OVARIAN TRANSPLANT (Need dissecting scope)

Use FVB/N x black or agouti F1 hybrids as recipients. Not only are hybrids better mothers that inbred strain but also this allows to identify the FVB/N-derived progeny (i.e., white vs. black or agouti). You will still need to genotype later on to rule out ovulation from the recipient's ovarian residual tissue. Recipients should be about 6 weeks old at the time of transplant.

Preparation of the donor mouse:

Survival ovariectomy under isoflurane is possible. The procedure described below is for euthanized donors. After euthanasia with CO2 or isoflurane overdose, shave the back of the mouse using a #40 clipper blade. Alternatively, use Nair hair removing cream by applying to area and wiping off after 5 seconds. Prep the shaved skin with 3 alternating antiseptic scrubs of Betadine or Chlorhexidine, followed by 70% ethanol, preferably in a different room than that where the surgery will be performed. Drape the area with sterile drapes to prevent hair from entering the surgical field. Using fine sterile scissors, make a small paralumbar incision in the lateral dorsal flanks under the kidneys. The ovaries are found just under the peritoneal wall. Make a small incision in the peritoneal wall over the ovarian fat pad. Grasp the ovarian fat pad with blunt forceps, and using two pairs of a 45¡ tipped forceps (#5-45 Dumont; FST No. 11251-35) open the ovarian bursa surrounding the ovary to expose the ovary. In survival procedures, use a drop of lidocaine w/ epinephrine to reduce bleeding. Keep the site perfused with warm saline to maintain a clear visual field. Using fine Vannas spring scissors (FST No. 15100-09), remove the ovary from the ovarian bursa and place it in a sterile 35-mm dish containing warm sterile PBS. The donor ovaries may be split in half with fine scissors in order to implant into 4 recipient mice.

Recipient mouse:

Proceed exactly as described for the donor mouse up to the ovary removal step except that you do not euthanize the recipient! Do this procedure under general anesthesia with isoflurane instead.

After removing the recipient’s own ovary as described above, introduce the ovary or hemiovary from the donor animal in the recipient’s ovarian bursa using Dumont 5-45 forceps. Pull the bursa over to cover the transplanted tissues. Using forceps, tent up the abdominal incision and allow the ovarian fat pad and uterine horn to return to the abdominal cavity. Close the peritoneal incision with a single suture (5-0 Vicryl) and close the skin incision with one or two wound clips. Repeat the procedure on the opposite side (second skin and abdominal incisions). While the female is still anesthetized, inject 0.3 ml of saline IP to maintain good hydration. Place the mouse in a clean, unbedded cage and keep warm during recovery. Observe mice continuously until recovery from anesthesia and observed at least once a day in the week after surgery. Remove wound clips 7-10 days after surgery. Recipients may be mated 2 weeks after surgery. Expected pregnancy rate: 75%

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