Standard Operating Procedures	
UNIVERSITY OF GUELPH **Animal Care Services**	Title: **Rodent Ovariectomy Surgery**
	SOP.ACC.822. Rodent.Ovariectomy **Approval Date: Mar 14, 2025** **Revision Date:**

1. ***Purpose:*** To provide technical instructions for performing ovariectomy surgeries in rodent species.

2. ***Responsibilities:*** Animal care staff, veterinarians, and trained individuals listed on an approved Animal Utilization Protocols (AUPs). All animal users performing procedures in animals must have successfully completed relevant training courses and mentor-facilitated training.

3. ***Introduction:***

The ovariectomy procedure in rodents refers to the surgical removal of both ovaries. It may be performed to study the impacts of gonadal hormones, or lack thereof, in biomedical research. It may also be used to allow mixed-sex housing without generating offspring or to decrease the risk of mammary tumours.

The following instructions are specific to mice and rats, but the same guiding principles apply to other rodent species as well.

4. ***Procedures:***

This procedure involves placing an animal under general anesthesia using isoflurane and surgically entering its abdomen. For complete instructions on anesthetizing rodents, aseptic practices for surgery, and providing appropriate pain control, see SOP.ACS. 817. Rodent Survival Anesthesia-Inhalant, SOP.ACS.818.Asepsis for Rodent Survival Surgery, and SOP.ACS.814.Administration of pain control to rats and mice, respectively.

Equipment list for the ovariectomy procedure:

This list does not include equipment for anesthesia (induction, maintenance, monitoring) or the maintenance of asepsis (e.g., PPE, drapes, sterilizing equipment).

Microsurgical instruments with miniaturized tips are easier to handle than ophthalmic instruments, but may not be accessible.

- Autoclaved surgical instruments:
  - #3 scalpel handle
  - Mosquito hemostatic forceps (curved or straight)
  - Atraumatic thumb forceps (e.g., Debakey thoracic tissue forceps)
  - Small needle drivers (e.g., small Mayo/Olsen Hegar, ophthalmic)

  - Small tissue scissors (e.g., Iris, tenotomy)
  - Small sharp-blunt scissors for cutting suture (not essential but prevents the dulling of delicate, expensive scissors meant for cutting tissue)
- Scalpel blades (#10 and #11 or 15)
- Bead sterilizer
- Autoclaved gauze (ideally of known weight in case of blood loss)
- Size 4-0 to 6-0 sterile absorbable suture (e.g., Monocryl, Biosyn, Vicryl) for muscle and intradermal/external skin closure
  - Non-absorbable suture (e.g., Ethilon, Prolene) or staples (using an autoclaved stapler) can also be used for external skin closure.

Preparation for surgery:

1. Plug in the bead sterilizer and allow to heat to 250˚C.

2. Set up the surgical and prep areas, anesthetize the animal, and provide systemic analgesics as per ACS SOPs 817, 818, and 814. A focal light source (vs. overhead light only) is recommended for this procedure.

3. Position the anesthetized animal on the nose cone in sternal recumbency with a neutral spine. If the anesthetic set-up allows, this procedure is more easily performed with surgeon positioned at the animal's side, versus at the animal's tail.

4. Visualize the incision site to guide shaving and administration of local anesthetic.

5. Shave a rectangular area on both sides of the spine that extends from the last rib to the hip (where the hind leg meets the abdomen). Clip 1 cm each side of the dorsal spinous processes for mice and 2-3 cm for rats (see Figure 1).

6. Remove hair, change lab coats/gloves and prepare the skin as per ACS.SOP.818.

7. Administer bupivacaine subcutaneously as per ACS.SOP.814. The needle is inserted at the caudal end of the planned incision and advanced to the cranial end. The plunger is then slowly depressed as the needle is gradually withdrawn, so that the drug is distributed along the entire length of the planned incision.

8. Don surgical PPE and drape the animal with Press'n Seal as per ACS.SOP.818. If performing external skin sutures or staples, cut a window in the drape to expose the planned incision site.

Surgical procedure

Rodent ovariectomies can be performed using a ventral midline or dorsal approach. This SOP describes the dorsal approach, which causes more muscle trauma but is technically easier to perform, as the ovaries are dorsal to other abdominal viscera.

1. Using a #10 scalpel blade, make a dorsal skin incision parallel to the spine from the mid-thoracic curvature to the end of this curvature (usually 1-2 cm, see Figure 2). If performing intradermal sutures, the drape and skin can be incised together.

2. Gently use tissue scissors to bluntly dissect the skin away from the subcutaneous tissue. This is done by inserting the closed scissor underneath the skin perpendicular to the incision and opening the jaw in a 'stretching' motion without cutting (see Figure 3).

    a. Only blunt dissect the minimum amount to allow positioning of the skin incision over the ovarian fat pad. Excessive dissection may result in seroma formation.

3. The ovarian fat pad can be located by visualizing a white patch under the abdominal wall (see Figure 4). In mature rats, the abdominal wall can be too thick to visualize this fat pad.

4. Tent the abdominal wall over the ovarian fat pad (or where the ovarian fat pad is likely to be) with thumb forceps and take a bite of the abdominal wall using absorbable suture. Tenting the abdominal wall ensures the needle does not grab any viscera (see Figures 5 and 6).

5. Now use the hold suture to tent the abdominal wall and make a stab incision through the muscle using a #11 or 15 blade (cutting edge facing up, see Figure 7).

6. If necessary, extend the muscle incision using tissue scissors to allow the ovary to be exteriorized.

7. Place the hemostats in the bead sterilizer to heat up.

8. Gently, while applying slight downward pressure to the body wall, use thumb forceps to lift the ovarian fat pad out of the abdomen (see Figure 8). To avoid tearing the fat, it may be necessary to release the fat pad onto the skin/drape, grasp a more proximal portion, and repeat until the ovary is visible.

9. Only manipulate the ovarian fat pad, not the ovary itself, to avoid ovarian fragments re-entering the abdomen. Ensure no other abdominal tissues have been exteriorized.

10. Retrieve the hemostats and clamp them across the ovarian fat pad proximal to the ovary, ensuring they do not touch skin or muscle (see Figure 9). This clamp should cauterize both ovarian vessels and the proper ligament of the ovary. A sizzling noise is audible if the hemostats are sufficiently hot.

    a. Alternatively, these vessels can be ligated with absorbable suture.

11. Use a scalpel blade to transect the ovarian fat pad proximal to the ovary and remove the ovary and associated fat from the surgical area (see Figure 10).

12. Grasp a portion of the stump of the ovarian fat pad proximal to the hemostats with thumb forceps and gently unclamp the hemostats. The cauterized edge should appear greyish black (see Figure 11). Observe it for bleeding. If any bleeding is observed, a ligature should be placed or the stump re-cauterized more proximally.

13. Continue to hold the stump with thumb forceps and observe it for bleeding as you slowly release the tension, as some bleeding is not evident while blood vessels are taut. Gauze can be dabbed on the cauterized edge to reveal active bleeding.

14. Replace the stump in the abdominal cavity by gently lifting the body wall to engulf it.

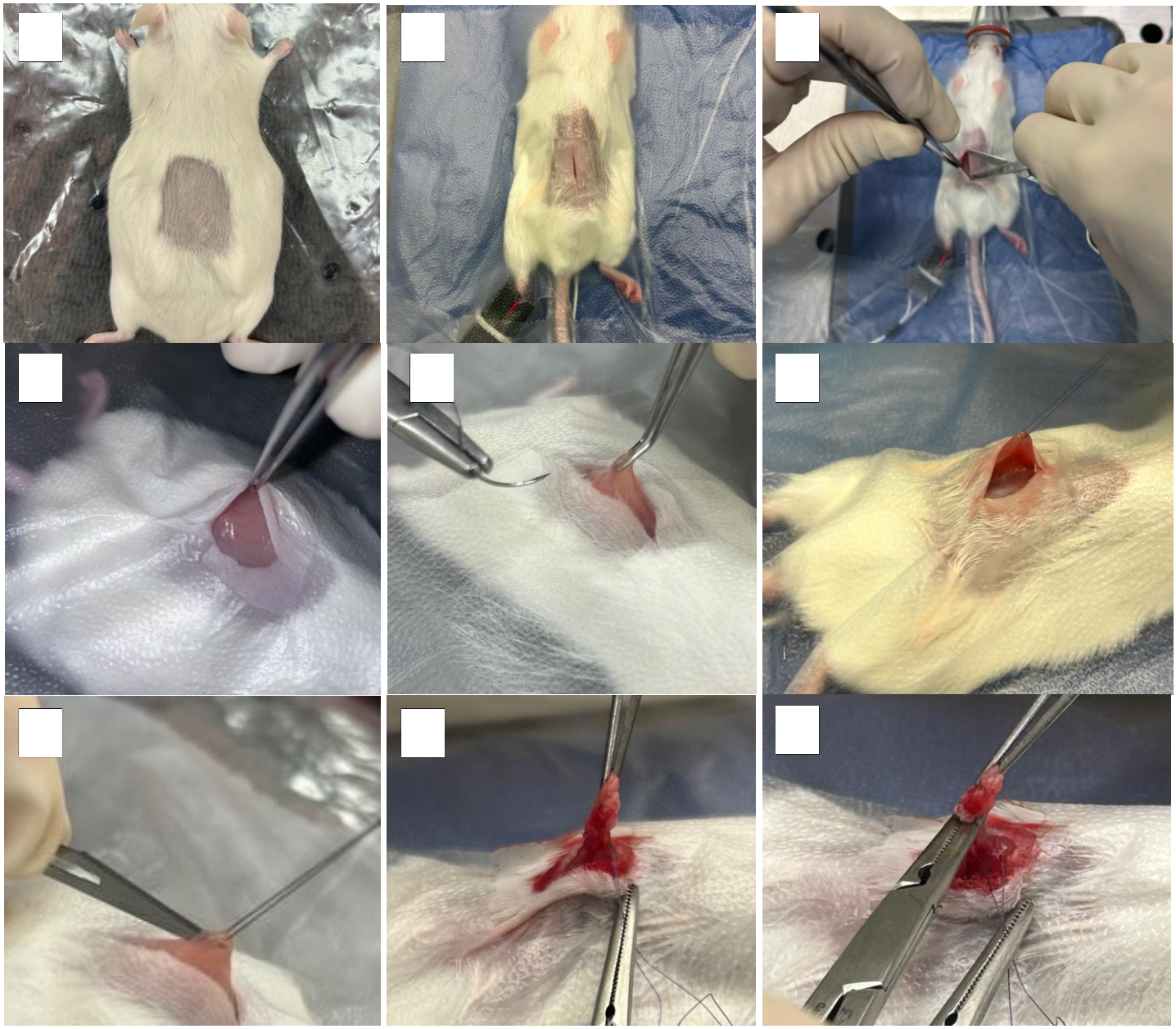
15. Suture the muscle layer with absorbable suture. A single cruciate suture is often appropriate.

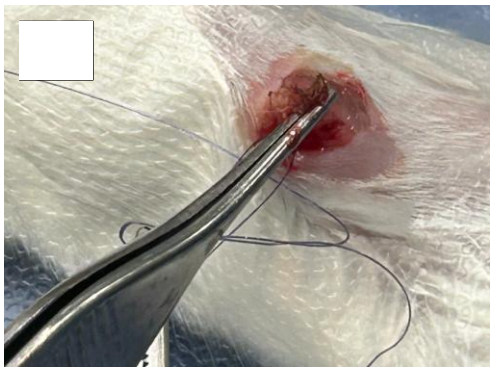
16. Re-position the skin over the spine and repeat steps 2 – 15 for the opposite ovary via the same skin incision.

17. Close the skin using a continuous intradermal pattern (absorbable suture) or interrupted or cruciate external skin sutures (absorbable, non-absorbable, or staples – see Figure 12).

18. Use gauze and sterile saline to wipe the skin clean of blood and skin prep solution.

19. Provide analgesics/fluids and recover and monitor the animal as per ACS.SOP.817 and 814.





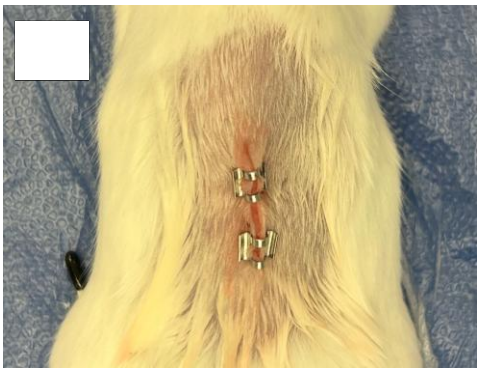


Figure 1. Shaving from the last rib to the hip.
Figure 2. Dorsal skin incision parallel to the spine from the mid-thoracic curvature to the end of this curvature (about 1-2 cm).
Figure 3. Bluntly dissection of the skin away from the subcutaneous tissue using small tissue scissors. Forceps are used to lift (vs. grip) the skin.
Figure 4. Visualization of the ovarian fat pad (a white patch under the abdominal wall).
Figure 5. Tenting the abdominal wall over the ovarian fat pad with thumb forceps to take a bite using absorbable suture.
Figure 6. Using the hold suture to tent the abdominal wall.
Figure 7. Making a stab incision through the muscle using a #11 or 15 blade (cutting edge facing up).
Figure 8. Using thumb forceps to lift the ovarian fat pad from the abdomen (the hold suture can be kept out of the way with hemostats).
Figure 9. Clamping and cauterizing the ovarian pedicle and fat pad with hot hemostats.
Figure 10. Transecting the ovarian pedicle and fat pad with a #10 blade.
Figure 11. Visualizing the cauterized cut edge and slowly releasing the fat pad into the abdomen while observing for hemorrhage.
Figure 12. Closing the skin incision with staples (after removing both ovaries and closing the muscle incisions).

5. References:

Queen's University Animal Care Committee: SOP 7.29 - Dorsal Approach Ovariectomy (Mice). https://www.queensu.ca/animals-in-science/policies-procedures/sop/mice/7-29

Queen's University Animal Care Committee: SOP 10.29 - Dorsal Approach Ovariectomy (Rat). https://www.queensu.ca/animals-in-science/policies-procedures/sop/rats/10-29

Stout Steele, M., & Bennett, R. A. (2011). Clinical Technique: Dorsal Ovariectomy in Rodents. Journal of Exotic Pet Medicine, 20(3), 222–226. https://doi.org/10.1053/j.jepm.2011.04.008