

1. Mouse Reproduction

A. General Mouse Information

- i. The average mouse lives approximately 2.5 years; however, the reproductive life span of mice is significantly shorter at 7-8 months.
- ii. Most mice reach sexual maturity (males and females) at 4-7 weeks of age. Younger mice generally produce smaller litters and therefore are not typically mated until they reach 6-8 weeks, of age. Mice that have been housed alone or in same-sex pairs will usually not breed successfully if they are older than 6-8 months.
- iii. The mouse estrous cycle is 4-5 days in length. Mice cycle continuously throughout the year (non-seasonal breeders). Female mice are only receptive to males when they are in estrus. Mating typically occurs at night (lights off). Ovulation occurs 8-12 hours after the onset of estrous.
- iv. If fertilization occurs, fetuses can be palpated by day 14.
- v. Gestation in mice is typically 19-21 days (strain dependent).
- vi. Parturition in mice may last 1-3 hours and frequently occurs at night. Females will go into estrus within 24 hours of parturition and are sexually receptive during this time.
- vii. Litter size varies among strains, but averages 4-12 pups. Inbred mice tend to have smaller litters than outbred mice.
- viii. Mice are typically weaned at 21-28 days or at 10g of body weight.

The Purdue Animal Care and Use Committee requires that mouse pups be weaned at 21 days unless PACUC approval is given on an approved animal use protocol. See Policy attached.

B. Factors that influence reproduction

i. Strain

- 1) Reproductive performance is highly affected by inbreeding. Most inbred strains have an expected reproductive life span of 7-8 months, producing an average of 4-6 litters.**
- 2) Genetic mutations may affect phenotype, including breeding performance. Some induced mutations are embryonic lethal, some cause infertility or reduced fertility or affect females ability to produce insufficient milk for the pups.**

ii. Age

- 1) Mice will usually not breed if they are younger than 4 weeks of age.**
- 2) Younger mice produce smaller litters and therefore are not typically bred until 6-8 weeks.**
- 3) It is not unusual for a first time mother to cannibalize her litter.**
- 4) Mice that have been housed alone or in same-sex groups will usually not breed successfully if they are older than 6-8 months.**
- 5) Occasionally, older mice may breed successfully, but they may have significant problems with the delivery of live pups and nursing.**
- 6) Older mice have infrequent litters and the litters are often small in number.**
- 7) Breeding pairs, no matter what the age, that have not produced a litter for 4-8 weeks should be replaced.**

iii. Environment

- 1) Laboratory mice breed best when the temperature is between 64-79F and the humidity is between 40-60%.**
- 2) Breeding typically occurs at night (lights off) and therefore breeding performance is best when a consistent and uninterrupted light-dark cycle is maintained.**
- 3) Disturbances such as noise and vibrations can decrease breeding performance and may induce mothers to cannibalize pups.**
- 4) Strong odors, noxious fumes and perfumes can decrease breeding performance.**
- 5) Breeding mice require calm and consistent handling. They should be handled as infrequently as possible, especially when new litters are present.**
- 6) Remember that all mouse facilities are different. Strains that breed well in one facility may not breed well in another.**

iv. Diet

- 1) Some mice breed better when fed diets high in fat (11% compared to 4%)**

v. Health

- 1) Laboratory mice stop breeding if they are unhealthy. Conditions detrimental to breeding include ulcerative dermatitis.**

vi. Pheromones

- 1) Lee-Boot Effect: Housing female mice in close density in isolation from male mice will result in the suppression of estrus. This phenomenon in combination with the Whitten effect, is used to synchronize estrus in females.**

- 2) **Whitten Effect:** Male mouse urine contains a pheromone that induces estrus in previously suppressed females (see Lee-Boot effect) within three days.
- 3) **Bruce effect:** Also known as pregnancy block, this is the phenomenon by which female mice terminate their pregnancies during the preimplantation period, following exposure to the scent of an unfamiliar male. During the first 5 days of pregnancy, females should not be exposed to foreign males or their soiled bedding.

vii. Timed Pregnancies

- 1) **Method of breeding when exact day of mating required**
- 2) **Advantages of timed pregnancies**
 - a) **Produce embryos of a certain age**
 - b) **Accurate aging of litters**
 - c) **Helpful for planning age dependent projects**
- 3) **Disadvantages**
 - a) **Females must be checked daily for “plugs”**
 - b) **Requires more females**
 - c) **Requires detection of estrus**
- 4) **Estrus detection**



- a) Notice the swollen, moist and open appearance to the vaginal opening on the left. This mouse is in Estrus. The mouse on the right has a closed vaginal opening and pale mucus membranes and is NOT in estrus.
- b) The female on the left should be mated and checked the following morning before 9AM to check for a “plug.”

5) Checking for Copulatory Plug in female mice

- a) The copulatory plug or vaginal plug is made of several components....sperm, ejaculate fluids, and mucus are the primary substances that harden within the cervix of the female mouse forming the plug.
- b) The plug forms following copulation and is visible within, or slightly protruding from the vaginal opening. The plug's presence indicates a successful mating but does not guarantee that conception has occurred.
- c) Again the females should be checked for plugs the following morning before 9AM if housed under a typical 12 on 12 off light cycle.



This female mouse has a copulatory plug. Notice the opaque, whitish, waxy structure within the vaginal opening. Sometimes the plug is more difficult to

visualize but by gentle palpation of the vaginal area the plug can be partially exposed. Do not use forceps and do not try to remove the plug. Occasionally the plug may be found in the bedding near the nesting area.

C. Breeding Systems

i. Monogamous-One male bred to one female (can be continuous or non-continuous)

1) Pros –

- a) Ease of accurate record keeping**
- b) Continuous breeding, i.e., male and female always together – takes advantage of postpartum estrus**
- c) Parents are known**
- d) Don't have to separate parents**
- e) Reduces male burnout from excessive breeding**
- f) Exact date of birth for each litter**
- g) Continuous breeding allows for the generation of a maximum amount of litters per female during her breeding life span**

2) Cons –

- a) Higher number of males required. Male mice that have been used for breeding must never be removed from the breeding cage and placed in a cage with other male mice. THEY WILL FIGHT!**
- b) Higher number of cages required**
- c) If the male is aggressive he may cannibalize pups and/or injure the female – not common**
- d) If one of the breeding pair dies, it can be difficult to replace**

ii. Polygamous (harem), non-continuous – One male bred to two females

1) Pros –

- a) Requires fewer male mice. Again, if the male is removed from a breeding pair, he must not be placed in a cage with other male mice. THEY WILL FIGHT!**
- b) Fewer numbers of cages needed**

- c) Can reduce exposure of pups and females to aggressive males
- d) Allows for the production of a large number of litters quickly

2) Cons-

- a) Can be labor intensive
- b) Accurate record keeping is more difficult
- c) PACUC only allows one female and one litter per cage so second female must be removed when either or both female are determined to be pregnant.
- d) Unable to always take advantage of postpartum estrus and therefore fewer litters per female
- e) Higher incidence of male burnout

In the case of either monogamous pair breeding or harem breeding as described above, older litters must be weaned if female is bred at postpartum estrus and has a second litter. The presence of the older litter can cause the parents to cannibalize the newborn pups or be trampled by so many older mice in the cage.

D. Weaning pups

- i. Separation of sexes at weaning
 - 1) Male and female pups are separated at weaning into separate cages with NO more than 5 mice per cage.
 - 2) If a litter contains a single animal of one sex then place a gel cup in the cage with the pup.
- ii. Provision of food and water
 - 1) Again, if a litter contains a single animal of one sex then place a gel cup in the cage with the pup.
 - 2) Place a few pieces of rodent chow on the floor of the cage with ALL newly weaned pups in addition to filling the hopper with rodent chow and providing a clean water bottle.

E. Record Keeping

- i. Accurate records are very important!
- ii. Maintaining a rodent breeding colony requires a great deal of time and commitment and can require observations 7 days a week.
- iii. Breeder cages must be observed frequently but excessive handling of breeding mice can inhibit breeding and cause parents to cannibalize or ignore newborn pups.
- iv. Breeding card information
 - 1) Strain and date of birth (DOB) of both female and male
 - 2) Date of birth of litter
 - 3) Wean date – 21 days after date of birth
 - 4) Include how many pups are weaned
 - 5) If PACUC has given approval for extended weaning up to 28 days the cage card must be labeled with that information.

Again, you must have PACUC approval to extend the weaning age past 21 days. If a second litter is born, the older litter must be weaned even if PACUC approval for extended weaning age has been given.

F. Sex determination in mice

- a) In the adult mouse, male and female mice are easily differentiated. The mouse on the right is male. The mouse on the left is female. Note the anogenital distance is clearly longer in the male than in the female. Nipples are also easily identified in the female.



- b) At weaning (21 days) mouse pups must be weaned by sex into separate cages. Female pups have a shorter anogenital distance. Nipples in a female mouse pup may be more difficult to visualize. The female pup is on the right and the male is on the left.



- c) Sex two pups at a time. If they look the same, try again until you get one that looks different. Again the one with the longer anogenital distance is male. The other is female. Keep one pup of known sex and use it to compare against the rest of the pups.
- d) It is always a good idea to recheck the sex of the mice about a week after weaning to be sure that only males or females are in a single cage.
- e) Be sure to provide newly weaned pups with a few pieces of food on the floor of the cage along with filling the food hopper and providing a clean water bottle.
- f) Record the strain and date of birth on the cage card.

Mouse Housing and Cage Density Policy

Purpose: The purpose of this policy is to ensure safe population densities in mouse colonies at Purdue University. Overcrowded mouse cages can be a significant animal welfare concern and violate federal policies which require that space recommendations of 8th edition *The Guide for the Care and Use of Laboratory Animals (The Guide)* be appropriately applied.

Policy:

For standard cages measuring (11" x 7"x 5") or (11" x 6.25"x 5")

- Breeding animals may be housed one adult male and one adult female mouse or one adult male with two adult female mice.
- If one adult male and two adult females are housed together for breeding, female mice must be placed in separate cages as soon as pregnancy is apparent unless approved otherwise by PACUC. The male mouse may remain with one of the females.
- An adult female mouse plus her own pups will be considered a single entity until the pups are 21 days old. At that time, the space requirements in *The Guide* must be applied.
- Pups must be weaned by 21 days unless PACUC approval for delayed weaning has been given.
- If a female mouse delivers a second litter of pups prior to the older pups weaning date. The older litter must be weaned.
- Overcrowded cages are those standard mouse cages that have greater than 5 adults of the same sex or 5 weanlings of the same sex in a cage. Overcrowded cages must have the number of animals reduced immediately.

For large breeder cages measuring (11" x 19" x 5")

- Breeding animals may be housed one male plus up to three females.
- An adult female mouse plus her own pups will be considered a single entity until the pups are 21 days old. At that time, the space requirements in *The Guide* must be applied.
- Any pups may remain until weaning at 21 days of age at which time they must be weaned unless PACUC approval for delayed weaning has been given.
- As the potential for newborn pups being in the cage along with litters of 2-3 week old mice exists, extra care may be needed to assure that ample food and water are available at all times and that cages do not become excessively soiled.

Note: Breeding colonies must be attended to every day by investigative staff including weekends and holidays. Investigators not complying with the population policy will receive a Non-Compliance PACUC Housing Policy notification and be given 24 hours to correct the situation unless the welfare of the animals requires immediate separation. Failure to correct the situation in the time allotted will result in EVPRP staff weaning the cage and charging tech time (separation of overcrowded cages is not covered

under the per diem). A report of Non-Compliance PACUC Housing Policy occurrences will be sent to PACUC monthly.