OLAR Veterinary Staff

Ida Washington, DVM, PhD, DACLAM
Katie Knapek, MS, DVM
Courtney Cecil, RVT, RLAT
Brandi Underwood, RVT, RLAT
Naymalis La Santa, RVT, RLAT
Samantha Glaspell, RVT
Rodent Breeding & Weaning Outline

- Estrous cycle
- Mating schemes
- Pheromone Effects
- Pregnancy
- Parturition
- Environmental Factors
- Dystocia
- Neonatal period
- Cross-fostering
- Weaning
- Care of Weanlings
Estrous Cycle

- Puberty mice: m - 5 - 7 wks; f - 4 - 5 wks
  rats: m - 5.5 - 8.5 wks; f - 5.5 - 7 wks

- First occurs at 4 - 5 weeks old

- Occurs every 4 - 5 days

- Polyestrous, no seasonal variation

- Mice and Rats - spontaneous ovulation
  - Ovulation does not accompany every estrus
  - Estrus may not coincide with every ovulation
  - Estrus dependent on gonadal hormones
  - Occurs ~10 hrs after beginning of estrus.
  - Receptivity lasts ~10-13 hrs.

A. proestrus - wide opening, swelling, moist
B. estrus - reduced swelling
C. metestrus - minimal opening
D. diestrus - no swelling, small opening
Vaginal Cytology

A. Proestrus
B. Estrus - cornified epithelial cells
C. Metestrus
D. Diestrus

Approx. 1 day in each stage

https://embryology.med.unsw.edu.au/embryology/index.php/Mouse_Estrous_Cycle
Mating Schemes

Pairs
One sire (♂)
One dam (♀)

Trio
One sire (♂)
Two dams (♀♀) - must remove 1 preg dam by d15 (IACUC Policy)

Harem (all members should be siblings)
One sire (♂)
Three or more dams (♀♀♀) - must remove all but 1 dam + litter
Mating Schemes

- Females must be at least 6-weeks old
- Put female in male cage
- Mate more frequently during dark periods
- Detect mating
  - Vaginal plug (12-24 hrs)
- Separate male prior to birth (JAX says male should stay)
- Don’t re-house males after breeding

## Pheromone Effects

### What are pheromones?
- Species-specific signals that trigger behavioral reactions in organisms
  - Expressed mainly via urine or odor

<table>
<thead>
<tr>
<th>Pheromone</th>
<th>Laboratory procedures</th>
<th>Effects on rodents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruce effect(^8)</td>
<td>Addition of a foreign male</td>
<td>Blocks pregnancy in females</td>
</tr>
<tr>
<td>Hoover–Drickamer effect(^\text{16})</td>
<td>Presence of urine from a foreign pregnant or lactating female</td>
<td>Prolongs estrus</td>
</tr>
<tr>
<td>Lee–Boot effect(^\text{24})</td>
<td>Females are housed together and isolated from males</td>
<td>Suppresses or prolongs estrus; decreases luteinizing hormone; increases prolactin</td>
</tr>
<tr>
<td>Vandenbergh effect(^\text{31})</td>
<td>Accidental exposure of prepubescent female mice to male urine</td>
<td>Accelerates female puberty</td>
</tr>
<tr>
<td>Whitten effect(^\text{32})</td>
<td>Females exposed to male animal or urine</td>
<td>Induces estrus in a group of females</td>
</tr>
</tbody>
</table>

[Link to PubMed article](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624779/)
Pregnancy

- **Gestation**
  - Mice: 18 - 21 days
    - Varies by strain
    - 19 - 21 days for wild mice
  - Rats: 21 - 23 days

- **Detecting pregnancy**
  - Monitor weight and abdominal distension
    - Observable at 10 days
    - Clearly observable at 12 days
    - Gentle palpation at 14 days
      - "string of pearls"
  - Imaging

- **Enrichment/nesting material paramount**

- **Litter size**
  - Mice: 4 - 12
  - Rats: 8 - 14
Parturition

- **Minimize disturbance**
  - Disturbing the cage ~2-3 days prepartum and 3-5 days postpartum may lead to cannibalism or pup rejection
    - OLAR husbandry staff counts pups 5 days postpartum

- **Postpartum estrus**
  - Females enter estrus 24 hours following parturition
    - If successful, second litter due in 3-4 weeks
      - Wean first litter before second one is born
    - If unsuccessful, must wait until litter is weaned to breed again

- **Role of male**
  - Females may benefit from male companionship
Environmental Factors

- Noise & Vibration
- Seasonal Changes
- Handling
- Strains
- Light/Dark Cycle
- Enrichment
- Diet
- Temperature & Humidity

Rodent Reproductive Diet - Sterile
Dystocia

**Definition:** abnormal or difficult birth.

**Causes:**
- Maternal factors (uterine inertia, inadequate size of birth canal)
- Fetal factors (oversized fetus, abnormal orientation as fetus enters birth canal).
- Knockout strains more susceptible
- Age related - replace breeders after 7-9 months of age

**How do we recognize Dystocia?**
- 1-2 hours have passed between birth of pups
- Enlarged abdomen with bloody discharge but no evidence of pups
- Expecting mothers present with clinical signs of pain/distress
  - Rough hair coat
  - Squinty eyes
  - Hunched or abnormal gait
  - Dehydration or weakness due to overexertion
  - Depressed behavior
Current Treatment: SQ Fluids, Calcium Gluconate, Extra Nesting material, and Heat Support
In the future, we will be trying different “dystocia cocktails” as treatment options!
Neonatal period

Mouse and rat pups are born altricial
- Born in undeveloped state, requires care & feeding by parents

Dam sensitive to stress
- Dam is easily stressed in early neonatal period
  Sources of stress: noise, vibration, disturbance, male (+/-)
- Maternal care accounts for 70% of neonatal body wt in mice
  Milk production increases to P12, then declines
- Stressed dam may reject pups
  May neglect pups, or even kill and eat them

Nest is important
- Quality of nest associated with survival of litter
  Creation of dome = quality nest
JAX® Mice Pup Appearance by Age

<table>
<thead>
<tr>
<th>Days of Age</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/cJ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **pigment**
- **milk spot**
- **ears back**
- **hair**
- **nipples**
- **eyes open**
- **eat food**

The approximate age of mouse pups can be determined by their physical attributes during the first two weeks of life. Examples of the developmental stages of albino, agouti, and black pups are shown.
Cross-fostering
To remove an offspring from its biological mother to be reared by another

Reasons to Cross-Foster
- Eliminate certain pathogens like Helicobacter and MNV
- Mother not taking care of pups
- Death of mother

Selecting Foster Mothers
- Proven mother (a few successful litters)
- Should have already had pups within a week of foster pups being born
- Best to have pups that are different colors for identification of litters at weaning
- Strain: Swiss Webster and CD-1 make great foster mothers

Notes
- Foster mother litter size should remain close to the same, meaning you should remove some of the foster mother’s pups
- Once pups are fostered, do not disturb cage for several days
- Cross-fostering should be in your IACUC protocol unless performed by OLAR vet staff
Procedures

The foster transfer should occur as soon as possible after donor mother parturition.

1. Place the foster mother’s cage on a heating pad (low setting) and remove the foster mother to a clean static cage.
2. Take foster pups and donor mother pups and immerse them in dilute iodine solution. (The solution should be roughly the color of dilute iced tea)
3. Massage the pups with sterile gauze until you are confident they are breathing well and place them in the nesting material in the donor mother’s cage.
4. Replace the wire lid and place the donor mother thereon with the plastic lid over her for 10 minutes.
5. Replace the donor mother in the cage with all pups and observe for at least 5 minutes. IF you note signs of rejection or anxiety by the mother, repeat step 4.
6. Once the donor mother is calm with the pups, place the cage back on the rack and do not disturb.
7. Monitor the progress of the foster family daily and report any questions, concerns, or problems to OLAR vet staff.
Weaning

Age at weaning:
mice & rats: 21 days
can be up to 28 d if approved in protocol, or clinical reason

Avoid overcrowding:
limit = 1 litter/cage
weight limitations: Guide

Separate before puberty:
avoid accidental pregnancies

Genotyping:
up to 5mm tail; or use ear punch/notch
recommend tail snip <17 days
> 17d use anesthetic
Care of Weanlings

Care of newly weaned pups
- provide water bottle
- provide 1-2 pellets moist chow in Petri dish for 1 day
- 1-2 pellets per mouse on cage floor
- gel pack for small or orphaned pups

Housing male pups
- house males together only if housed together at weaning
- males removed for surgery or handling often fight when reunited
- males removed for breeding should never be reunited
References

JAX site

IACUC Policy: Breeding & Weaning of Rats and Mice

IACUC Policy: Tail biopsy for DNA extraction in Mice

IACUC SOP: Prevention and Management of Fighting (Aggression) in Mice

Guide
Questions?
Thank you!