Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the technique for performing an ovariectomy using the dorsal approach in the rat.

1. **Introduction and Definitions:** Ovariectomy (surgical removal of the ovaries) is a common procedure in rodents. The single incision dorsal approach is the least invasive method used.

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI**, subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP,** intramuscular **IM**, per os **PO,** per rectum **PR**

2. **Materials:**
   - Anesthetics (refer to SOP 10.6)
   - Gas anesthesia machine (calibrated within the last 12 months and with active or passive scavenging)
   - Transparent induction chamber
   - Nose cone
   - Analgesics (refer to SOP 10.1)
   - PPE (cap, gloves, mask, clean lab coat or surgical gown)
   - Standard surgical gloves
   - Clippers
   - Scale
   - Eye lubricant and sterile cotton tip applicator
   - Heat lamp
   - Lactated Ringers solution (fluid therapy)
   - Surgical scrub (4% chlorhexidine scrub, 70% isopropyl alcohol and iodine solution)
   - Alcohol (for cleaning surgical equipment/gloves between surgeries)
   - Bead sterilizer
   - External heat source for maintaining body temperature
   - Sterile surgical kit

- Suture material

3. Procedures:

Preparation for Surgery:

- Blood collection performed prior to the surgery for measuring hormone concentration, if required as per protocol.
- Analgesics may be given preoperatively as per protocol. Refer to SOP 10.1 "Pain Management (Rat)"
- Anesthetize and prep rat as per SOP 10.3 "Aseptic Surgical Techniques (Rat)"
- Place the rat on the prep area in sternal recumbency.
- Shave an area on the rat bounded dorsally by the spine, laterally from the last rib to the hindleg and ventrally to the sides of the rat. Shave both sides of the rat (Figure 1).

Surgical Procedure using a Single Dorsal Incision:

- Place a sterile drape over the rat with the opening situated over the surgery site.
- A dorsal skin incision is made parallel and lateral to the spine from the mid-thoracic curvature to end of curvature (Figure 2). The skin incision can then be shifted to either side of the abdomen until a white patch becomes visible in the area under the skin. The white patch corresponds to the fat pad that surrounds the ovary (Figure 3). This fat pad will be below the kidney.
- Continue blunt dissection with mosquito hemostats to tunnel through the subcutaneous tissue and muscle to find the site to enter the abdominal cavity (Figure 4).
- It is recommended to place a hold suture in the muscle wall when you cut through, this will make it easier to close after you remove the ovary.
- Gently, using forceps and slight pressure, lift the fat pad outwards to exteriorize the ovary (Figure 5). Use mosquito hemostats to crush the oviduct and cranial part of the uterine horn distal to the ovary (Figure 6). Only grasp the fat pad. If you clamp the ovary, you could possibly fragment it resulting in ovarian fragments.
- The ovary is removed by cutting or applying pressure between the ovary and cranial edge of the hemostats ensuring that all the ovarian tissue is removed (Figure 7 and Figure 8).

- The hemostats should be left clamped for 15-20 seconds to minimize hemorrhage. Gently holding the fat pad, release the hemostats and check for bleeding. If bleeding persists, apply pressure again for an additional 15-20 seconds or apply a ligature of absorbable suture (6-0). Return the uterine horn to the abdomen.
- The muscle can be closed with 6-0 absorbable interrupted suture(s) (Figure 9).
- Displace the skin to the opposite side of the abdomen and repeat the procedure as outlined above.
- Close the skin incision using sutures in a simple interrupted pattern or staples.
- Remove the drape and gently clean the surgery site as needed.
- Follow postoperative care and monitoring as per SOP 10.4 "Rodent Post-Operative Care (Rat)"
- Rats need to be left for approximately 3 weeks to see physiological changes.
  a. Perform vaginal swabs 2-3 weeks after surgery. Should not see nucleated or cornified epithelial cells. Should see immune cells and inflammatory cells.
  b. Perform blood test to confirm low hormone levels.

Notes:
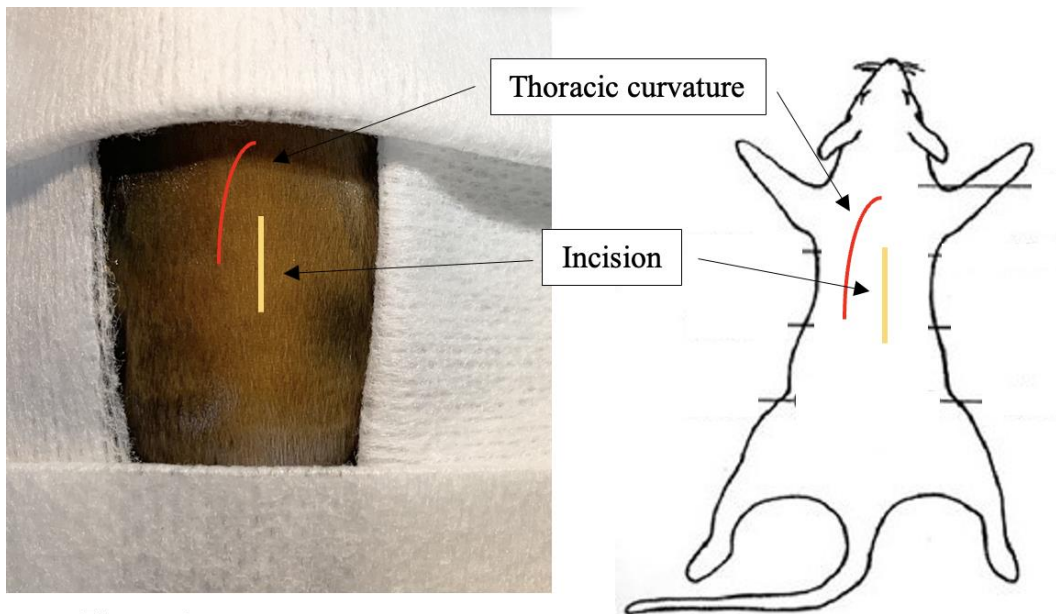*Do not use older or obese female rats as fat contains estrogen.*

Figure 1.
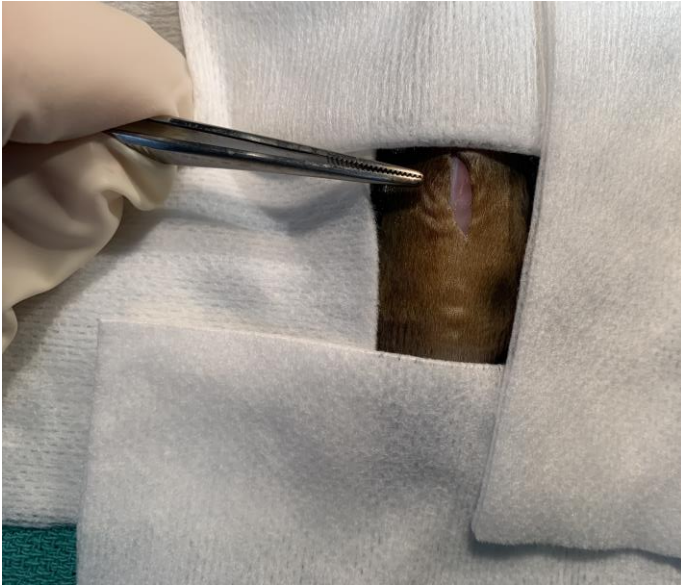
Figure 2 – Skin Incision

Figure 3 – Shifting skin to lateral aspect of the thorax to find white patch

Figure 4 – Incision through muscle wall to expose fat pad, single suture on muscle wall

Figure 5 – Fat pad gently lifted outwards to exteriorize the ovary

Figure 6 – Mosquito hemostats to crush the oviduct and cranial part of the uterine horn distal to the ovary

Figure 7 – Removal of the ovary

Figure 8 – Ovaries removed

Figure 9 – Muscle wall sutured

References:

SOP Revision History:

Date	New Version