



1. PURPOSE

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

2. CONSIDERATIONS

- 2.1. Breeding of marmosets requires scientific justification in an Animal Use Protocol approved by the Facility Animal Care Committee and a breeding permit delivered by the Canadian Food Inspection Agency (CFIA) for the animals involved.
- 2.2. Breeding must be carefully managed to fulfill the objectives of the breeding program, prevent inbreeding, and prevent overpopulation. Any marmoset not approved for breeding should be on a birth control program.
- 2.3. Generally, marmosets will reach sexual maturity at approximately one and a half years of age. The average life span for breeding females is around six years; the range of reported average lifetime number of litters for a breeding pair is 3.45 to 4.0.
- 2.4. The average gestation period is estimated to be 143 to 144 days. A fertile postpartum ovulation generally occurs within 10 to 20 days after delivery. Simultaneous lactation may prolong gestation.
- 2.5. Lactation lasts for 65 to 90 days. If the original dam-sire pair remain intact, offspring can remain in family groups indefinitely, unless conflict arises.

3. PROCEDURES

- 3.1. Diagnosis of pregnancy:
 - 3.1.1. Ovulation occurs approximately 10 to 14 days post-partum. Therefore, delivery date + 10 days to ovulation + 144 days gestation = estimated due date.
 - 3.1.2. Uterine palpation:
 - 3.1.2.1. Manual palpation of the uterus of female marmosets is a good way to estimate and follow pregnancy.
 - 3.1.2.2. Always wait 6 weeks post-partum to palpate a new pregnancy.
 - 3.1.2.3. One person manually restrains a female marmoset (marmoset should be as relaxed as is possible), 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.
 - 3.1.2.4. 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.
 - 3.1.2.5. The due date can be estimated based on 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.
 - 3.1.3. Ultrasonography:
 - 3.1.3.1. Anesthetize female marmoset as per NHP Anesthesia SOP and place animal in dorsal recumbency. Cooperative animals may be imaged without the use of anesthesia.
 - 3.1.3.2. Remove fur from abdomen and apply ultrasound gel generously.
 - 3.1.3.3. Perform transabdominal ultrasound to confirm pregnancy.
 - 3.1.3.4. Uterine and/or fetal measurements can be used to estimate gestational age.

3.2. Birth control methods:

- 3.2.1. The veterinarian, in consultation with the Principal Investigator, will select the most appropriate birth control method. Selected method must be described in the approved Animal Use Protocol.
- 3.2.2. Cloprostenol sodium, 250 µg/mL (Estrumate®).
 - 3.2.2.1. Cloprostenol works by inducing termination of pregnancies. Doses must be repeated every 28 days to maintain contraception.
 - 3.2.2.2. Prepare a 1:10 dilution of Estrumate in sterile saline or sterile water for injection.
 - 3.2.2.3. For females that are 21 to 45 days post-conception, administer 0.75 µg cloprostenol (30µL) intramuscularly in the quadriceps muscle.
 - 3.2.2.4. For female marmosets that are 45 to 60 days post-conception administer 1.0 µg cloprostenol (40µL) for 2 consecutive days.
 - 3.2.2.5. Palpate the female marmoset one week later and if uterine size has decreased, the cloprostenol dose has worked.
 - 3.2.2.6. If the uterine size is the same or larger, administer 1.0 µg cloprostenol (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 cloprostenol. This is the maximum dosing that should be administered.
 - 3.2.2.7. Once a cloprostenol dose has worked, schedule another palpation in 4 weeks. Female marmosets will ovulate approximately 7 to 10 days after the cloprostenol dose.
- 3.2.3. Etonogestrel, 68 mg (Nexplanon®)
 - 3.2.3.1. The etonogestrel implant works by preventing ovulation. The implant is effective for 3 years; it may be removed as needed or replaced after 3 years.
 - 3.2.3.2. Cut the implant into three equal sections using a sterile scalpel blade. One 22 mg section is implanted per female. Refrigerate unused portions of the implant for up to 6 months.
 - 3.2.3.3. Prior to implantation, determine pregnancy either by manual palpation or ultrasound. Pregnancy does not prevent the implantation.
 - 3.2.3.4. Under general anesthesia, make a small incision between the scapulae, insert the implant in the subcutaneous space, close the incision with tissue glue. Refer to NHP Anesthesia and Analgesia SOPs.
- 3.2.4. Surgical vasectomy of males
 - 3.2.4.1. The vasectomy works by prohibiting sperm from passing into the ejaculate.
 - 3.2.4.2. Refer to the NHP Anesthesia and Analgesia SOPs and the Large Animal Surgery SOP.
 - 3.2.4.3. Under general anesthesia and analgesia, make a 1 cm ventral midline incision approximately 1 cm cranial to the scrotum. Perform a ligation of the vas deferens and repeat on the contralateral side. Close the incision.
 - 3.2.4.4. Post-vasectomy semen analysis should be performed 8-16 weeks after the procedure. Vasectomy is considered successful if no motile sperm are seen.
- 3.2.5. Surgical ovariectomy or ovariectomy of females
 - 3.2.5.1. Removal of the ovaries and uterus permanently prevents pregnancy.
 - 3.2.5.2. Refer to the NHP Anesthesia and Analgesia SOPs and the Large Animal Surgery SOP.
 - 3.2.5.3. Under general anesthesia and analgesia, make a 1-2 cm midline abdominal skin incision is made over the palpable uterus. Excise both ovaries or the ovaries and uterus. Close the incision.

4. REFERENCES

- 4.1. Tardif, S. D., Smucny, D. A., Abbott, D. H., Mansfield, K., Schultz-Darken, N., & Yamamoto, M. E. (2003). Reproduction in captive common marmosets (*Callithrix jacchus*). *Comparative medicine*, 53(4), 364–368.
- 4.2. Roubos, S., Louwerse, A. L., Langermans, J. A. M., & Bakker, J. (2021). Retrospective Analysis of the Effectiveness and Reversibility of Long-Acting Contraception Etonogestrel (Implanon®) in Common Marmosets (*Callithrix jacchus*). *Animals : an open access journal from MDPI*, 11(4), 963. <https://doi.org/10.3390/ani11040963>.
- 4.3. Anesthesia and Select Surgical Procedures. Robert P. Marini, Jennifer Haupt, in *The Common Marmoset in Captivity and Biomedical Research*, 2019.

SOP REVISION HISTORY

DATE	NEW VERSION
2023.02.06	<p>2. BACKGROUND</p> <p>2.1. Marmoset breeding is necessary for two reasons:</p> <p>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.</p> <p>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.</p>
2023.02.06	<p>3. HOUSING CONSIDERATIONS</p> <p>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.</p> <p>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.</p> <p>3.2.1. Floor space = 0.15sq. m. per animal</p> <p>3.2.2. Minimum height = 0.51m.</p> <p>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).</p> <p>3.3. It is optimal to divide the cage systems between breeding and research colonies. When possible, experiments should be avoided on breeding animals.</p>
2023.02.06	<p>2. CONSIDERATIONS</p> <p>2.1. Breeding of marmosets requires scientific justification in an Animal Use Protocol approved by the Facility Animal Care Committee and a breeding permit delivered by the Canadian Food Inspection Agency (CFIA) for the animals involved.</p> <p>2.2. Breeding must be carefully managed to fulfill the objectives of the breeding program, prevent inbreeding, and prevent overpopulation. Any marmoset not approved for breeding should be on a birth control program.</p> <p>2.3. Generally, marmosets will reach sexual maturity at approximately one and a half years of age. The average life span for breeding females is around six years; the range of reported average lifetime number of litters for a breeding pair is 3.45 to 4.0.</p> <p>2.4. The average gestation period is estimated to be 143 to 144 days. A fertile postpartum ovulation generally occurs within 10 to 20 days after delivery. Simultaneous lactation may prolong gestation.</p> <p>2.5. Lactation lasts for 65 to 90 days. If the original dam-sire pair remain intact, offspring can remain in family groups indefinitely, unless conflict arises.</p>
2023.02.06	<p>3.1. Diagnosis of pregnancy:</p> <p>3.1.2. Uterine palpation protocol</p> <p>3.1.2.1. Prior adaptation of the marmoset to the procedure is necessary.</p> <p>3.1.2.3. One person manually restrains a female marmoset using the safe hold then the marmoset's legs are extended. (the marmoset should be as relaxed as is possible) (tight abdominal muscles can make palpation difficult).</p> <p>3.1.2.4. 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.</p> <p>3.1.2.5. 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.</p>
2023.02.06	<p>4.2 Infant survival procedures</p> <p>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.</p> <p>4.2.1.1. Care should be taken when removing or replacing infants in cages, as the adults may get aggressive.</p> <p>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.</p> <p>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.</p>
2023.02.06	<p>4.3 Infant euthanasia procedures:</p> <p>4.3.1. Inject pentobarbital at a dose of 100mg/kg intraperitoneally.</p> <p>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.</p>
2023.02.06	<p>3.2 Termination of pregnancy Birth control methods:</p> <p>3.2.1. The veterinarian, in consultation with the Principal Investigator, will select the most appropriate birth control method. Selected method must be described in the approved Animal Use Protocol.</p> <p>3.2.1. 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.</p>
2023.02.06	<p>3.2.2.1. Cloprostenol works by inducing termination of pregnancies. Doses must be repeated every 28 days to maintain contraception.</p> <p>3.2.2.3. For Female marmosets that are 45 to 60 days post-conception may also be stopped, but will need an initial larger dose of administer 1.0µg cloprostenol Estrumate® (40µL) for 2 consecutive days.</p>

2023.02.06	<p>3.1.3. Ultrasonography:</p> <p>3.1.3.1. Anesthetize female marmoset as per NHP Anesthesia SOP and place animal in dorsal recumbency. Cooperative animals may be imaged without the use of anesthesia.</p> <p>3.1.3.2. Remove fur from abdomen and apply ultrasound gel generously.</p> <p>3.1.3.3. Perform transabdominal ultrasound to confirm pregnancy.</p> <p>3.1.3.4. Uterine and/or fetal measurements can be used to estimate gestational age</p>
2023.02.06	<p>3.2.3. Etonogestrel, 68 mg (Nexplanon®)</p> <p>3.2.3.1. The etonogestrel implant works by preventing ovulation. The implant is effective for 3 years; it may be removed as needed or replaced after 3 years</p> <p>3.2.3.2. Cut the implant into three equal sections using a sterile scalpel blade. One 22 mg section is implanted per female. Refrigerate unused portions of the implant for up to 6 months.</p> <p>3.2.3.3. Prior to implantation, determine pregnancy either by manual palpation or ultrasound. Pregnancy does not prevent the implantation.</p> <p>3.2.3.4. Under general anesthesia, make a small incision between the scapulae, insert the implant in the subcutaneous space, close the incision with tissue glue. Refer to NHP Anesthesia and Analgesia SOPs.</p>
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2023.02.06	<p>4. REFERENCES</p> <p>4.1. Tardif, S. D., Smucny, D. A., Abbott, D. H., Mansfield, K., Schultz-Darken, N., & Yamamoto, M. E. (2003). Reproduction in captive common marmosets (<i>Callithrix jacchus</i>). <i>Comparative medicine</i>, 53(4), 364–368.</p> <p>4.2. Roubos, S., Louwerse, A. L., Langermans, J. A. M., & Bakker, J. (2021). Retrospective Analysis of the Effectiveness and Reversibility of Long-Acting Contraception Etonogestrel (Implanon®) in Common Marmosets (<i>Callithrix jacchus</i>). <i>Animals : an open access journal from MDPI</i>, 11(4), 963. https://doi.org/10.3390/ani11040963.</p> <p>4.3. Anesthesia and Select Surgical Procedures. Robert P. Marini, Jennifer Haupt, in <i>The Common Marmoset in Captivity and Biomedical Research</i>, 2019.</p>