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| University Animal Care Committee Standard Operating Procedure | | |
| Document No: 7.24 | Subject: Mice Breeding and Weaning | |
| Date Issued: February 1 st , 2022 | Revision: 2 | Page No: 1 |

Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the standard procedure to breed mice and wean mouse pups.

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI**, subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP**, intramuscular **IM**, per os **PO**, per rectum **PR**, animal use protocol **AUP**

- 1. Introduction and Definitions:** In the laboratory, the traditional breeding age of mice begins at 3-4 months and ends at 7-12 months of age. Male mice are capable of impregnating females as early as 5 weeks of age, although they normally reach sexual maturity at 6 weeks. There are two breeding schemes: polygamous -placing a single male with multiple females, and monogamous - a single male with a single female. Additionally, one can implement timed or untimed breeding. In timed breeding, mates are placed together for a predetermined period of time (usually several days). Mating is usually nocturnal, the female mice are checked daily for a copulatory plug, which indicates breeding has occurred. Alternatively, a vaginal flush can be performed to monitor for the presence of spermatozoa and determine the female's stage in her estrus cycle. This is a more sensitive and reliable method for determination of mating and allows for a more precise estimate of the expected date of parturition. Refer to SOP 7.25 "Estrus Cycle Monitoring" for details on this procedure. With untimed breeding, mating pairs (or groups) are placed together for 10 days and then separated, so the date of breeding is unknown.
 - Monogamous:* one male housed with a single female for breeding. Mice are not separated until female is pregnant or delivers pups. Postpartum estrus allows female to become pregnant and nurse at the same time. The 3-week-old litter must be weaned before the new litter is born.
 - Trio Breeding:* one male is housed with 2 females. Only acceptable for strains with average litter sizes of 7 pups or less. Lactating females may be left in the same cage when the number of pups is 7 or less. If pregnancy occurs the pups need to be weaned at 21 days of age,
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prior to the birth of the new litters.

- *Copulatory plug*: a secretion passed from male to female during mating which hardens to form a plug at the entrance to the vagina. It is a white or cream-coloured plug that stays for 8-24 hours after breeding. The vaginal plug does not guarantee pregnancy.
- *Gestation period*: 19-21 days for mice.
- *Cannibalism*: consumption of a pup by the same species (such as the doe or buck).
- *Weaning*: process of removing a pup from the doe (thereby withdrawing it from the doe's milk supply). Occurs at 21 days of age in mice, unless exemption has been approved from the UACC. Pups with low growth rate can be weaned around 10-12g

2. Materials:

- Mouse cage setup (sterile cage/food/enrichment)
- Breeding cage cards

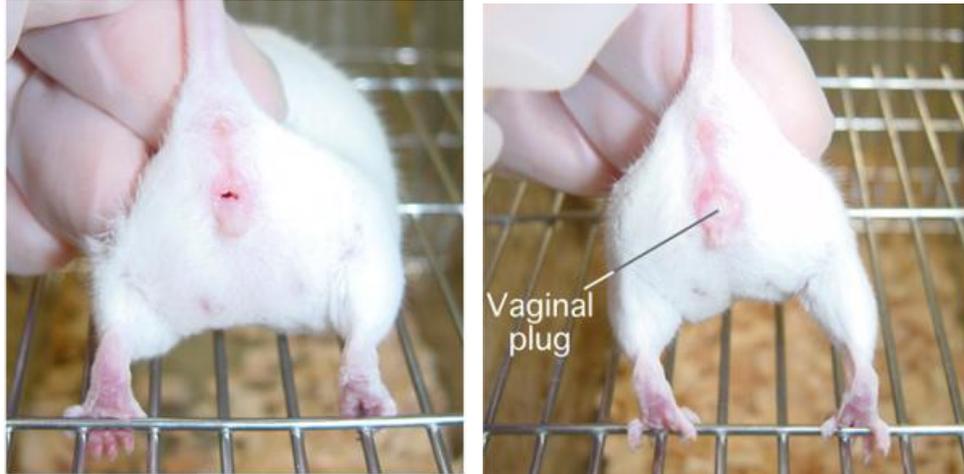
3. Procedures:

a. Breeding:

- Pair-house animals (same sex) for 1 week to habituate to the colony.
 - Place the female mouse/mice into the home cage of the male mouse, with food and water available ad libitum. A ratio of 1 male: 1 female or 1 male: 2 females give the optimal results. The exposure of the females to the male's dirty bedding (specifically, pheromones in the urine), can induce estrous in the females.
 - Write the date the male is added with the females on each breeding cage card (and in a separate logbook if required), and identities of the animals.
 - Presence of a copulatory plug ~12hr after pairing is indicative of mating; however, this does not ensure success (although in most cases a plug does result in a viable pregnancy). Additionally, absence of a plug does not indicate non-pregnancy, as the plug may have been absorbed or may be deeper in the mouse's vaginal canal.
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Absence of Plug



Laboratory Animal Care BOSTON UNIVERSITY Animal Health Monitoring
(Photo of a mouse plug)

- If after 14 days there is no vaginal mucous plug or signs of pregnancy, switch out the breeders.
- During the breeding period, clean cages as per standard cage cleaning procedures and monitor animals daily for grooming, appearance, posture, activity, and food and water intake.
- At the end of the breeding period and depending on the breeding scheme will dictate whether animals are separated or left in cages together.
- Females may be weighed on the day of copulation and at gestational day 8, pregnant females will increase weight by a minimum of 2g.
- For a minimum of 24-48 hours following birth, mothers are not to be disturbed as even minor disruption can alter pup growth or result in cannibalism.
- Following this period, pups and mom should be monitored for grooming, appearance, activity, food. They should be minimally handled unless problems arise including (but not limited to): cannibalism, inability of a pup/s to nurse, death of the mother or rejection of the litter by the mother.
- Any breeding issues should be tracked for phenotypic trends.

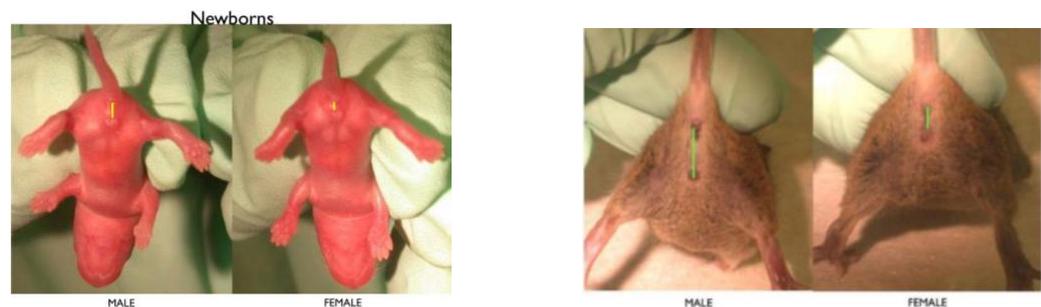
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b. Weaning:

- Approximately one week before weaning add a few pellets of rodent chow to the bottom of the cage to introduce the pups to an adult diet. Ensure all pups can access the waterspout.
- At 21 days of age, robust pups that are an appropriate size are removed from the mother's home cage, sexed and grouped or singly housed according to protocol.
- If the female has a new litter of pups the first litter must be weaned as the female cannot nurse two litters.
- Cage cards are generated through Queen's University Lab Animal Census Software for each weanling.
- Diarrhea or constipation are periodic complications of weaning but should resolve within a few days. If there are any concerns regarding the health of the weaned pups (such as marked anorexia or signs of dehydration) notify the veterinary staff, and/or Animal Care Services.

c. Sex Differentiation:

- In the adult mice, males and females are more easily differentiated than when young. Note the anogenital distance is longer in the male than it is in the female. Nipples are also easily identified in the female. Be aware that sexing is more challenging in younger animals. If uncertain, hold pups up to compare genitalia and anogenital distance. Confirm sexing after one week (recheck weaned pups).



<https://www.research.uky.edu/uploads/mouse-breeding-colony-management>

d. Female Mouse Breeding Endpoint:

Mice should be retired:

- At 8 months of age
- If 6 litters produced

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- Dystocia *refer to SOP 7.34 Treatment Protocol for Sick Mice
- Not bred in 1 month despite males being rotated.
- If more than 2 litters have been born but pups have not survived to weaning age.
- If physical health of mouse has deteriorated or negative phenotypes are observed.
- If there has been a large decrease in litter size.

References: <https://www.purdue.edu/research/regulatory-affairs/animal-research/docs/Mouse%20Breeding%20Colony%20Management%20Document.pdf>
[608 -mouse breeding colony management - dec 2018.pdf \(mcgill.ca\)](https://www.mcgill.ca/608/-mouse-breeding-colony-management-dec-2018.pdf)

SOP Revision History:

| Date | New Version |
|-----------|--|
| 3/27/2023 | Update format and add updated endpoints for breeders |
| 1/22/2025 | Triennial Update – Breeding endpoint updated, updated language |
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