1. PURPOSE

This SOP describes the set up and record keeping for timed mating procedures of mice used at Carleton University.

2. CONSIDERATIONS

2.1. Timed mating is very useful to have females come into estrus at the same time to have a similar date of birth for pups. This may be used experimentally or to cross-foster pups.

2.2. The first procedure takes advantage of the use of pheromones. It is important to remember that mice are very sensitive to smells and the introduction of other smells or dirty bedding from other animals may affect the success of this breeding scheme.

2.3. The use of cytology may improve the success of timed mating, as outlined in the second procedure, however a reference must be used to ensure accurate interpretation.

2.4. Refer to SOP COL-01 Colony Management – Mice for other considerations when breeding mice.

3. GENERAL TIPS

3.1. Minimize the amount of noise and traffic in the animal holding room.

3.2. Try to always have the same personnel take care of the mice.

3.3. Don't bang cages around or jostle the mice any more than necessary.

3.4. Handle mice calmly and quietly.

3.5. Provide environmental enrichment, particularly nesting material (nestlets with Envirodry). Including a plastic house or cardboard Shepherd Shacks.

3.6. Pup production may improve if cages are placed low on racks, far from room doors, and in rooms with limited traffic and minimal cage or rack disruption.

4. PROCEDURE

4.1. House females together in a group of 3 for up to two weeks prior to the desired mating date.

   4.1.1. Females should be at least 8 weeks old to increase the likelihood that they will become pregnant. Avoid using virgin females older than 15 weeks because often they mate less reliably.

4.2. Isolate stud males for at least 1 week after a previous mating to maximize fertility.

   4.2.1. Use experienced, proven stud males, if possible. Otherwise, pick older males (3-4 months old) over younger males.

4.3. Add dirty bedding from the male breeder cage into the female cage.

   4.3.1. Do not introduce bedding or scent from other male cages, wipe hands with Accel between cages to prevent the spread of odours.
4.4. Place a green flag on the cage indicating that timed mating is occurring so the cage is not to be changed

4.5. On the third afternoon after adding male bedding, place females into the male cage or all animals into a clean cage
   
   4.5.1. Breeding females on occasion become territorial, adding the females to the male cage reduces the chances of aggression

4.6. Monitor females daily for vaginal plugs
   
   4.6.1. Plugs are best seen first thing in the morning, after the lights switch on (8am)
   
   4.6.2. The presence of a vaginal plug does not indicate pregnancy

4.7. When a plug is observed, remove the female from the male cage and record the date as E0
   
   4.7.1. Mice should give birth 21 days later
   
   4.7.2. If signs of pregnancy are not visible after two weeks (weight gain, bulge in abdomen), the female may need to be rebred

5. **PROCEDURE – ESTRUS DETECTION**

   5.1. Once female mice are at an appropriate breeding age, use a sterile swab with sterile saline (NOT sterile water) to swab the vaginal canal
   
   5.2. This procedure requires care to avoid injury to the mice
   
   5.3. Roll the swab onto a microscope plate and stain
   
   5.4. Examine the cells under a microscope and determine the phase of estrus
   
   5.5. Females should be paired with males during proestrus
   
   5.6. This may be paired with the above procedure to introduce dirty bedding
   
   5.6.1. Introduce dirty bedding when all females are in anestrus then continue as above

6. **SAFETY**

   6.1. In the event of an injury seek medical attention (x4444) and notify your supervisor. In the case of an animal bite refer to SOP: Animal Related Injury.

7. **REFERENCES**

SOP OHS-02: Animal Related Injury

SOP COL-01: Colony Management – Mice
