A Salt With a Deadly Weapon:
Effect of High Salt Diets on Reproductive Organs of the Rat
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Background
Research
Dr. Dori Miller’s Research

High salt diets effect when rats go through puberty.

Graph depicting the percentage of rats that have gone through vaginal opening recorded daily. LFLS represents rats fed low fat and low salt diets, LFHS represents rats fed low fat and high salt diets, HFLS represents rats fed high fat and low salt diets, and lastly HFHS represents rats high low fat and high salt diets.
Does a High Salt Diet Effect Fertility?
Abstract

Infertility rates among women all over the world are on the rise.

With the promotion of westernized cuisine people are consuming more salt than ever before.

Dr. Miller’s research determined a high salt diet can postpone puberty in rats.

By observing specific features in the ovaries of rats that have been fed a control diet and those that have been fed a high salt diet, a greater understanding of morphological differences caused by a high salt diet could be observed.
Oocytes

Very lightly stained from H&E. Will be significantly larger than granulosa cells they are surrounded by.
Primary, Preantral, Early Antral, and Preovulatory (Graafian) follicles:

Very similar in size ranging between 50-200 μm. Indistinguishable through basic analyses. Was counted if there was a clear Oocyte tightly surrounded by granulosa cells. Granulosa cells appeared darker than surrounding cells. Some appeared ruptured and looked “tomato like” after egg release. Preovulatory (Graafian) follicles were counted if they surpass 200 μm across and contain no oocytes.
Corpus Luteum

Approximately 500 μm across and relatively circular. Rarely contains cyst in early development of rat, typically off center in the feature. Lighter cells will surround the entire feature, forming a circular structure. The cyst has granulosa like cells along with large, less stain sensitive luteal cells.

A corpus luteum was counted if the feature was larger than 400 μm across. It was distinguished from follicles by the luteal cells present around the cyst, or by containing no space.
Methods
Methods

Rats were weaned from their parents on post-natal day 21. From this point on they were separated into two groups and fed:

- **Control diet**: 0.3% NaCl diet
- **Treatment diet**: 8% NaCl diet

Animals were euthanized on day 39 and ovaries were collected.

Ovaries were sliced 8μm thick using a cryostat, and every 5th slice was placed on a polysine coated slide slide.

Collected slices were stained using Mayer-Hematoxylin and Eosin Y.

Slices were photographed under 40x microscope.

Ovarian features including corpora lutea, follicles, and oocytes were quantified.
What did I find?
<table>
<thead>
<tr>
<th>Ovary Image</th>
<th>Corpora Luteum</th>
<th>Primary, Preantral, and Early Antral Follicles</th>
</tr>
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<tbody>
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**Corpus Luteum**

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**Primary, Preantral, and Early Antral Follicles**

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**Total**

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<tr>
<td>Percent Salt Diet</td>
<td>Ovary Image 1</td>
<td>Corpora Lutea</td>
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<tr>
<td>------------------</td>
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</tr>
<tr>
<td>0.08% NaCl Treatment Diet</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
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**Ovary Image 1**

- Ovary Image 1 represents various stages of ovarian follicles and corpora lutea.
- The follicles are categorized into different sizes and stages.
- The numbers indicate the count of each category.

**Corpora Lutea**

- Corpora lutea are the yellow structures within the ovary that develop during the later stages of ovulation.
- The image includes detailed counts of corpora lutea in different conditions.

**Follicles and Oocytes**

- Follicles are the structures that contain oocytes (eggs).
- Oocytes are the immature reproductive cells within the follicles.
- The follicles per CL column indicates the average number of follicles per corpora lutea.

**Percent Salt Diet**

- The comparison is made between 0.08% NaCl Treatment Diet and 0.3% NaCl Control Diet.
- The table shows a decrease in follicles and oocytes in the treated diet compared to the control diet.

**Average CL**

- The average CL is calculated based on the observations from the ovary images.
- The averages reflect the overall health and development of the ovaries.

**8% NaCl Treatment Diet**

- The 8% NaCl Treatment Diet shows a significant reduction in follicles and oocytes compared to the control diet.
- The treatment appears to impair ovarian function.

**8% NaCl Control Diet**

- The control diet maintains healthy ovarian function without evident reduction in follicles or oocytes.
But Luckily...
Results

Quantification Results

![Bar chart showing results for Average CL, Average Follicles, and Average Oocytes under 0.3% NaCl Control Diet and 8% NaCl Treatment Diet.](chart.png)

- **Average CL:** Low values for both diets.
- **Average Follicles:** Moderate increase for 8% NaCl Treatment Diet compared to 0.3% NaCl Control Diet.
- **Average Oocytes:** Significant increase for 8% NaCl Treatment Diet compared to 0.3% NaCl Control Diet.

**Legend:**
- **Green Bar:** 0.3% NaCl Control Diet
- **Orange Bar:** 8% NaCl Treatment Diet
Additional findings

Average Number of Follicles per Corpus Luteum

- **0.3% NaCl Control Diet**
- **8% NaCl Treatment Diet**
Conclusion

- Through the research conducted, rats fed a high salt diet have fewer corpora luteum, follicles, and oocytes on average than rats fed a control diet.

- Additionally, the average number of follicles per corpus luteum seemed to be substantially less in the control diet than in the treatment diet. This may be partially responsible for the difference in the quantity of ovarian features, as the corpus luteum is an endocrine structure in the ovary.

- With further investigation, a link between the reduced number of ovarian features and infertility could be discovered.
Moving to the Future

• What’s next?
Tacr3 Knockout
Thanks!
Questions?