Estrous Cyclicity of Mice During Simulated Weightlessness

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Motivation for studying estrous cyclicity in simulated weightlessness

• STS-131, STS-133, STS-135 revealed cessation of estrous cycle in female mice (Tash 2012 & Ronca 2014)
• Spaceflight leads to loss of corpora lutea and significantly reduced estrogen receptor mRNA levels in the uterus

Goals of this study
• Assess whether female endocrine signaling biomarkers are altered in simulated weightlessness via hindlimb unloading model in both reproductive and non-reproductive organs
Experimental Design

- Normally Loaded (n=10)
- Hindlimb Unloaded (HU) (n=10)
- Vivarium Control (n=10)

Primary endpoints:
1. Did mice maintain/return to normal estrous cycling?
2. Were there structural changes to reproductive organs (ovaries, uterus, vaginal wall)?

Hindlimb Unloading (HU) 16 wk C57BL6 female mice

3 day cage acclimation

Begin HU
HU
12
Dissection
Methods: Daily lavage and Imaging


Cora, Michelle C., Linda Kooistra, and Greg Travlos. "Vaginal cytology of the laboratory rat and mouse: review and criteria for the staging of the estrous cycle using stained vaginal smears." *Toxicologic pathology* 43.6 (2015): 776-793.
Methods: Cytology Analysis

• Translate qualitative data into an experiment timeline for each mouse defining Day 0 as start of treatment.

• Graph each animal’s estrous cycle in relation to other experiment landmarks

Hypothesis

• Hindlimb unloading will cause mice to arrest estrous cyclicity in the diestrous stage

<table>
<thead>
<tr>
<th>Experiment Landmark</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>HU Cage Acclimation Begins</td>
<td>-3</td>
</tr>
<tr>
<td>HU Treatment Begins</td>
<td>0</td>
</tr>
<tr>
<td>Euthanasia/Tissue Collection</td>
<td>11/12</td>
</tr>
</tbody>
</table>
Result: Pair-feeding/Cage effect observed
Result: Differences observed in HU reproductive organs and adrenals
Cytology Results

- Average cycle length of vivarium cage control animals was 4.7 days.
  - In line with reported literature\(^1,2\)
  - Suggests our technique did not impede normal cycling

- Normal cycling did not present in many animals during the acclimation to HU cages, or even throughout remainder of experiment.

- Some occurrences of cycling did return to HU cage mice.

- Infection-like symptoms resulted in missing data in HU mice.

Conclusions

• Cage effect/Pair Feeding effect present
• Validated model for observing estrous stage in VIV control
• Longer acclimation period may allow control cage animals to return to normal estrous cyclicity
• Longer HU period may allow HU animals to acclimate and return to normal estrous cyclicity
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