Female Reproductive System

Justin D. Vidal
“If you cannot identify the tissue, then it is probably part of the female reproductive system!”
Introduction

– The female reproductive system is constantly changing, so it is normal to see huge variation in the size, shape, color, and function of the reproductive organs
– HUGE species differences
– Pathology is often linked to fertility/infertility and not necessarily the overall health of the animal
Introduction

– Part I
  – Reproductive Cycles
  – Evaluation of the Female Reproductive System in a General Toxicology (GT) setting
    – Role of the pathologist
    – Limitations and areas/things to look out for
    – Additional Endpoints

– Part II
  – Basic Mechanisms and Patterns
    – Hormonal effects
    – Direct ovarian effects
    – Mixed pattern
<table>
<thead>
<tr>
<th>Organ</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td>- Oocyte development</td>
</tr>
<tr>
<td></td>
<td>- Produce hormones</td>
</tr>
<tr>
<td>Oviducts (Fallopian tubes, uterine tubes)</td>
<td>- Transport of oocytes and sperm</td>
</tr>
<tr>
<td></td>
<td>- Site of fertilization</td>
</tr>
<tr>
<td>Uterus</td>
<td>- Development of embryo/fetus</td>
</tr>
<tr>
<td></td>
<td>- Parturition</td>
</tr>
<tr>
<td>Cervix</td>
<td>- Functional gate for the uterus</td>
</tr>
<tr>
<td>Vagina</td>
<td>- Intromission</td>
</tr>
<tr>
<td></td>
<td>- Deposition of semen (species dependent)</td>
</tr>
<tr>
<td></td>
<td>- Parturition</td>
</tr>
</tbody>
</table>
Endocrinology

- Female reproductive tract is made up of several organs all of which need to work in unison
- The changes seen are largely driven by ovarian steroids
Endocrinology

- Hypothalamus
  - Gonadotropin Releasing Hormone (GnRH)
- Pituitary
  - Follicle Stimulating Hormone (FSH)
  - Luteinizing Hormone (LH)
  - Prolactin
- Ovary
  - Estradiol
  - Progesterone
  - Inhibin(s)
GnRH

+ FSH and LH

+ Inhibin

- Estradiol and Progesterone

- (but sometimes +)
Steroidogenesis

- Steroids are produced by enzymatic reactions of precursors (cholesterol and other steroids)
- Very little storage of hormones
- Levels are controlled by the balance of production and metabolism
Steroidogenesis

- Reproductive and adrenal steroids begin as cholesterol
Steroidogenesis

Cholesterol (C27)

- Side Chain Cleavage

Progestins (C21)

- P450c17

Androgens (C19)

- Aromatase

Estrogens (C18)
Steroidogenesis

Enzymes

- 3βHSD
- P450c17
- Arom
- 17βHSD
Androgens produced in the theca are converted to estrogens in the granulosa cells.
Prolactin

- Produced by the anterior pituitary
- Dopamine is the major inhibitory factor
  - Centrally acting compounds may impact prolactin levels via changes in dopamine
- Prolactin has a diverse (and complicated) set of functions
  - Stimulates lactation
  - Major regulatory of corpus luteum “lifespan” in rats
    - In the normal estrus cycle proestrus prolactin surge is luteolytic
    - If rats are mated, then prolactin production is altered and is luteotrophic
- Changes in prolactin levels can lead to a profound change in reproductive function
  - Increased prolactin: Leads to increased ovarian weight, increased size and number of corpora lutea; increased mammary gland development; and vaginal mucification. These changes are similar to a pseudopregnancy
  - Decreased prolactin: Increased ovarian weight and number of corpora lutea.
Reproductive Cycles

- What are they?
- How are they defined?
### Reproductive Cycles

<table>
<thead>
<tr>
<th>Menstrual Cycle</th>
<th>Estrous Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Old World Monkeys</td>
<td>- Rodents</td>
</tr>
<tr>
<td>- Apes</td>
<td>- Dogs</td>
</tr>
<tr>
<td>- Humans</td>
<td>- Pigs</td>
</tr>
<tr>
<td>- Cycle defined by menses</td>
<td>- Cycle defined by standing heat</td>
</tr>
<tr>
<td>- Menopause</td>
<td>- Reproductive senescence</td>
</tr>
</tbody>
</table>
Menstrual Cycle

– Follicular Phase
  – Development of a dominant follicle
  – High estradiol levels
– Ovulation
– Luteal Phase
  – Corpus Luteum
  – High Progesterone levels
  – Estradiol levels can be elevated too (species dependent)
– Menses
Human Menstrual Cycle

Menses

Follicular Phase

Luteal Phase

Menses

Anterior Pituitary Hormones

FSH

LH

Ovarian Hormones

Estradiol

Progesterone

Ovulation

Follicle

Corpus Luteum

Corpus Albicans

Ovary

Recruitment

Selection

Dominance

Recruitment

Uterine Endometrium

Days
Cynomolgus macaque

Inhibin B: Highest during the follicular phase
Inhibin A: Highest during the luteal phase

Weinbauer et al. Physiology and Endocrinology of the Ovarian Cycle in Macaques
Toxicol Pathol 2008 36: 7S-23S.
Estrous Cycle

- Proestrus
  - Follicular development
- Estrus
  - Standing heat
  - Approximate time of ovulation
- Metestrus
  - Early CL development
- Diestrus
  - CL
  - High Progesterone (species dependent)
Canine Cycle

The image represents the canine cycle, showing the changes in LH (luteinizing hormone), Estradiol, and Progesterone levels over time. The diagram highlights the following phases:

- P: Proestrus
- E: Estrus
- Diestrus
- Anestrus

Ovulation occurs near the peak of LH levels. The graph illustrates the typical hormonal fluctuations during the cycle, with a peak in Estradiol followed by Progesterone, indicating the different stages of the cycle.
Rat Estrous Cycle

- 4-5 day cycle
- Need cervical stimulation for significant progesterone production
Pig Estrous Cycle

How do we evaluate the female reproductive system in a general toxicology study?

- **Standard Endpoints**
  - Organ weights
  - Macroscopic evaluation
  - Microscopic evaluation

- **Additional Endpoints**
  - Vaginal Cytology
  - Hormone Measurements
Limitations

- Necropsy is a single time point and animals are not prescreened or synchronized
  - Will have a random mix of different cycle stages at necropsy including abnormal cyclers
  - Don’t forget to consider age at necropsy

- Rat:
  - 4-5 day cycle with significant variability in histologic appearance
  - Need cervical stimulation for significant progesterone production
    - CLs in GT are not the same as in FF/EFD!!!!
  - Reproductive senescence

- Dog:
  - 100+ day cycle
    - Most will be in diestrus or anestrus and not even ovulate during a 14-28 Tox study

- Nonhuman Primate
  - Most are pre or peripubertal
  - Significant social effect on reproduction

- Minipig
  - ???
## Impact of Puberty

<table>
<thead>
<tr>
<th>Species</th>
<th>Age at Sexual Maturity</th>
<th>Age Range at Study Start</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>8-10 Weeks</td>
<td>6-12 Weeks</td>
</tr>
<tr>
<td>Mouse</td>
<td>7-8 Weeks</td>
<td>6-12 Weeks</td>
</tr>
<tr>
<td>Dog</td>
<td>7-12 Months</td>
<td>5-12 Months</td>
</tr>
<tr>
<td>Monkey</td>
<td>3.5-4.5 Years</td>
<td>1-6 Years</td>
</tr>
</tbody>
</table>
Impact of Age in Rat General Toxicity Studies

Study Start at 12 Weeks Old

- 26 weeks
- 13 weeks
- 4 weeks
- 2 weeks

Study Start at 6-7 Weeks Old

- 26 weeks
- 13 weeks
- 4 weeks
- 2 weeks
- Puberty

Reproductive Senescence*

*Sprague Dawley- other strains going into repro senescence later
NHP Menstrual Cycle

- Do NHP in Gen Tox studies cycle regularly?
  - In the cyno, Weinbauer et al. reported mean cycle length as 30.4 days +/- 4.7 days with a range of 19-69 days (determined by daily vaginal smears)
  - Resko et al. reported only 15% of rhesus macaques ovulated in the first five cycles following menarche
  - In the cyno, Weinbauer et al. reported an increase in cycle length from 31 days to 46 days for the 6 months following transfer from single housing to group housing
Social Stress and Cyclicity in Macaques

Adams et al., 1985

- Normal
- Luteal Defect
- Anovulatory

Dominant:
- Normal: 88%
- Luteal Defect: 9%
- Anovulatory: 3%

Subordinate:
- Normal: 54%
- Luteal Defect: 23%
- Anovulatory: 23%

Slide courtesy of Dr. Mark Cline, WFU.
Case 1: What is the stage of the estrous cycle?
INHAND Recommendations

- At a minimum, histopathologic examination of the ovary, uterus and vagina should be conducted.
- Recording of the stage of the cycle for routine screening toxicity studies is not necessary; however, it is important that the pathologist evaluate the female reproductive tract tissues with an awareness of normal cyclicity and understanding of the morphologic features consistent with each phase.
- If alterations are detected, it is recommended that morphologic diagnoses be used to detail the spectrum of changes present in each of the organs of the female reproductive system, as estrous cycle stages are not suitable standalone morphologic diagnoses.
- In studies where recording of the stage of the cycle is deemed necessary, it is recommended that the stage of the estrous cycle along with any specific morphologic diagnoses be recorded for all animals being evaluated when possible, recognizing that perturbations of the estrous cycle often make it difficult or inappropriate to assign a 'stage' of the cycle to these animals. In these situations, a term such as 'unable to determine stage' or 'indeterminate stage' can be used, but the morphologic alterations that are apparent in the reproductive organs should be recorded in the individual organs. Interpretation of these changes can be described further in the pathology report.
Role of the Pathologist

- Evaluation of end organ toxicity
- Evaluate pituitary, ovary, uterus, cervix, vagina, and mammary gland together
- Correlation of organ weights
- Interpretation and discussion of systemic effects
- NOT cycle dynamics and physiologic effects
- Additional reproductive toxicology studies will be performed
Vaginal Cytology

Late Diestrus/Early Proestrus  Proestrus  Estrus  Metestrus  Diestrus
### Vaginal Cytology

**Days**

|   | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | Necropsy |
|---|----|----|----|----|----|----|----|----|----|----|----|----|----|--------|
| 1 | D  | P  | E  | M  | D  | D  | P  | E  | M  | D  | D  | P  | E  | M  | D     |
| 2 | D  | D  | E  | M  | D  | D  | P  | E  | D  | M  | D  | D  | P  |    | P     |
| 3 | M  | D  | D  | P  | E  | M  | D  | D  | E  | M  | D  | D  | E  | M  | D     |
| 4 | E  | M  | D  | D  | E  | M  | D  | D  | E  | M  | D  | D  | E  | M  | D     |
| 5 | D  | E  | M  | D  | D  | E  | M  | D  | D  | E  | M  | D  | D  | E  | M     |
| 6 | M  | D  | D  | P  | E  | M  | D  | D  | D  | E  | M  | D  | D  | D  | E     |
| 7 | E  | E  | M  | D  | D  | E  | M  | D  | D  | E  | M  | D  | D  | D  | E     |
| 8 | M  | D  | D  | E  | M  | D  | D  | E  | M  | D  | D  | D  | E  | M  | D     |
| 9 | D  | D  | E  | M  | D  | D  | D  | D  | D  | D  | D  | E  | M  | D  | D     |
| 10| M  | D  | D  | E  | M  | D  | D  | E  | M  | D  | D  | D  | E  | M  | D     |
| 11| M  | D  | D  | E  | M  | D  | D  | E  | M  | D  | D  | D  | E  | M  | D     |
| 12| E  | E  | M  | D  | D  | D  | E  | M  | D  | D  | D  | E  | M  | D  | D     |

*Note: The highlighted cells indicate the necropsy day.*
### Vaginal Cytology

#### Days

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | Necropsy |
|---|---|---|---|---|---|---|---|---|---|----|----|----|----|---------|
| 1 | E | E | E | E | E |   |   |   |   |    |    |    |    | D       |
| 2 | E | E | E | E |   |   |   |   |   |    |    |    |    | P       |
| 3 | E | E | E | E |   |   |   |   |   |    |    |    |    | D       |
| 4 | E | E | E | E | E |   |   |   |   |    |    |    |    | D       |
| 5 | E | E | E | E | E |   |   |   |   |    |    |    |    | M       |
| 6 | E | E | E | E | E |   |   |   |   |    |    |    |    | E       |
| 7 | E | E | E | E | E |   |   |   |   |    |    |    |    | P       |
| 8 | E | E | E | E | E |   |   |   |   |    |    |    |    | D       |
| 9 | E | E | E | E | E |   |   |   |   |    |    |    |    | D       |
| 10| E | E | E | E | E |   |   |   |   |    |    |    |    | D       |
| 11| E | E | E | E | E |   |   |   |   |    |    |    |    | D       |
| 12| E | E | E | E | E |   |   |   |   |    |    |    |    | P       |
Table 2. Power calculation for ovarian and pituitary hormones in female rats.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Fold change in hormone</th>
<th>Ovarian hormones</th>
<th>Pituitary hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P4</td>
<td>E2</td>
</tr>
<tr>
<td>No. observations^a</td>
<td>30</td>
<td>42</td>
<td>20</td>
</tr>
<tr>
<td>CV (%)^b</td>
<td>97</td>
<td>32</td>
<td>25</td>
</tr>
<tr>
<td>Power (%)</td>
<td></td>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>32</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>104</td>
<td>211</td>
</tr>
</tbody>
</table>

*Note: CV, coefficient of variation. Shown here are the numbers of animals needed to detect 3-, 2-, 1.5-, and 1.25-fold changes (increase or decrease) in P4, E2, LH, and PRL, in a single-point sample during diestrus-1, with power values of 50% and 80%.
^aHormone data came from intact Sprague-Dawley rats 10–12 weeks of age, sampled by decapitation.
^bLog-normal transformation was performed before analysis.

Table 3. Power calculation of AUC of P4 and E2 in female rats.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>P4</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. observations</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
<td>CV (%)</td>
<td>47</td>
<td>81</td>
</tr>
<tr>
<td>Power (%)</td>
<td>50 60 70 80 90</td>
<td>50 60 70 80 90</td>
</tr>
<tr>
<td>3.0</td>
<td>3 3 4 4 5</td>
<td>5 6 7 8 10</td>
</tr>
<tr>
<td>2.0</td>
<td>5 &gt;6 7 8 10</td>
<td>10 12 14 18 24</td>
</tr>
<tr>
<td>1.5</td>
<td>11 13 17 21 27</td>
<td>25 32 39 50 66</td>
</tr>
<tr>
<td>1.25</td>
<td>32 41 51 64 86</td>
<td>79 101 127 161 214</td>
</tr>
</tbody>
</table>

Note: AUC = area-under-the-curve; CV = coefficient of variation. Shown here are the numbers of animals needed to detect 3-, 2-, 1.5-, and 1.25-fold changes (increase or decrease) in the AUC of the P4 and E2 pattern during proestrus/early estrus with power values of 50%, 60%, 70%, 80%, and 90%.

*aHormone data came from intact Wistar Hsd:Brown rats 10–45 weeks of age, sampled by tail vein.

*Log-normal transformation of the AUC was performed before analysis. In case of missing values, imputation was used to estimate AUC.
Table 4. Power calculation of P4 and E2 in adult female cynomolgus macaques.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Fold change in hormone</th>
<th>Follicular phase</th>
<th>Luteal phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P4</td>
<td>E2</td>
</tr>
<tr>
<td>No. observations(^a)</td>
<td></td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>CV (%)(^b)</td>
<td></td>
<td>74</td>
<td>113</td>
</tr>
<tr>
<td>Power (%)</td>
<td></td>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td>3.0</td>
<td></td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td>22</td>
<td>43</td>
</tr>
<tr>
<td>1.25</td>
<td></td>
<td>69</td>
<td>139</td>
</tr>
</tbody>
</table>

Note: CV = coefficient of variation. Shown here are the numbers of animals needed to detect 3-, 2-, 1.5-, and 1.25-fold changes (increase or decrease) in P4 and E2 levels during the follicular and luteal phase with power values of 50% and 80%.

\(^a\)Hormone data came from intact adult female cynomolgus macaques 8–20 years of age, housed in all-female social groups of 3–5 (Stute et al. 2004). Follicular and luteal phase samples were paired from the same group of animals. Mean day of cycle (±SD) based on vaginal cytology was 11.2 ± 1.5 for follicular phase samples and 21.2 ± 1.6 for luteal phase samples.

\(^b\)Log-normal transformation was performed before analysis.
Additional Endpoints

- Vaginal cytology and endocrine measurements tend to be most helpful in follow-up studies to further characterize a known toxicity
Part II

– Mechanisms and Patterns of Toxicity in the Female Reproductive System
Hormonal effects

- Most important thing to remember is to evaluate pituitary, ovary, uterus, cervix, vagina, and mammary gland together!!!
Rabbit McPhail Assay (1934)

Performed in prepubertal rabbits - no ovarian input

No Treatment

Estradiol
Rabbit McPhail Assay

Estradiol

Estradiol/MPA
McPhail Score 4
Hormonal effects

  - Type I: atrophic vagina, uterus and ovary
    - GnRH agonists/antagonists
    - Don’t see very often in pure form
  - Type II: atrophic ovary with hyperplastic/hypertrophic uterus and vagina
    - Estradiol
    - Compounds with ER, PR, or AR activity
  - Type III: hyperplastic/hypertrophic ovary, uterus and vagina
    - LH, FSH
    - See most commonly with compounds that increase prolactin

- Helpful to understand basic concepts, but many compounds in pharmaceutical development are not this straightforward.
  - Weak off-target effects at high doses, direct ovarian effects, stress at high doses, mixed patterns, impact of age, partial agonists, selective receptor modulators, etc.
GnRH
+ FSH and LH
+ Estradiol and Progesterone
- (but sometimes +)
Novel Progestogen
Novel Progestogen
Novel Progestogen
Novel Progestogen
Rat Vagina: Hormonal Effects

Progestogenic
Androgenic

Estrogenic
Rat Vagina: Hormonal Effects

Estrogenic

Vaginal Cytology

Vaginal Histology
Prolactin

- Produced by the anterior pituitary
- Dopamine is the major inhibitory factor
  - Centrally acting compounds may impact prolactin levels via changes in dopamine
- Prolactin has a diverse (and complicated) set of functions
  - Stimulates lactation
  - Major regulatory of corpus luteum “lifespan” in rats
    - In the normal estrus cycle proestrus prolactin surge is luteolytic
    - If rats are mated, then prolactin production is altered and is luteotrophic
- Changes in prolactin levels can lead to a profound change in reproductive function
  - Increased prolactin: Leads to increased ovarian weight, increased size and number of corpora lutea; increased mammary gland development; and vaginal mucification. These changes are similar to a pseudopregnancy
  - Decreased prolactin: Increased ovarian weight and number of corpora lutea.
Prolactin IHC in the female rat pituitary. Majority of cells in the female anterior pituitary are prolactin positive.
Prolactin: Increased
Decreased LH/FSH

FSH and LH +

GnRH +

Inhibin -

Estradiol and Progesterone - (but sometimes +)
LH IHC in the female rat pituitary
FSH IHC in the female rat pituitary
FSH and LH

IHC double labeling for FSH (DAB) and LH (Fast Red) in the female rat pituitary.
Decreased number of CL
Luteinized Follicular Cysts
Differential Diagnoses: Follicle, luteinized, cystic
Luteinized Follicular Cyst
Increased Gonadotropins
Inhibition of Steroidogenesis

- FSH and LH (+) stimulate GnRH (+) in the hypothalamus, leading to the production of Inhibin (-) which inhibits steroidogenesis in the ovaries.
- Estradiol and Progesterone (-) (but sometimes +) also inhibit steroidogenesis.
Inhibition of Steroidogenesis
Aromatase Inhibition

Enzymes:
- 3βHSD
- P450c17
- Arom
- 17βHSD

Androgen Levels

Estrogen Levels
Reproductive Senescence
Reproductive Senescence

Control

High dose
Direct Effects

- More common with oncology compounds
Degeneration, CL

Note the central necrosis. Angiogenesis inhibitors. Compare to proestrus ovary, luteal cyst, and luteinized follicular cyst.
CL: Degeneration vs. Proestrus
CL: Degeneration vs. Luteinized Follicular Cyst
Mineralization
Hemorrhage
Mixed pattern?
Things to Remember…

- Evaluate pituitary, ovary, uterus, cervix, vagina, and mammary gland together!!!
- Use diagnostic terms and try to stay away from cycle stages as a diagnosis
- Ovarian changes can be difficult to identify in short-term studies
- Not always straightforward
Questions?
“All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.”