The Estrous Cycle

- GnRH
- E₂
- LH
- P₄
- PR
- L
- FSH
- DA

DE  PE  E  ME
Hypothalamus

Anterior Pituitary

Ovary

Hypothalamus

GnRH

DA

FSH

LH

PRL

Higher brain centers

Behavioral estrus

Cervical stimulation

1° follicle → Preantral follicle → Graafian follicle → Corpus Luteum

E₂ → P₄

Ovulation

E₂ → P₄

Higher brain centers

Behavioral estrus

Cervical stimulation
Timing Mating with Ovulation

• $E_2$ peaks to indicate that the follicle has reached maturity and is ready to ovulate.
  – $E_2 = 100 - 200$ pg/ml of blood

• This peak triggers events leading to
  – Behavioral estrus
  – Ovulation
E₂ peaks when follicle matures
E₂ causes GnRH to peak
GnRH stimulates LH

LH surge

Ovulation

LH

Luteinization of follicle

↑P₄ + ↑E₂

Behavioral Estrus

Ovulation
Timing is critical to assure fertile mating

Fertile life of gametes in the reproductive tract in hours:

<table>
<thead>
<tr>
<th>Species</th>
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<th>Ova</th>
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<tr>
<td>Mouse</td>
<td>6</td>
<td>8 - 12</td>
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<tr>
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<td>14</td>
<td>12 - 14</td>
</tr>
<tr>
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<td>8</td>
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<tr>
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Timing is critical to assure fertile mating

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Effect of time of insemination on fertility in rats:

- PE-4 pm: 50%
- E-4 am: 83%
- E-4 pm: 4%
The Estrous Cycle Mouse Model

• Cycle Stages
  – Diestrus (DE): Resting stage
  – Proestrus (PE): Preovulatory stage
  – Estrus (E): Ovulatory stage
  – Metestrus (ME): Degenerative stage

• Spontaneous ovulation

• Cycle is considered “incomplete”
Diestrus

GnRH → FSH → Early Graafian follicle (Started dev. 5 cycles ago) → E2

Proliferation of the endometrium
Increase of cells & blood vessels

GnRH → FSH → E2

GnRH → +
Proestrus

GnRH inc. production (hyp.)
  inc. receptors (ant. pit.)

FSH  LH  luteinization

Early Graafian follicle

Increasing levels of $E_2$

Mature Graafian follicle
**Proestrus**

**Early Graafian follicle**
- GnRH inc. production (hyp.)
- inc. receptors (ant. pit.)

- FSH
- LH
- luteinization

**Increasing levels of E₂**

**Mature Graafian follicle**
- GnRH (high levels)
- FSH
- LH surge

- E₂ reaches 100pg/ml
- P₄
Proestrus

GnRH

E₂

FSH

LH
Proestrus

- GnRH inc. production (hyp.)
- inc. receptors (ant. pit.)

- FSH
- LH luteinization

Early Graafian follicle

- Increasing levels of E₂

- Mature Graafian follicle

- GnRH (high levels)
- FSH
- LH surge

- E₂ reaches 100pg/ml
- P₄

- LH surge
- BH begins

Inhibits DA

PRL (Released from inhibition from DA when E₂ is high)

Behavioral Estrus

0 4 8 12 16 20 24
Proestrus

- GnRH
- FSH
- LH
- E₂
- P₄
- PRL
- DA

Behavioral estrus
Estrus

- GnRH
- LH surge
- Mature Graafian follicle
- Ovulation
- E2, P4

Inhibits DA
- PRL (Released from suppression)
- Maintains CL
- Corpus Luteum
- P4, low E2

GnRH inhibited by High P4 + low E2

High P4 stimulates Secretions in the oviduct to nurture ovulated ovum
Estrus

Mature Graafian follicle

GnRH
LH surge

Inhibits DA
PRL (Released from suppression)
Maintains CL

Corpus Luteum

Ovulation

Inhibits GnRH
Inhibits FSH

GnRH released from inhibition

Low E2 releases DA from suppression
PRL is suppressed by DA

CL regresses

P₄ decreases

16 20 24 4 8 12 16 20 24

Behavioral Estrus

Secretory activity in oviduct & endometrium due to P₄

E₂, P₄

E₂, P₄

Low E₂
Estrus

- LH
- E₂
- FSH
- PRL
- P₄
- DA

Behavioral estrus

timetable:
20 24 4 8 12 16 20 24
Metestrus

GnRH (Released from P₄ inhibition)

FSH

Preantral follicle

P₄ levels have dropped due to returned inhibition of PRL by DA

E₂

Resorption of the endometrium
Estrous cycle hormones
Vaginal Cells

• Leukocyte (neutrophil)
  – 5-7 µm
  – Round w/ large multilobular nucleus

• Nucleated Epithelial cell
  – 30 µm
  – Oval w/ oval nucleus

• Cornified epithelial cell
  – 60-80 µm
  – Hexagonal shape, no nucleus
Vaginal Cytology

- Vaginal cytology/Saline Lavage/Vaginal Smear
  - Collect at a fixed time of day, usually morning
  - Collect cells from vagina using an eyedropper filled with saline or deionized water, or a moist cotton swab.
  - Drop or smear on slide
  - Stain with New Methylene Blue and observe cell types under a microscope

- Recording
  - Include all cell types present indicating predominate cells vs those with fewer numbers
  - Example: L/ec, E/c, C/e, LC
**ESTROUS CYCLE**

- **Ovary**
  - Follicular development
  - Follicular maturation
  - Ovulation
  - Secretions

- **Uterus**
  - Proliferation of endometrium
  - Secretory activity

- **Vagina**
  - Leukocytes
  - Epithelial cells

- **Cornified cells**

- **Resorption of endometrium**

- **Resorption of CL**

- **GnRH**
- **LH**
- **P4**
- **PRL**
- **FSH**

- **Behavioral estrus**

- **E2**

- **DE** (Diestrus)
- **PE** (Proestrus)
- **E** (Estrus)
- **ME** (Metestrus)

- **Ovulation**
Diestrus

• Predominantly leucocytes
  – white blood cells; neutrophils
  – 5 – 7 µm in diameter
  – Round
  – Large multi-lobular nucleus
Proestrus Cytology

- Nucleated Epithelial cells
  - May be called epithelial or nucleated cells
  - 30 µm in diameter
  - Ovoid
  - Relatively small oval nucleus
Estrus Cytology

• Cornified Epithelial Cells
  – 60 – 80 µm in diameter
  – Roughly hexagonal in shape and flat
  – No nucleus
Metestrus Cytology

• Leukocytes and cornified cells in equal number.
Spontaneous Ovulation

- GnRH leads to an LH surge.
- LH surge inhibits DA and releases PRL (released from suppression).
- PRL maintains CL.
- P4, low E2.

Ovulation occurs with each cycle.

Mature Graafian follicle

E2, P4

Behavioral Estrus

Ovulation occurs with each cycle.
Spontaneous Ovulation

Cervical stimulation sets up a neural reflex loop to inhibit dopamine

GnRH

LH surge

Mature Graafian follicle

GnRH

E2, P4

Inhibits DA

PRL (Released from suppression)

Maintains CL

Corpus Luteum

Corpus Luteum

P4

low E2

Ovulation

Behavioral Estrus

16 20 24 4 8 12 16 20 24
Initiation of Pregnancy

Spontaneous Ovulators

- Cervical stimulation rescues the CL
- Prolactin has a biphasic release pattern

Copulation during behavioral estrus stimulates the cervix.

DA is again inhibited, releasing PRL from inhibition.

PRL maintains the CL which produces P₄.
Initiation of Pregnancy

Induced Ovulators

E₂ does not directly cause the GnRH/LH surge or the inhibition of DA. E₂ does cause behavioral estrus without the help of P₄.
Induced Ovulation

17 day cycle
no mating

17 day cycle
mating

ovulation

GnRH

LH

Cervical stimulation

P4

PRL

E2

BE

DA

E2

BE
Ovulation

• Spontaneous
  – Rodents
  – Ovulation occurs during each cycle
  – Cervical stimulation maintains pregnancy
  – Short behavioral estrus

• Induced
  – Rabbits
  – Ovulation occurs following copulation
  – Cervical stimulation triggers ovulation
  – Long behavioral estrus

Changes in vaginal opening with cyclicity

Changes in vulva with cyclicity
Factors affecting the estrous cycle

- Photoperiod
- Nutritional variables
- Pheromonal influences
- Stress
- CNS depressants
Photoperiod

• Reproductive activity vs. Light
  – 12 to 14 hours: increased breeding
  – < 12 hours: anestrus and gonadal atrophy
  – Continual light: persistent estrus and failure to cycle

• Sensitivity: most to least
  – Hamsters
  – Rats and Mice
  – GP and Rabbit
Pineal Gland

- Key organ in neuroendocrine pathway between photoperiod and reproduction
- Secretes melatonin during dark phase
Pheromones

• Chemicals released from one individual that influence the physiology or behavior of a conspecific.
• Stimulated by reproductive hormones
• Direct contact is not necessary
Pheromonal Effects

• Cycle Length vs. Housing Density
  – Rats: Housing in large groups causes synchrony of estrous cycle.
  – Mice: Housing in large groups causes persistent diestrus (Van der Lee/Boot)
Pheromonal Effects

• Whitten effect in mice
  – Mice in persistent diestrus will synchronize upon exposure to a male
  – 30% or more females will be in estrus on day 3 following exposure to the male
  – Can be used for timed mating
**Whitten Effect**

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>♂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>9%</td>
<td>11%</td>
<td>32%</td>
<td>18%</td>
<td>6%</td>
</tr>
<tr>
<td>Castrated</td>
<td>10%</td>
<td>10%</td>
<td>9%</td>
<td>14%</td>
<td>7%</td>
</tr>
<tr>
<td>Saline</td>
<td>16%</td>
<td>8%</td>
<td>13%</td>
<td>14%</td>
<td>6%</td>
</tr>
<tr>
<td>No ♂</td>
<td>12%</td>
<td>11%</td>
<td>11%</td>
<td>13%</td>
<td>6%</td>
</tr>
<tr>
<td>Preputial gland fluid</td>
<td>10%</td>
<td>17%</td>
<td>22%</td>
<td>14%</td>
<td>8%</td>
</tr>
<tr>
<td>Preputialectomized</td>
<td>12%</td>
<td>10%</td>
<td>16%</td>
<td>14%</td>
<td>7%</td>
</tr>
</tbody>
</table>

- Percent of females in estrus
- Dn represents number of days following exposure to a male.
- Intact and Preputial Gland Fluid are the only groups with a probability of non-random distribution.
- The preputial gland plays an important role in the Whitten effect influence.

Data from: R. K. Chipman and E. D. Albrecht

*The relationship of the male preputial gland to the acceleration of oestrus in the laboratory mouse*

Timed Mating

• Used whenever timing or number of pregnancies is important: Lab classes, Cesarean section or embryo transfer, cyclicity and gestational studies.

• Group house females to initiate Van der Lee/Boot

• Place females into male cage 3 days prior to desired mating. You can use male urine for the first 2 days but remember to put the female in with an intact male on day 2 if you want a fertile mating.