1. **PURPOSE**

This Standard Operating Procedure (SOP) describes the breeding program for colony-housed marmosets.

2. **BACKGROUND**

2.1. Marmoset breeding is necessary for two reasons:

2.1.1. To maintain a steady supply of animals for various behavioral and biological experiments with this species. Marmosets are not easily and often available and further, they are very expensive animals to acquire, transport, and quarantine. They are also susceptible to stress and zoonotic exposure, thereby making them delicate from a handling point of view.

2.1.2. Marmosets are normally housed in social family groups. Reproductive behavior and child rearing is a natural part of their social function. Therefore, colony-housed marmosets are allowed to breed to ensure that social cohesion and parenting behaviors are maintained.

3. **HOUSING CONSIDERATIONS**

3.1. Marmosets are typically maintained in social family groups of 2 to 12 animals per cage in research colonies. The main limiting factor is cage size.

3.2. The following recommendations have been made by the Canadian Council on Animal Care (CCAC) for housing non-human primates with weights of less than 1.0 kg.

3.2.1. Floor space = 0.15 sq. m. per animal

3.2.2. Minimum height = 0.51 m.

3.2.3. Given the need for marmosets to have vertical flight, the minimum height requirement for the present colony should be doubled (i.e. 1 meter or approximately 6 feet).

3.3. It is optimal to divide the cage systems between breeding and research colonies. When possible, experiments should be avoided on breeding animals.

4. **PROCEDURES**

4.1. Uterine palpation protocol:

4.1.1. Manual palpation of the uterus of female marmosets is a good way to estimate and follow pregnancy:

4.1.1.1. Prior adaptation of the marmoset to the procedure is necessary.

4.1.1.2. One person manually restrains a female marmoset using the safe hold and the marmoset’s legs are extended. The marmoset should be as relaxed as is possible (tight abdominal muscles can make palpation difficult).

4.1.1.3. The other person gently palpates the abdominal area with the thumb and index finger going down the abdomen until the uterus can be felt. Do not confuse it with fecal contents.

4.1.1.4. A non-pregnant marmoset uterus feels like a hard pea-sized mass near the lower end of the abdomen (approximately 0.5 cm). As the pregnancy develops, this will become larger and softer. Near the end of pregnancy, one will be able to feel at least one infant head. The uterine limits will not be well-defined at this point.

4.1.1.5. To obtain an estimate of uterine size, it is best to place the thumb and index finger on either side of the uterus, and then carefully transfer them to a ruler without moving them apart. This procedure requires some practice. Measure the space between thumb and index finger to give you an estimate of the uterine size.

4.1.1.6. The due date can be estimated on the basis of uterine size. Monthly palpations help verify that the pregnancy is continuing. Once the uterine size is over 3.0 cm, use the size to estimate due date.
4.1.2. Ovulation occurs approximately 10 to 14 days post partum. Therefore, delivery date + 10 days to ovulation + 144 days gestation = estimated due date.

4.1.3. Always wait 6 weeks post partum to palpate a new pregnancy.

4.2. Infant survival procedures

4.2.1. The typical infant mortality rate for colony-housed marmosets ranges from 30 to 70%. The following protocol should be followed when dealing with weak or injured marmoset infants. It is important that these situations are dealt with in a consistent and humane way that conforms to CCAC standards.

4.2.1.1. Care should be taken when removing or replacing infants in cages, as the adults may get aggressive.

4.2.1.2. Infants with serious injuries should be euthanized immediately (see Section 4.3). If it is not known how serious the injury is, a veterinarian or other qualified person should be consulted.

4.2.1.3. When an infant is noticeably weak and hanging low from a parent, the 3-strikes rule applies. A weak infant should be watched closely. If it falls, give the older animals a chance to pick it up. Otherwise, attempt to place the infant back on. If the adults refuse to care for the infant, or it has been dropped 3 times in a row, it is probably too weak to survive. The infant will have to be euthanized.

4.3. Infant euthanasia procedures:

4.3.1. Inject pentobarbital at a dose of 100mg/kg intraperitoneally.

4.3.2. To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change or opacity in eyes.

4.4. Termination of pregnancy:

4.4.1. Because of considerations of cost, space limitations, or a particular breeding family’s health condition, there may occur a need to actively control the rate of births of breeding pairs.

4.4.2. Use Estrumate® (cloprostenol sodium, 250µg/ml).

4.4.3. Prepare a 1:10 dilution of Estrumate® in sterile saline or sterile water for injection.

4.4.4. For females that are 21 to 45 days post-conception administer 0.75µg Estrumate® (30µL) intramuscularly in the quadriceps muscle.

4.4.5. Female marmosets that are 45 to 60 days post-conception may also be stopped, but will need an initial larger dose of 1.0µg Estrumate® (40µL) for 2 consecutive days.

4.4.6. Follow-up:

4.4.6.1. Palpate the female marmoset one week later and if uterine size has decreased, the Estrumate® dose has worked.

4.4.6.2. If the uterine size is the same or larger, give 1.0 µg Estrumate® (40µL) for 2 consecutive days. Follow up with another palpation 1 week later and if uterine size is still the same or larger, repeat dosing again, this time for 3 days of 1.0 µg Estrumate®. This is the maximum dosing that should be administered.

4.4.7. Once an Estrumate® dose has worked, schedule another palpation in 4 weeks. Female marmosets will ovulate approximately 7 to 10 days after the Estrumate® dose.