



ACC-6 Rat Breeding and Colony Management

1. PURPOSE

The purpose of this policy is to present general guidelines for breeding colony management in keeping with best practices and standard of care for laboratory animal use at the University of Ottawa. These guidelines are aimed at ensuring the well-being of rodents and as a result facilitate the production of healthy offspring. This policy is consistent with the Ontario Animals for Research Act, R.S.O. 1990, c.A22 and Canadian Council on Animal Care standards and policies.

2. GENERAL

Before animals may be bred, the University of Ottawa Animal Care Committee must review and approve the Animal Use Protocol (AUP) for breeding. Experimental procedures are generally not conducted on the breeding protocol unless they are a necessary component of producing offspring with the desired genotype or phenotype (e.g. administration of tamoxifen to pregnant females).

It is the responsibility of the Principal Investigator named on the approved breeding protocol to ensure that all persons working under his/her supervision that are responsible for colony management and monitoring breeding animals, have the knowledge and training necessary to do so competently. Careful calculation of anticipated research needs, and proper colony management will prevent the unnecessary production of animals that are ultimately euthanized (overbreeding).

Rats have short gestation times and large litters. Therefore, cages may quickly become overcrowded if the individual responsible for managing the breeding colony and separating animals at weaning age does not do so in a timely fashion. When this happens, the animals become uncomfortable and stressed. Newborn pups can be trampled and die. In static micro-isolator cages, the air quality quickly deteriorates with a high animal count and may predispose them to respiratory disease. Overcrowding of cages is an animal welfare concern and can have a deleterious effect on research.

Breeding cages should be observed at least twice a week for a general health check of the animals and to monitor for newborn animals, litters that are ready for weaning and separating males and females as described below.

Pair or group housing is the default housing recommendation for rats. Requirements for single housing must be justified in the AUP and approved by the ACC.

3. BREEDING

Male rats may be bred from 10-12 weeks of age and females rat may be bred from 8-9 weeks of age. To optimize breeding performance, delay breeding until female is at least 90 days old and approximately 200-275g, depending on strain. Young males should not be used until at least 3 months of age or until they weigh 275-350g.

The reproductive lifespan of rats is an average between 12 and 15 months. Breeders should be removed from the breeding colony by 10 months of age or when reproductive performance decreases, as evidenced by no litter production

with a young or proven female or the lack of a vaginal plug in the bred female. In female rats, reproductive performance may decline by 7-9 months of age.

Rats are polyestrous and breed throughout the year. The estrous cycle averages four to five days in length. The cycle consists of four stages: proestrus, estrus, metestrus, and diestrus. Where timed pregnancies are required, the stages of the cycle are easily identified cytologically from vaginal smears. Successful mating may be confirmed by observing the copulatory (vaginal) plug in the female or by identifying spermatozoa in the vaginal smear. Fetuses may be palpated as early as 10 days of gestation.

The gestational period is 21-23 days and the weaning age is 21 days.

Many variables affect litter size, including stock, strain, and maternal age. The second litter is typically the largest, and reproductive function declines after nine months

Post-partum estrus occurs within 48 hours of giving birth and matings at that time are better than 50% successful. Failure to conceive at that time will delay breeding until two to four days after the litter is weaned.

There are three possible breeding schemes that may be approved at the University of Ottawa. The breeding scheme should be described within the Animal Use Protocol (AUP) form. Principal investigators (PI) that wish to deviate from the ACC-approved breeding schemes listed below must provide justification to the ACC.

3.1. BREEDING PARADIGM

3.1.1. MONOGAMOUS PAIR

- One (1) male and one (1) female per cage.
- Both female and male can remain together as a pair after the female is confirmed pregnant and may remain together after she has delivered the pups (see Pregnancy and Parturition section).
- Takes advantage of postpartum estrus and allows the female to become pregnant and nurse at the same time.
- The 21 days old litter must be weaned prior to the birth of the new litter.
- For strains that require pups to be weaned later than 21 days of age, female must be separated to avoid post-partum estrus and overcrowding.

3.1.2. TRIO BREEDING

- One (1) male and two (2) females are housed together for mating.
- One of the females must be separated when pregnancy is confirmed, before delivery of pups, to avoid overcrowding. One of the lactating females may be left in the same cage with the male.
- The 21 days old litter must be weaned prior to the birth of the new litter.
- For strains that require pups to be weaned later than 21 days of age, female must be separated to avoid post-partum estrus and overcrowding.

3.1.3. TIMED MATING

- One (1) male + up to three (3) females per cage.
- If the females will be harvested during the gestational period, the adult cage group may remain intact.
- However, if the intention is to harvest pups during the post-natal period, prior to weaning age, the females must be separated before parturition (when/if a litter is born, there can be no more than 3 adults in the cage, as described above in Trio breeding).

3.2. PREGNANCY AND PARTURITION

Following copulation (or the detection of a vaginal plug), the females should be monitored closely for the next 10 days for evidence of pregnancy.

Clinical signs of pregnancy include:

- Non-receptivity to the male
- Increased appetite and weight gain

- Abdominal enlargement
- Mammary gland enlargement (in later stages of pregnancy)

The females should be assessed for the anticipated birthing date (parturition) based on a calculation of a gestational period of 21 days. When parturition is imminent, the removal of the male may be considered to avoid crowding, conflict and possible cannibalization of the offspring that may result due to conflict. Removal of the male is also helpful to avoid breeding during the post-partum estrus cycle and lactation period, thus preventing the perpetual pup production where such production is not desirable. If the male is to be removed it is recommended that the animal not be housed with other males as fighting may ensue. In the event that the animal must be singly housed added environmental enrichment is required to avoid the development of stereotypic behaviours. Female cage mates scheduled to give birth at the same time should not exceed two in number. Maintaining a maximum of one dam with her pups per cage prevents cage overcrowding and reduces maternal and neonatal stress. This cage density will allow for optimal hygiene level and proper animal visualization.

4. COLONY MANAGEMENT AND WEANING

Typically, the weaning age of rats is 21 days of age. Smaller or less robust pups should remain with the dam until they are healthy enough to be separated. In cases when a strain is known to produce weaker pups, the delayed weaning procedure should be approved by the ACC as part of the animal use protocol submission. Cages must be identified as “extended weaning period”.

Where animals are not weaned in a timely fashion, the PI or designate responsible for colonies will receive notification from the Animal Care and Veterinary Service (ACVS) once the cage is 1 day overdue and will be asked to wean their rats the same day. If no action is taken, the animals will be weaned by ACVS staff the following day (2 days overdue) and technical service fees will be charged to the protocol.

Note: In some instances, due to animal welfare concerns, a 2-day delay in weaning may not be appropriate (presence of newborn animals in a cage containing weanling age pups). In this case, ACVS will make every attempt to contact the research team but ACVS staff will have to proceed with the weaning if the research team cannot be reached or cannot do the weaning themselves on the same day.

5. HOUSING AND ANIMAL IDENTIFICATION

5.1. CAGE DENSITIES

The CCAC requires that cages provide at least 800 cm² of floor space, and occupancy should be based on the minimum floor space required per animal. Floor area should be sufficient to provide the animals with a comfortable resting area, including shelter, and allow the animals to express normal behaviours. The CCAC’s Rat Housing Assessment Tool is used to evaluate whether space provided permits rats to carry out behaviours essential for their welfare. The cage densities outlined below are a guideline only and ACVS may recommend alternate arrangements if cage size appears to be preventing animals from expressing natural behaviours.

The height of cages should accommodate typical postures of rats, including the ability to stretch vertically, which has been shown to be a common and important behaviour for rats. To determine the appropriate cage height, the length of the rat from the nose to the base of the rump should be estimated, and then approximately four centimetres added to account for the additional distance required for upright stretching with partial leg extension.

5.1.1. TECNIPLAST DOUBLE DECKER CAGE (1862 CM²)

- Up to 150g: 5 rats
- 150-400g: 4 rats
- 400-600g: 3 rats
- Over 600g: 2 rats
- Breeding:
 - Maximum 1 male and 2 females (NO litter)
 - Maximum 1 male and 1 female with litter

5.1.2. TECNIPLAST REGULAR CAGE (1147 CM²)

- Up to 150g: 4 rats as long as height requirements are met as they grow.
- These cages are not suitable for adult rats or breeding.

5.1.3. CONVENTIONAL STATIC RAT CAGE (968 CM²)

- Up to 150g: 3 rats as long as height requirements are met as they grow.
- These cages are not suitable for adult rats or breeding.

5.1.4. CONVENTIONAL STATIC GUINEA PIG CAGE (1776 CM²)

- Up to 150g: 5 rats as long as height requirements are met as they grow.
- These cages are not suitable for adult rats or breeding.

5.2. CAGE IDENTIFICATION

Cage cards are medical and experimental records and are used to track the history of animal manipulations and their outcomes as well as animal health (as indicated in CCAC standards and the Animals for Research Act, R.S.O.,c.A.22).

Breeding cage card information related to pup production and survival to weaning is to be provided and regularly updated in order for the ACVS to monitor the breeding colony performance and/or to assist with colony production, including the determination of the causes of reproductive non-performance. Along with Investigator and Protocol identification, cage cards should include:

- Breeder female date of birth, strain and identification
- Breeder male date of birth, strain and identification
- Date of pairing
- Date of breeder male removal (if applicable)
- Litter birth date and other information: number of pups and status (born alive, dead, total, abnormalities and sex)

6. HOUSING AND ANIMAL IDENTIFICATION

Accurate record keeping is critical to the proper maintenance of a breeding colony. Breeding cage cards along with a centralized record keeping system should be used to keep track of mating set-ups and breeding outcomes. Various types of colony management systems exist including commercial products, free databases and templates, or labs can create their own. Labs are also encouraged to keep calendars to note when litters are born or are due to be weaned. Information to be included on the breeding cage cards and in a centralized breeding record include:

- Dam and sire ID's, birthdates, and genotypes
- Date paired//set up (with updated Topaz cards if breeders are changed and older cards placed behind the new updated card)
- Date of birth and number of pups
- Disposition of the litter (wean/died)
- Noted problems

The following information should be maintained in the breeding record database but does not need to appear on the breeding cage card:

- Pup IDs
- Pup genotype information
- Designated use of pup (e.g. experiment, breeding, euthanasia)

The Animal Care Committee will approve animal use based on the number of animals used for breeding and the number of offspring produced. Properly maintained breeding records will:

- 1) Facilitate the required reporting of animal numbers to the Animal Care Committee at the time of the protocol's annual renewal.
- 2) Will allow a determination of the total number of animals produced versus the number of animals used experimentally or as replacement breeders.

REFERENCES

- Suckow, M.A., Weisbroth, S.H. & Franklin, C.L. (2005). The Laboratory Rat, 2nd Edition. San Diego, CA: Elsevier Academic Press.
- Guide to the Care and Use of Experimental Animals, Vol. 1 (2nd ed). Canadian Council on Animal Care, Canada, 1984: <http://ccac.ca/Documents/Standards/Guidelines/Vol2/rats.pdf>

POLICY HISTORY

DATE	NEW VERSION
August 2020	Policy creation (v1)
October 2023	Policy revised (v2)