Embryo Transfer - Oviduct

1.0 Equipment

1.1 Dissecting microscope

1.2 IVC recovery rack

1.3 Anaesthetic equipment

1.4 Cold light source

1.5 Angle poised lamp

1.6 Heat pad

1.7 Red heat pads

1.8 Top pan balance

1.9 Needle Block

1.10 Sterile Kidney Dishes

1.11 Safety Glasses

1.12 Cling Film Dispenser

1.13 EZ grip handheld pipette

1.14 Plain Cloth Reusable Drapes

1.15 Sterile surgical kit

1.16 Nose cones for anaesthetic mask

2.0 Supplies

2.1 Pseudopregnant recipient mice

2.2 Mouse embryos

2.3 Sterile Disposable Scalpel

2.4 1ml syringes

2.5 25 gauge, 5/8 inch needles

2.6 135uM/170uM EZ grip pipette tips

2.7 Water repellent sheet

2.8 Basic dressing pack

2.9 Sterile Non Woven Swabs

2.10 Ethicon vicryl suture

2.11 Cling Film

2.12 Aldasorber

2.13 Sharps bin

2.14 Anaesthetic: Isoflurane

2.15 Analgesic: Vetergesic

2.16 Analgesic: Torbugesic

2.17 M2 medium

2.18 Culture Dish 35:3004

2.19 P1000 Gilson pipette

2.20 100-1000µl pipette tips

2.21 10% Hibiscrub

2.22 5% Distel wipes

2.23 Chemgene wipes

2.24 2% Trigene

2.25 70% Alcohol

2.26 Tissues

2.27 Sterile surgical gloves

2.28 FFP2 Masks

2.29 Gloves

2.30 Purified water

2.31 Plastic bags

2.32 Blue Roll

2.33 Hyaluronidase

2.34 0.3% Hypromellose Eye Drops/gel

2.35 Sterets

2.36 Wound Glue

2.37 Sterile cohesive Bandage

2.38 Autoclaved foil

3.0 Procedure

3.1 General Information

3.1.1 Use an assistant, and a separate preparation area to the surgical area, where possible.

3.1.2 For 2-cell embryos, transfer into 0.5d.p.c. recipients.

3.1.3 For 4-cells/8-cells/Morula embryos transfer into 0.5d.p.c. or 1.5d.p.c. recipients.

3.1.4 Morula/Blastocyts can be transferred into 0.5d.p.c., 1.5d.p.c. or 2.5d.p.c. recipients.

3.1.5 Embryos should be treated with hyaluronidase prior to transfer if cumulus cells are still attached.

3.1.6 The number of embryos transferred depends on whether the transfer is unilateral or bilateral. The number of embryos for unilateral transfers can be between 9 and 15. The number of embryos for bilateral transfers can be between 18 and 25.

3.1.7 Mice should be recovered post surgery in an appropriate heated recovery rack.

3.1.8 A new surgical kit, scalpel, embryo transfer pipettes and surgical gloves should be used per recipient female. The same suture can be used for up to 5 females. New needles should be used for each mouse.

3.1.9 The nose cones for the anaesthetic masks should be replaced every cage.

3.1.10 After surgery, the work station should be cleaned the same way as prior to surgery, paying particular attention to areas that would have been touched during surgery.

3.1.11 EZ grip tips should be shortened by cutting off 4cm off the wide end with a scalpel and sterilised by Ethylene Oxide prior to use.

3.2 Preparing the Work Areas

3.2.1 Ensure the anaesthetic rigs and equipment have been set up correctly.

3.2.2 A separate work area should be assigned for both the animal preparation area and the surgical area where possible. Both areas require a heat pad, vaporiser, anaesthetic chamber and surgical stage with mask.

3.2.3 Weigh aldasorbers and replace if over the maximum weight.

3.2.4 Wearing gloves, mask and safety glasses, using the key fill adaptor, top up the isoflurane levels to the fill line.

3.2.5 Wearing gloves, clean entire work area with disinfectant wipes, with the exception of the vaporiser. The vaporiser, dials and connectors on the anaesthetic rig should be wiped using 2% Trigene sprayed sparingly onto blue roll.

3.2.6 Wipe entire area and all equipment with 70% alcohol with the exception of the anaesthetic chamber and

surgical stages. These should be wiped with purified water.

3.3 Animal Preparation Area

3.3.1 Open a pack of autoclaved swabs and place pack next to the preparation stage. The swabs should be left in the packaging.

3.3.2 Prepare the analgesia by pipetting equal amounts of Vetergesic and Torbugesic into an Eppendorf. Use this combined Vetergesic & Torbugesic solution to load into a 1ml syringe to administer 0.2ml per mouse.

3.3.3 Change the needle for every mouse. The needles should be re- sheathed using a designated block. Syringes and analgesia should be laid flat on the bench between transfers.

3.3.4 Place a bottle of 10% Hibiscrub, suitable eye lubricant and a shaver next to the preparation stage. Ear clippers may be needed for identification purposes if the mice are not already clipped (Picture 1).

Picture 1

3.3.5 Have one steret per transfer on the bench near the surgical area but do NOT put it on the drape.

3.4 Preparation of the surgical area

3.4.1 Place the cling film dispenser to the left hand side of the surgical stage, with the microscope on the other side. Put a sterile surgical drape to the left of the cling film dispenser or on a suitable disinfected surface (Picture 2).

Picture 2

3.4.2 Open out the following onto the drape (Picture 3);

3.4.2.1 an autoclaved surgical kit

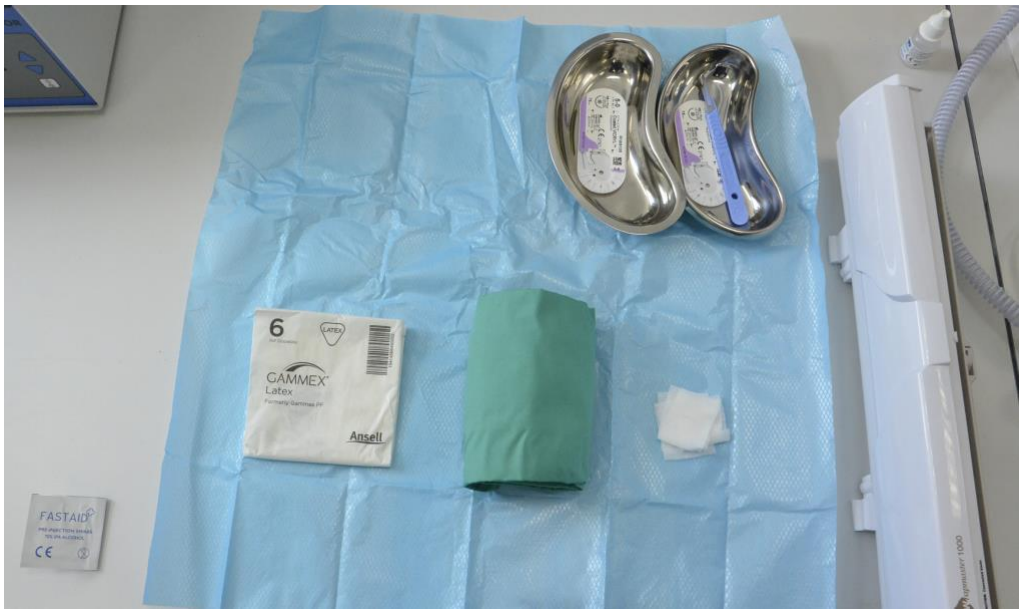
3.4.2.2 kidney dishes – at least x2, place x1 suture material in each, more dishes can be used if required

3.4.2.3 autoclaved swabs

3.4.2.4 sterile surgical gloves- open external packaging and drop contents onto drape

3.4.2.5 scalpel- open into one of the kidney dishes (the suture material in this dish should be designated for the body wall)

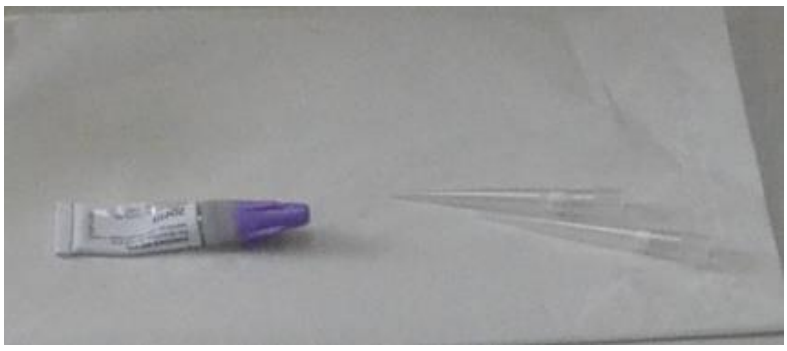
Picture 3



3.4.3 Open up a second sterile surgical drape and place it to the right of the microscope. This is for the embryo handling device(s) once they have been loaded with embryos (Picture 2).

3.4.4 Place the wound glue with any tips to be used onto a tissue near the drape. Do not place directly onto the drape (Picture 4).

Picture 4



3.4.5 Turn on heat pads for both the animal preparation and surgical work areas.

3.5 Loading transfer pipette with embryos

3.5.1 Treat the embryos with Hyaluronidase if required.

3.5.2 Wash embryos through two drops of M2 prior to transfer.

3.5.3 If using an EZ grip pipette:

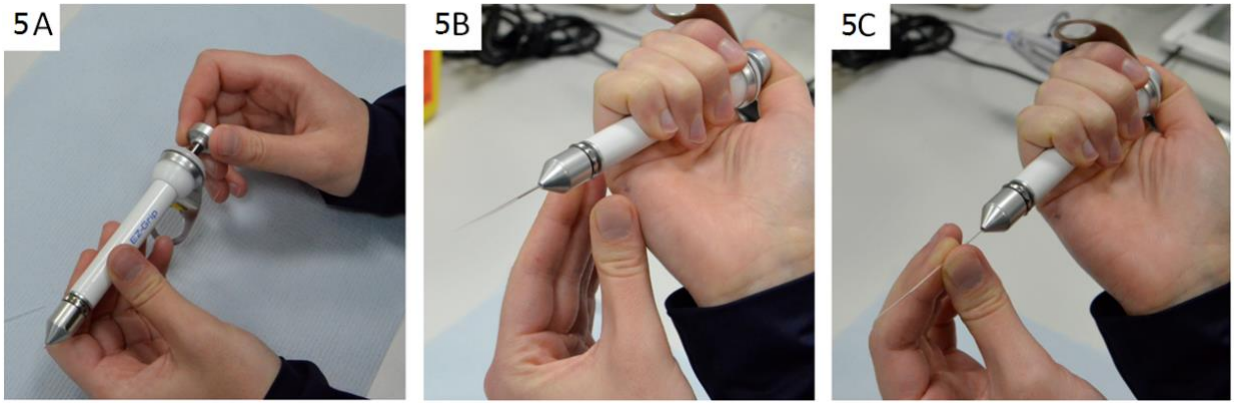
3.5.3.1 Set the pipette to 0.5µl for 135µl tips or 1µl for 170µl tips by turning the dial at the top (Picture 5A). Make sure the pipette tip size indicator dial is on the correct size for the tip being used.

3.5.3.2 Press down the plunger to the first stop and push a transfer tip onto the end until it cannot be pushed in any further (Picture 5B).

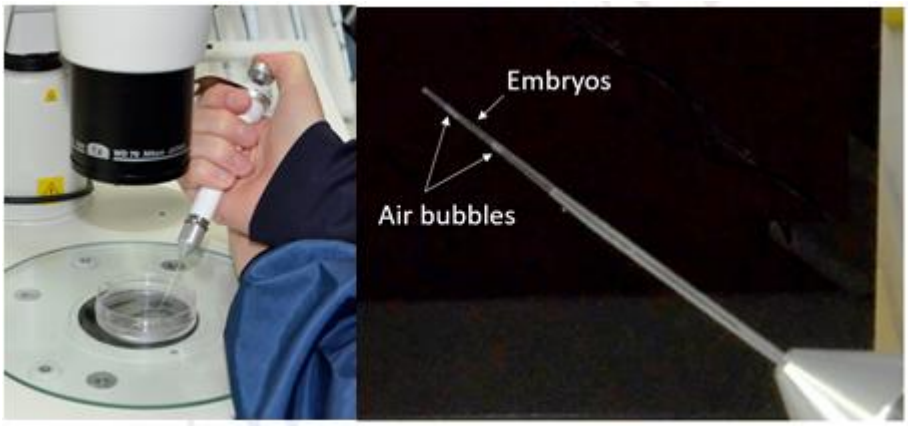
3.5.3.3 Draw up the plunger and check the the tip is securely in place (Picture 5C).

3.5.3.4 Press the plunger down to the first stop and aspirate up the full volume of media into the tip (Picture 6).

Picture 5



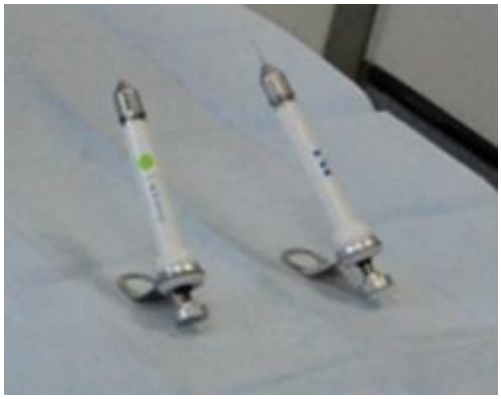
Picture 6



3.5.3.5 Expel approx. half the media and load a single bubble by drawing up air, then aspirate the embryos followed by a second air bubble and finish by drawing up M2 until the plunger reaches the top (Picture 6). The embryos should sit around the middle of the total volume held within the pipette.

3.5.4 Gently set aside the pipette loaded with embryos on the second sterile drape next to the microscope and repeat for second pipette if bilateral transfer is to be performed (Picture 7).

Picture 7



3.6 Preparation of Mouse for Surgery

3.6.1 For EZ grip transfers both surgeon and assistant should wear an FFP2 mask and gloves for the whole process.

3.6.2 Anaesthetise the mouse. Do not use any animals showing signs of stress.

3.6.3 Once anaesthetised, turn down the oxygen and isoflurane as required and insert the mask connector pipe into the vapouriser, and eject the chamber connector pipe.

3.6.4 Remove mouse from the chamber and lay the mouse on her front, with her nose in the nose cone mask, on the surgical stage of the animal preparation area.

3.6.5 To confirm the mouse is fully anaesthetised, check for a pedal reflex. Do not commence surgery until there is a lack of reflex response to this test.

3.6.6 Administer one drop of suitable lubrication to the eye, to prevent drying from the flow of oxygen/isoflurane, such as eye drops or eye gel.

3.6.7 Administer 0.2ml of the combined Vetergesic and Torbugesic solution, via subcutaneous injection.

3.6.8 Shave a patch of skin on the mouse's back for the incision site. Apply 10% Hibiscrub to a sterile swab and swab the shaved area by wiping from the head end to the tail end of the shaved patch. Repeat this action for a second time with a clean sterile swab. (Picture 8).

Picture 8

3.6.9 At this point, the mouse should be moved over to the surgical area. Turn on the oxygen and isoflurane on the surgical area vaporiser.

3.6.10 Turn off the isoflurane and oxygen on the vaporiser in the animal preparation area.

3.6.11 Ensuring the body of the mouse is supported and always holding the tail of the mouse, quickly and gently, move the mouse from the animal preparation area to the surgical area. Lay the mouse on her front, with her nose in the nose cone mask.

3.6.12 Drape the mouse and the surgical stage with a large piece of cling film. Ensure the piece is large enough to cover the handles attached to the surgical stage (Picture 9).

3.6.13 Place a Steret on top of the drape by tearing the packaging and letting the Steret fall onto the drape. The mouse is now ready for surgery.

Picture 9

3.7 Surgery

3.7.1 The surgeon should have donned a clean pair of sterile surgical gloves in the correct manner to maintain asepsis.

3.7.2 Open up the surgical kit already on the sterile drape, trim both the sutures to the required length and place the suture needle in the needle holder, then rest in kidney dish with the scalpel.

3.7.3 Hold the cling film drape up and away from the mouse above the destined incision site. Use a large pair of

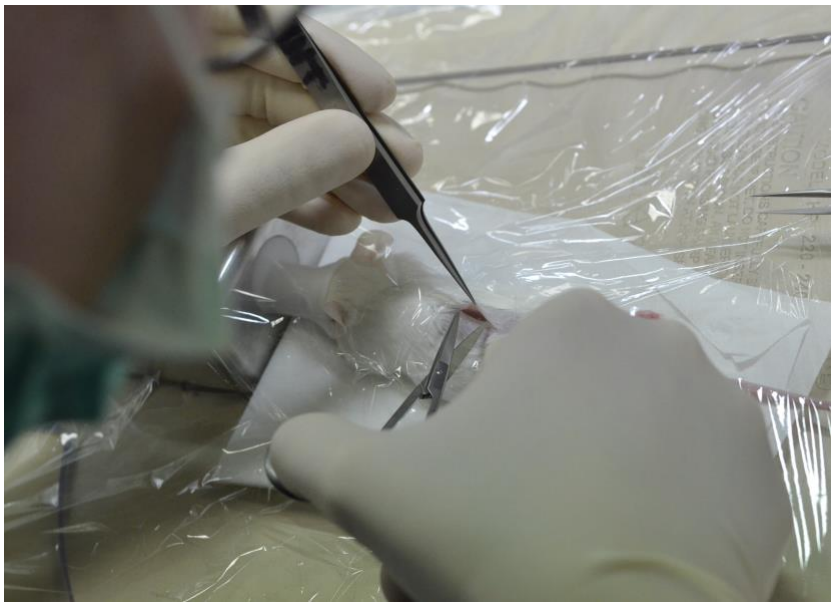
scissors to carefully cut a hole in the drape, then place these scissors to one side. Ensure the size of this hole is not larger than the shaved area on the mouse.

3.7.4 Using a sterile scalpel, make an approx. 1cm incision along the midline, between the natural curve of the back and the top of the hips (Picture 10). Discard the scalpel into a sharps bin.

Picture 10

3.7.5 Using a pair of watchmaker's forceps and fine scissors, carefully introduce the closed blades of the scissors between the skin and the body wall, at a depth of approximately 1cm. Open the blades to clear the connective tissue on the first side. Repeat as necessary to clear the connective tissue (See picture 11).

Picture 11



3.7.6 Move the skin around until the red ovary and the white ovarian fat pad can be seen under the body wall.

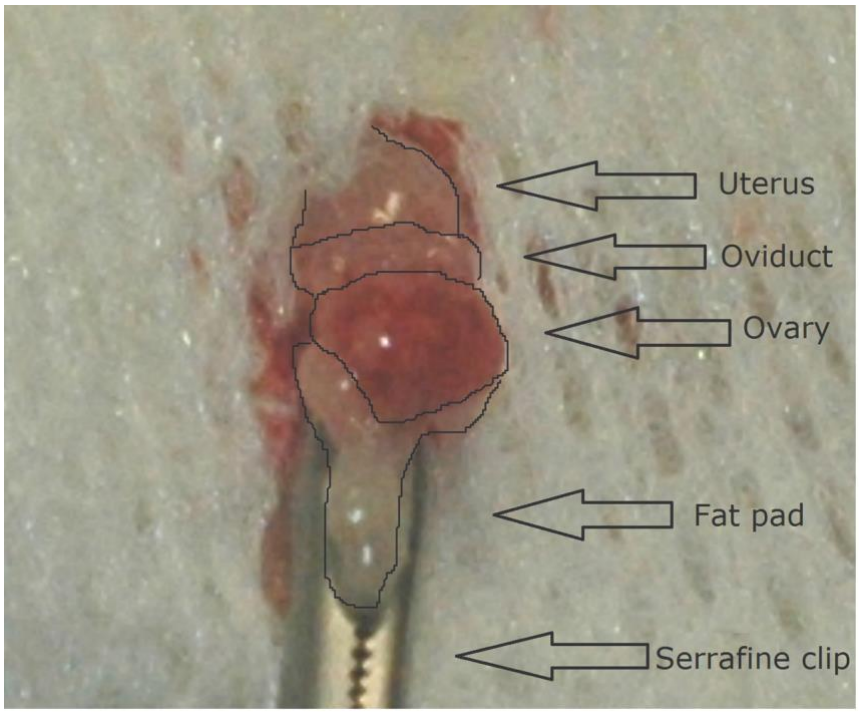
3.7.7 Using a pair of watchmakers forceps, pick up the body wall and use the fine scissors to make a small incision, approx. 5mm across, just above the ovary.

3.7.8 Hold open the incision in the body wall with one pair of watchmaker's forceps and use a second pair to reach into the abdominal cavity and grasp the ovarian fat pad.

3.7.9 Withdraw the ovarian fat pad, ovary, oviduct and some of the uterus and pull them through the hole in a pre-cut sterile swab.

3.7.10 Anchor the fat pad with a serrafine clip (Picture 12).

Picture 12



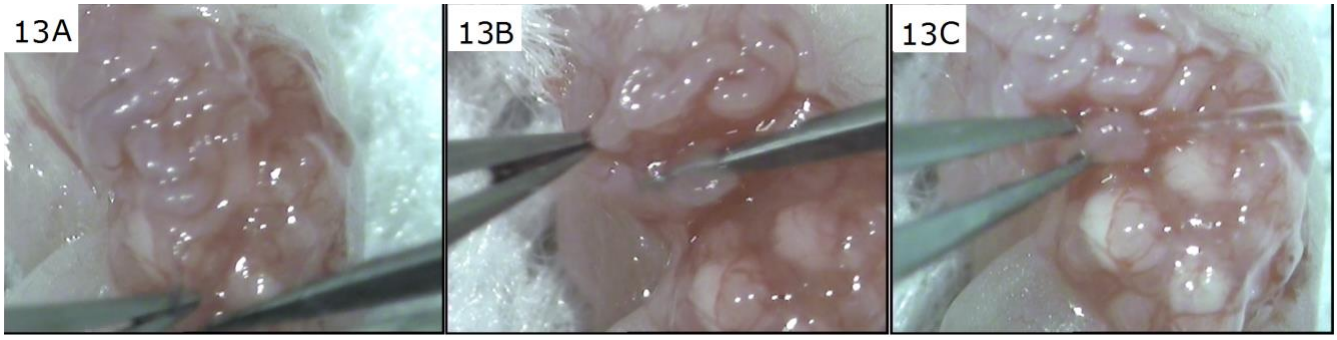
3.7.11 Using the handles of the surgical stage that are covered by the sterile drape, move the stage onto the dissecting microscope.

3.7.12 If any equipment needs adjusting at this stage, use an appropriate sterile implement i.e; swab, foil or bandage, to handle and maintain sterility.

3.7.13 In a 0.5d.p.c. recipient female, the swollen ampulla should be visible, and the lack of this is taken as a sign the animal is not psudopregnant. In this case the female is discarded and the procedure should be restarted with a fresh animal and surgical kit.

3.7.14 Gently tear the ovarian bursa using watchmaker's forceps to expose the infundibulum. Take care to avoid any blood vessels (Picture 13A).

Picture 13

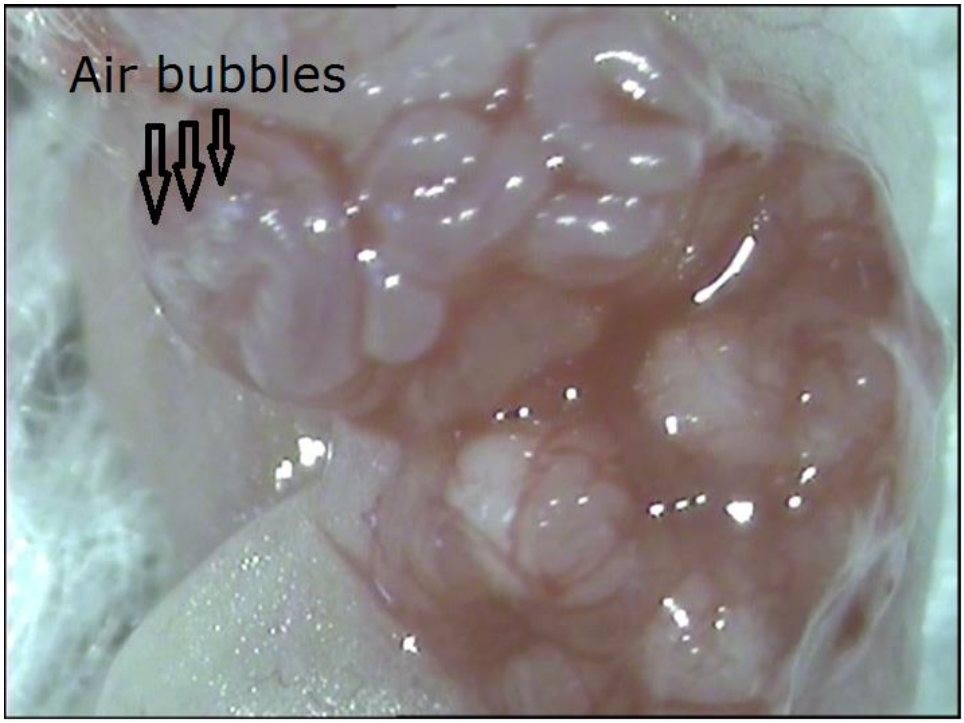


3.7.15 Gently insert one point of the watchmakers forceps into the infundibulum to locate the opening prior to inserting the pipette (Picture 13B).

3.7.16 Carefully place the pipette tip into the infundibulum. Hold the pipette in place using a pair of watchmaker's forceps (Picture 13C).

3.7.17 Gently expel the embryos and air bubbles. Stop expelling as soon as the air bubbles are visible in the ampulla. Picture 14.

Picture 14



3.7.18 After withdrawing the pipette, gently pinch the opening of the oviduct with a pair of watchmaker's forceps for approx. 5 seconds, to prevent the media from flowing back out. Place embryo handling device back on sterile drape.

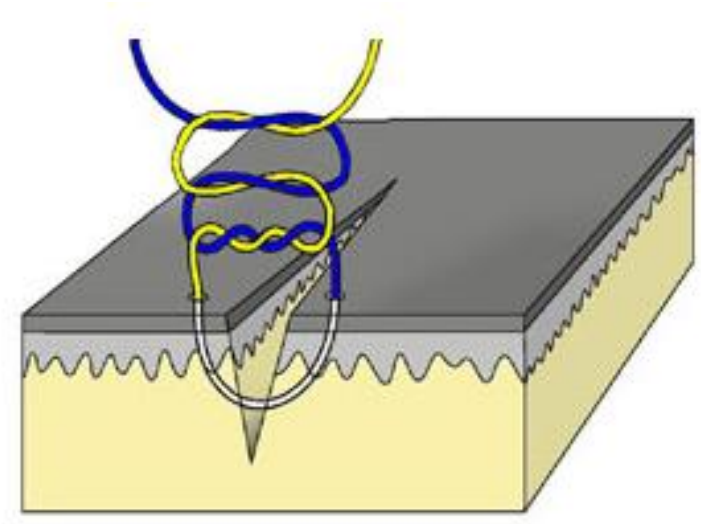
3.7.19 Move the surgical stage from the dissecting microscope and back onto the bench.

3.7.20 Remove the serrefine clip and the swab that has been holding the tissues in place.

3.7.21 Using watchmaker's forceps open the body wall incision site to allow the the uterus, oviduct, ovary and fat pad to re-enter the abdominal cavity. It may be necessary to manipulate the fat pad to encourage the structures to go back in, however, minimise any handling as it could have a detrimental effect on the birth rate.

3.7.22 Suture the incision in the body wall using a square reef knot/surgeons knot (Picture 15).

Picture 15



3.7.23 Use the scissors on the needle holder to trim the ends of the suture to 2mm in length.

3.7.24 If transferring embryos into both oviducts, repeat steps 3.7.5 through to 3.7.23.

3.7.25 Close the skin using a subcuticular stitch and wound glue (Picture 16).

Picture 16

3.7.26 At the end of the procedure, switch off the Isoflurane and allow oxygen only to pass through the mask to aid recovery of the mouse.

3.7.27 Prepare the home cage of the mouse by opening the lid/metal to allow the mouse to be easily placed back in the cage.

3.7.28 Remove the sterile drape. Administer suitable lubricant to each the eye. Turn off the oxygen.

3.7.29 Wrap the mouse in a tissue and place into the home cage.

3.7.30 Place the cage into an IVC recovery rack to allow the mouse to recover.

3.7.31 After 5mins has elapsed assess the welfare of the mouse. For further checks during recovery period see Table 1.

Table 1

	@ 5min check	@ 30min check	@ 1 hour check
Well	Leave and check @ 30mins.	No further checks required.	N/A.
Unwell	Remove cage – check for breathing. Replace cage into recovery rack.	Assess again at 1 hr post surgery.	Consult with lab manager and NACWO if necessary.

3.7.32 The mouse should be allowed to recover in a recovery rack for a minimum of approximately 15 minutes.

3.7.33 Never leave the mice in the recovery rack for an extended period of time (more than an hour). Monitor for signs of surgical complications.

3.7.34 When all surgeries have been completed, clean the work areas down as described in steps 3.2.5 & 3.2.6.

3.7.35 Turn off oxygen at vaporiser after each mouse. Ensure the supply to the vaporisers is turned off once all transfers are complete. Turn of heat pads after all surgeries have been carried out for the day.