be encouraged through the addition of pyruvate or other substance that will help increase follicular mitochondrial activity.

Overall, future studies into the survival of follicles as well as their correlated mitochondrial activity are indicated.

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