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H. Ovary Transplant (note, these sops are from Jax lab and need to be modified to meet ORNL requirements)

Procedure description:

(Include exact details of any/all chemical, biological, radiation, or physical agents as well as route(s)/dose(s)/volume(s)/frequency and duration)

1. Method of sterilization of instruments:

- a. Prior to surgery: Steam sterilization 250EF for 15 minutes or 270EF for 5 minutes
- b. Between animals: Wipe with sterile swab & 70% alcohol.
- c. Number animals/pack: 4 to 5 Or, wipe with sterile swab and saline and place tips in glass bead sterilizer.

A pack of instruments is prepared for the donor and a second pack of instruments is prepared for the recipient.

2. Procedure for removal of fur and preparation of skin:

DONOR: The donor mouse is euthanatized by carbon dioxide or cervical dislocation. The carcass is soaked with disinfectant (70% alcohol or quaternary ammonium) and placed on a paper towel. In the rare event that the donor mouse is not to be euthanatized, then procedures for survival surgery and ovariectomy are followed.

RECIPIENT: Fur on the back is plucked or clipped from an area bounded cranial-caudal by the thoraco lumbar junction and iliac crests and dorsal-ventral by lateral edges of the lumbar muscles. Loose hair is picked up with an adhesive tape. Skin is disinfected with 70% alcohol using a sterile swab. Application of 70% alcohol starts in the center of the clipped area and works outward in ever widening circles. The swab is discarded after it is used to spread fur away from the clipped area. Three applications of alcohol are made using a new sterile swab each time. Alternatively, two applications of 70% alcohol and one application of surgical iodine may be used.

3. Describe surgical procedure include incision site, manipulation to be done, skin closure:

DONOR: Ovaries are excised from the euthanatized donor mouse using sterile instruments and aseptic technique. Either of two alternative approaches may be used to excise ovaries from the donor.

RECIPIENT: The female is anesthetized using Isoflurane with a controlled precision vaporizer. Induction is performed in a chamber and maintained using a hose cone.

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Technique 1: The donor mouse is placed in dorsal recumbent position. The skin over the abdomen is pulled apart in a medial-lateral direction with fingers. One piece of skin is pulled cranially and the other piece of skin is pulled caudally to uncover the abdominal wall. Alternatively, sterile scissors are used to make an incision that starts at the pubic symphysis, turns laterally toward the left side of the mouse and ends near the xiphoid cartilage. Then the skin is bluntly dissected away from the abdominal wall. The long flap of skin is folded toward the right side of the mouse.

A second pair of sterile scissors and forceps are used to incise the abdominal wall from pubic tendon toward the xiphoid cartilage. One uterine horn is elevated out of the incision with another pair of sterile and the uterine horn is followed cranially until the ovary is exteriorized. The ovary and part of the fat pad are torn free of mesenteric attachments and placed in a dish of cold sterile PBS or tissue culture media. The second ovary is located, excised in the same manner and placed in the same dish of cold sterile PBS or tissue culture media. The dish and ovaries are placed under a dissecting microscope. Each ovary is dissected free of ovarian bursa and fat pad using sterile Dumont forceps and fine pointed scissors. Normal size ovaries are cut in half.

Technique 2: The donor mouse is placed in ventral recumbent position. The skin over the lumbar region is pulled apart in a medial-lateral direction with fingers and then one piece of skin is pulled cranially and the other piece of skin is pulled caudally to uncover the lumbar muscles and abdominal wall. Alternatively, sterile scissors are used to make an incision perpendicular to the vertebral column crest approximately midway between the iliac crest and the last rib. Then, the skin incision is rotated laterally beyond the ventral edge of the lumbar muscles to expose muscles of the abdominal wall.

A 3-6 mm incision is made in the abdominal wall. The incision in the abdominal wall is made between segmental blood vessels approximately 4-5 mm caudal to the last rib. A sterile pair of forceps and scissors are used to incise the left abdominal wall approximately midway between last rib and an imaginary line perpendicular to iliac crest. A second pair of sterile forceps is used to grasp and exteriorize the left ovarian fat pad. The ovary and part of the fat pad are torn free of mesenteric attachments and placed in a dish of cold sterile PBS or tissue culture media. The second ovary is located excised in the same manner and placed in the same dish of cold sterile PBS or media. The dish and ovaries are placed under a dissecting microscope. Each ovary is dissected free of ovarian bursa and fat pad using Dumont forceps and fine pointed scissors. Normal size ovaries are cut in half.

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After both ovaries are excised, the body of the donor is wrapped in paper towel and discarded. The table is disinfected with quaternary ammonium disinfectant or 70% alcohol and prepared for the recipient.

RECIPIENT: The recipient mouse is placed in ventral tending toward right lateral recumbent position. A right-handed surgeon should have the mouse's head pointed toward the surgeon's left hand and the mouse's body is parallel to the surgeon's body. A left-handed surgeon should have the mouse's pointed toward the surgeon's right hand.

A 0.5-1 cm skin incision is made perpendicular to the vertebral column crest approximately midway between the iliac crest and the last rib using sterile scissors and forceps. These instruments are used only on skin. The skin incision is rotated laterally beyond the ventral edge of the lumbar muscles to expose muscles to expose muscles of the left abdominal wall. A 3-6 mm incision is made in the left abdominal wall using a second pair of scissors and forceps. These instruments are used only on the abdominal wall. The incision in the abdominal wall is made between segmental blood vessels approximately 4-5 mm caudal to the last rib. It is parallel to the rib extending from the ventral edge of the lumbar muscles toward the ventrum.

A third pair of sterile forceps is used to grasp and exteriorize the left ovarian fat pad. This pair of forceps is used only to manipulate the fat pad. The fat pad is positioned on a 2x2 Versalon sponge or similar sterile surface so that the ovary is facing the surgeon and the oviduct is toward the surgeon's left hand. If desired, the ovarian fat pad can be held in position using a Schwartz temporary clip or baby Diffenbach serrefine. The ovarian capsule is incised at the junction of capsule and fat pad using a Vannas, DeWecker or Noyes ultra micro scissors. The incision in the ovarian capsule starts to the right of the oviduct at approximately 6 o'clock and extends cranially through 3 o'clock and ends near 12 o'clock. Dumont forceps are used to slide the capsule off of the ovary and place the capsule on top of the oviduct. One pair of Dumont forceps is placed under the ovary and used to clamp the ovarian blood vessels. A second pair of Dumont forceps is placed on top of the first pair and used to shear the ovary off of the ovarian blood vessels. The first pair of Dumont forceps remain clamped on the ovarian blood vessels to prevent bleeding. The excised recipient ovary is discarded on a sterile surface using the second pair of forceps and that pair of forceps is used to pick up a piece of donor ovary. If bleeding is observed from ovarian vessels, the Dumont forceps is removed and hemorrhage is controlled using direct pressure and a Surgical Spear or a fine tipped cotton wrapped toothpick. If bleeding is not observed, the piece of donor ovary is placed on top of the ovarian blood vessels as the first Dumont forceps is loosened and removed. The ovarian capsule is located near the oviduct, retracted over the donor ovary, and tucked back to its original position using one or both pairs of Dumont forceps. If bleeding is observed from

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ovarian vessels after the donor ovary is in place, it may be controlled by direct pressure on the ovarian capsule. Alternatively, it may be necessary to retract the capsule and temporarily remove the donor ovary in order to apply direct pressure and control hemorrhage. Dumont forceps must be banded carefully to avoid tearing the ovarian capsule or accidentally damaging the oviduct.

If used, the temporary clip is removed from the fat pad. The fat pad is returned to the abdominal cavity using the third pair of forceps. The "abdominal wall" forceps are used to approximate the edges of the incision in the abdominal wall. This incision is sutured with 4-0 or 5-0 absorbable suture with swaged on needle.

The mouse is rotated slightly to a ventral tending toward left lateral position. The skin incision is rotated dorsally to its original position and then rotated laterally beyond the ventral edge of the lumbar muscles to expose muscles of the right abdominal wall. A 3-6 mm incision is made in the right abdominal wall using a second pair of "abdominal wall" scissors and forceps. The right ovarian fat pad is exteriorized. The usual procedure is not to transplant a piece of donor ovary to the right ovarian bursa because of right ovary has a shorter pedicel and is more difficult to position. The right ovary is excised using one Dumont forceps to crush ovarian blood vessels as enter the base of the fat pad near the oviduct and a second Dumont forceps to shear off the ovarian capsule, ovary and piece of the oviduct. Alternatively, a transplant can be made to the right ovarian bursa using the technique described above.

The fat pad is returned to the abdominal cavity using the third pair of forceps. The "abdominal wall" forceps are used to approximate the edges of the incision in the abdominal wall. This incision is sutured with 4-0 or 5-0 absorbable suture with swaged on needle. The skin incision is rotated dorsally to its original location and closed with sutures or wound clip. Tissue adhesive may be used in combination with sutures for skin closure.

Complications: If the incision in the capsule is made in the middle of the capsule or the capsule is accidentally torn, the remaining bursa may not be large enough to retain the transplanted ovary. Also, the probability of scar tissue and adhesions from capsule to ovary is increased. If the incision made in the fat pad instead of the capsule/fat pad junction, there is increased risk of hemorrhage and the capsule cannot be entered to expose the ovary. If hemorrhage from ovarian blood vessels is not controlled, blood can push the transplanted ovary out of the capsule into the abdominal cavity. If the recipient's ovaries are not completely excised, they can regenerate and become functional. If the oviduct accidentally pinched with the Dumont forceps, an unwanted

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"tubal ligation? may occur. If the incision in the abdominal wall is not sutured, the ovary and oviduct or other viscera can herniate through the incision.

4. Anesthetic regimen:

a. Name of anesthetic: Tribromoethanol Diluent used: Sterile PBS or saline

Dosage of anesthetic: 250 mg/kg body weight

Route of administration: IP

Volume of anesthetic 0.2 ml/10g body weight

b. Supportive care while animal recovers from anesthesia: After surgery, the mouse is placed on a clean paper towel in ventral or lateral recumbent position in its cage. A 50-75 watt light bulb is placed over one end of the cage to provide supplemental warmth until the mouse recovers from anesthesia. Alternatively, the mouse may be placed on a water blanket for supplemental warmth.

5. Describe post-operative care: Include frequency of observations, schedule for removal of sutures/clips, and criteria to monitor well being of animals:

The mouse is observed daily for the first three or four days after surgery to assure that the incision is healing properly, and the mouse is eating, drinking and behaving normally. Wound clips or nonabsorbable skin sutures are removed between the five and seven days after surgery or the incision has healed normally.