Oviduct Transfer Protocol

Purpose: To transfer mouse 2-8 cell embryos into 0.5 day pseudopregnant recipient females for any of several procedures; embryo transfer rederivation, subsequent to in vitro fertilization, and following pronuclear injections and cryopreservation recovery.

1) Pipette three microdrops (~50 µl) of FHM or M2 media on the bottom of a 30 mm petri dish.
2) Cover the media with embryo-tested mineral oil and leave at room temperature.
3) After weighing a recipient female, inject the calculated dose of Avertin intraperitoneally.
4) The caudal dorsal area of the anesthetized female is shaved, then scrubbed using povidone iodine solution or scrub alternately with 70% alcohol on cotton tipped swabs (repeat two times).
5) Place the prepped pseudopregnant female under the dissecting microscope, ventral side down.
6) Surgical instruments should be either autoclaved or bead-sterilized. Using a small scissors and watchmaker forceps, make a 5 mm skin incision. Separate the skin from the body wall by blunt dissection using scissors tips. Make an incision through the body wall avoiding nerves and large blood vessels. Manipulate the incision until the white fat pad surrounding the ovaries is visible. Grasp the fat pad with a dull forceps and pull it through the incision.
7) Position the ovary for easy access to the oviduct.
8) Pipette enough embryos for one recipient into a microdrop of FHM.
9) Load a transfer pipette (the tip diameter should be only slightly larger than the embryos) with oil from transfer dish by using mouth suction. Oil level should be close to the beginning of the widest part of the pipette. Next, draw a 2-5 mm air bubble into the pipette, then media with embryos. Carefully press pipette barrel into the clay on the dissecting microscope until ready to transfer the embryos.
10) Gently tear open the bursa surrounding the oviduct with two pair of forceps allowing access to the infundibulum, which is the opening to the oviduct. In case of bleeding, use a sterile cotton swab to gently blot.
11) Once the infundibulum can be visualized, slide the tip of the loaded pipette in and blow into the pipette until the air bubble is visible within the oviduct.
12) Check the pipette tip underneath the microscope to make sure the embryos have been transferred.
13) Gently replace the ovary/fat pad and uterus into the abdominal cavity.
14) Close the body wall with one or two simple interrupted sutures of 5.0 silk. The skin is apposed with one or two sterile surgical clips.
15) The transfer can be done either uni- or bi-laterally. The pups are expected on day 20 following surgery.