



University Animal Care Committee Standard Operating Procedure

Document No:
10.24

Subject:
Rat Breeding and Weaning

Date Issued:
July 23, 2015

Revision:
2

Page No:
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Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

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

1. **Introduction and Definitions:** In the laboratory, the traditional breeding age of rats begins at 3-4 months and ends at 10-12 months of age. Male rats are capable of impregnating females as early as 5 weeks of age, although this is not common. 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) and female rats 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 10.25 "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.

- *Polygamous:* one male bred with multiple females.
- *Monogamous:* one male bred with a single female; alternatively, monogamous trios are commonly used in which one male is housed with 2-3 females. Each female is removed from the group when pregnancy is confirmed and remains housed separately until pups are weaned.
- *Copulatory plug:* a secretion passed from male to female during mating which hardens to form a plug at the entrance to the vagina.
- *Gestation period:* 21 days for rats (typically).
- *Cannibalism:* consumption of a pup by the same species animal (such as the mother or father).
- *Weaning:* process of removing a pup from its mother (thereby withdrawing it from the mother's milk supply). Occurs at ~21 days of age in rats.

Abbreviations: subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP**, intramuscular **IM**, per os **PO**

2. Materials:

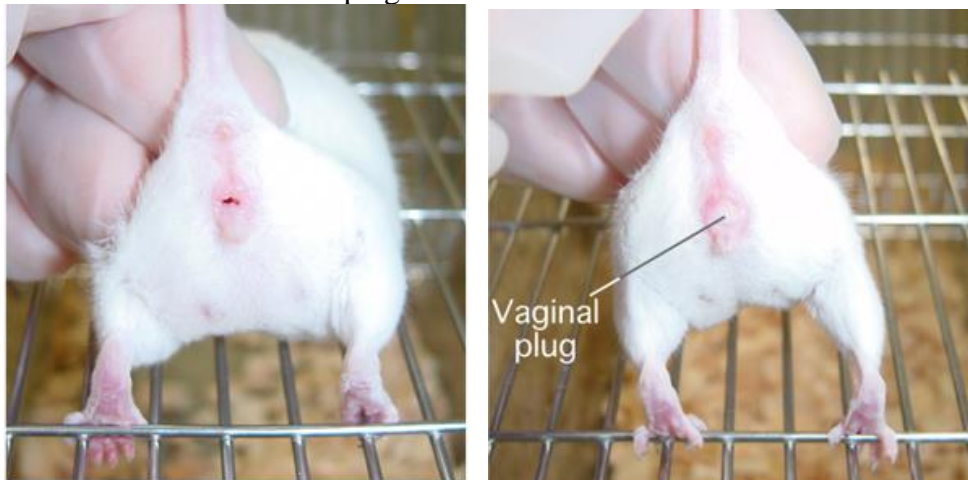
- Large cages
 - Nesting/bedding material
 - Breeding cage cards
 - Dissection probe
 - Rat chow pellets
 - Hydrogel (purified water gel with hydrocolloids, food acid, electrolyte mix)
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3. Procedures:

a. Breeding:

- Pair-house animals (same sex) for 1 week to habituate to the colony.
- Place the female rat(s) into the home cage of the male rat, with food and water available ad libitum. 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 log book if required), and identities of the animals.
- Presence of a copulatory plug ~12hr after pairing the couple 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 rat's vaginal canal. Gentle probing with a dissection probe will assist in visualizing.

Absence of a plug.



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- 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, remove the male and house individually in the main colony. Females may remain pair-housed until approximately 1 week before the expected date of parturition, at which time they are separated into individual cages (the dams may be given supplemental hydrogel on the veterinarian's recommendation).
- Females may be weighed daily to monitor for weight gain in order to diagnose pregnancy.
- Monitor dams once daily during pregnancy.
- For a minimum of 24-48 hours following birth, mothers are not to be disturbed as even minor disruption can alter pup growth.



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- Following this period, pups and mom should be checked twice daily for grooming, appearance, activity, food and water intake. 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.

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 (in some cases, T-Gel can be given to the pups). Ensure all pups can access the water spout.
- At 21 days of age, robust pups that are an appropriate size are removed from the mother's home cage, sexed and group or singly housed according to protocol. Undersized litters or pups can be left with the mother up to the age of 23 days.
- Cage cards are generated through Topaz Elements 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 rat, males and females are easily differentiated. The rat on the right is male. The rat on the left is female. Note the anogenital distance is clearly 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.purdue.edu/research/regulatory-affairs/animal-research/docs/Mouse%20Breeding%20Colony%20Management%20Document.pdf>

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