TCET Non- Surgical Embryo Transfer

1.0 Equipment

1.1 Dissecting microscope

1.2 IVC recovery rack Techniplast

1.3 Anaesthetic equipment

1.4 Cold light source

1.5 Top pan balance

1.6 Perspex surgical stage

1.7 Eppendorf 2.5µl pipette

1.8 Pipette stand

1.9 Heated stage

2.0 Supplies

2.1 TCET device

2.2 EZ Grip

2.3 135uM/170uM EZ grip pipette tips

2.4 Steret (alcohol wipe)

2.5 Aldasorber

2.6 Sharps containers

2.7 Anaesthetic: Isoflurane

2.8 0.3% Hypromellose Eye Drops/gel

2.9 M2 medium

2.10 60mm petri dish

2.11 P1000 Gilson pipette

2.12 100-1000µl pipette tips

2.13 5% Distel wipes

2.14 70% alcohol

2.15 Paper tissues

2.16 FFP2 mask

2.17 Gloves

2.18 Purified water

2.19 Rubbish bag

3.0 Procedure

3.1 General information

3.1.1 Recipient females should be at 2.5d of pseudopregnancy.

3.1.2 Embryos should be transferred at the compacted morula or blastocyst stage.

3.1.3 Aim to transfer 15 embryos where possible. If not possible, 10-15 embryos will be sufficient.

3.2 Preparation of the surgical area

3.2.1 Wearing gloves, clean entire work area with 5% Distel wipes. Follow this by wiping with 70% alcohol, or purified water for Perspex surfaces.

3.2.2 Place a folded tissue on the stage next to the anaesthetic mask.

3.2.3 Weigh aldasorber. Record weight on the label found on the aldasorber. Replace if over 1380g.

3.2.4 Fill isoflurane to fill line.

3.2.5 Attach a rubbish bag to the bench, close to the surgical area.

3.3 Loading the TCET device

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

3.3.2 Remove a TCET device from its sterile packaging and attach to a 2.5µl Eppendorf pipette that is set to 1.8µl (Picture 1).

NOTE: The device should be attached so that the comfort grip of the pipette is pointing to the left and the curve of the device is pointing straight ahead.

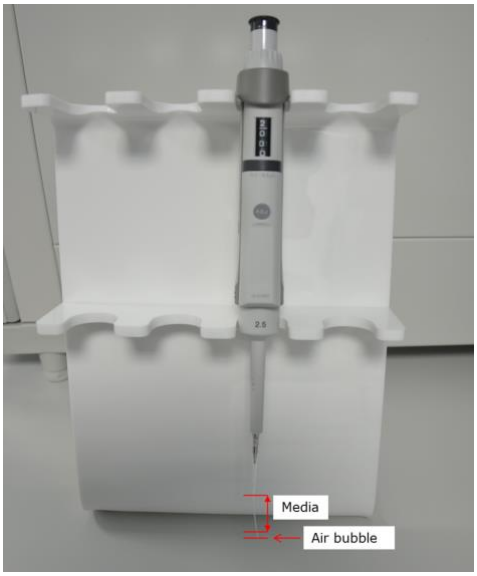
Picture 1

3.3.3 Retain the speculums that are provided in the sterile packaging for use later in the procedure.

3.3.4 Aspirate a small amount of media into the device to pre-wet the tip. Aspirate 15 embryos into the device.

3.3.5 Turn the dial on the pipette to 2.0µl to introduce an air bubble at the end of device (Picture 2).

Picture 2



3.3.6 Place the pipette on a pipette holder with the device tip pointing downwards, until required.

3.4 Transfer

3.4.1 Wearing an FFP2 mask and laboratory gloves, select a female at the correct stage of pseudo pregnancy, depending on the developmental stage of the embryos to be transferred.

3.4.2 Weigh the mouse and record the weight on the cage card.

3.4.3 Anaesthetise the mouse.

3.4.4 Lay the mouse on her front, on the surgical stage, with her nose in the mask. The surgical stage should be in the horizontal position at this point.

3.4.5 Check the mouse is fully anaesthetised by checking the pedal reflex. Check the level of oxygen and Isoflurane and adjust the concentration of Isoflurane if necessary.

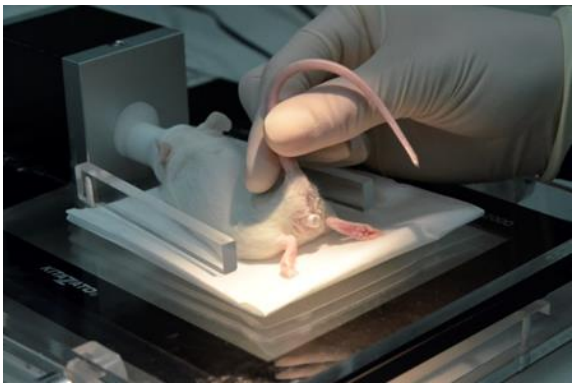
3.4.6 Administer one drop of Hypromellose to each eye.

3.4.7 Administer 0.1ml of Torbugesic via sub cutaneous injection.

3.4.8 Open the steret packaging and use the steret to clean the vaginal area of the mouse.

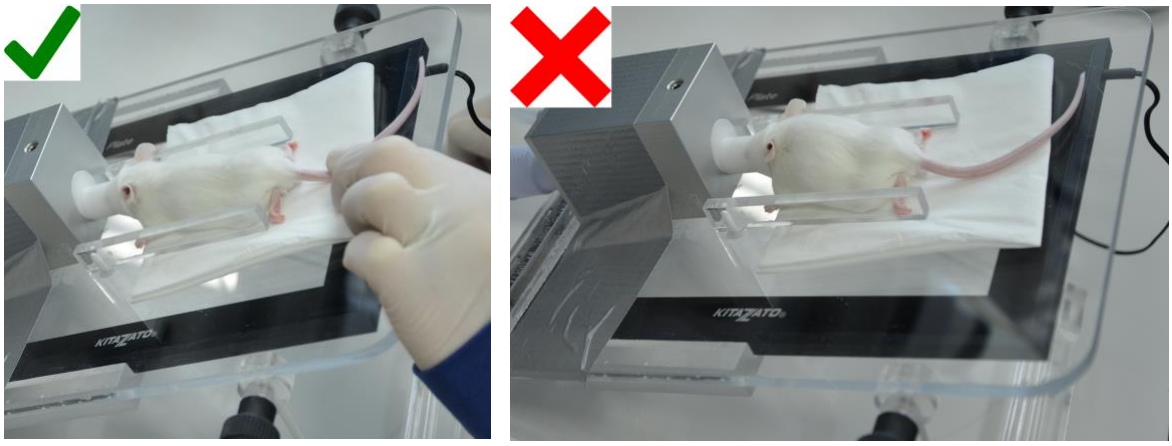
3.4.9 Gently insert the narrowest speculum into the vagina, ensuring the natural angle of the tract is followed (Picture 3).

Picture 3



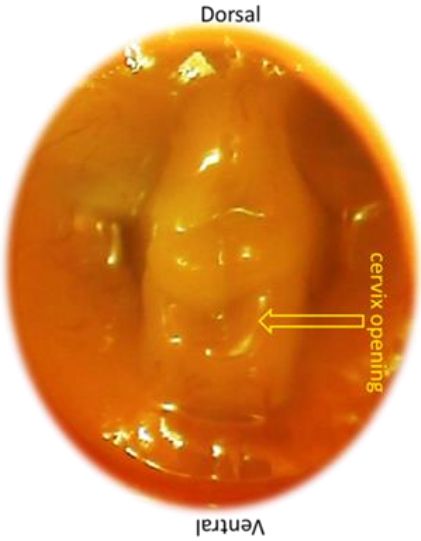
3.4.10 Raise the angle of the surgical stage to approximately 45°. This angle should be comfortable for the user and should give the best view of the cervix. While raising the stage, the mouse should be supported by pressing the tail of the mouse against the surface of the stage (Picture 4).

Picture 4



3.4.11 Adjust the position of the cold light source to ensure the cervix can be easily located (Picture 5).

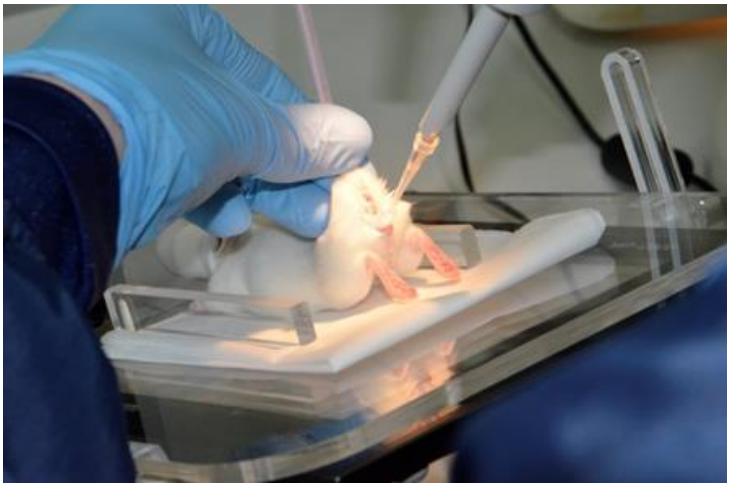
Picture 5



3.4.12 Pick up the pipette with the pre- loaded TCET device attached. Position the pipette so that the comfort grip of the pipette is pointing to the left and the curve of the device is pointing straight ahead.

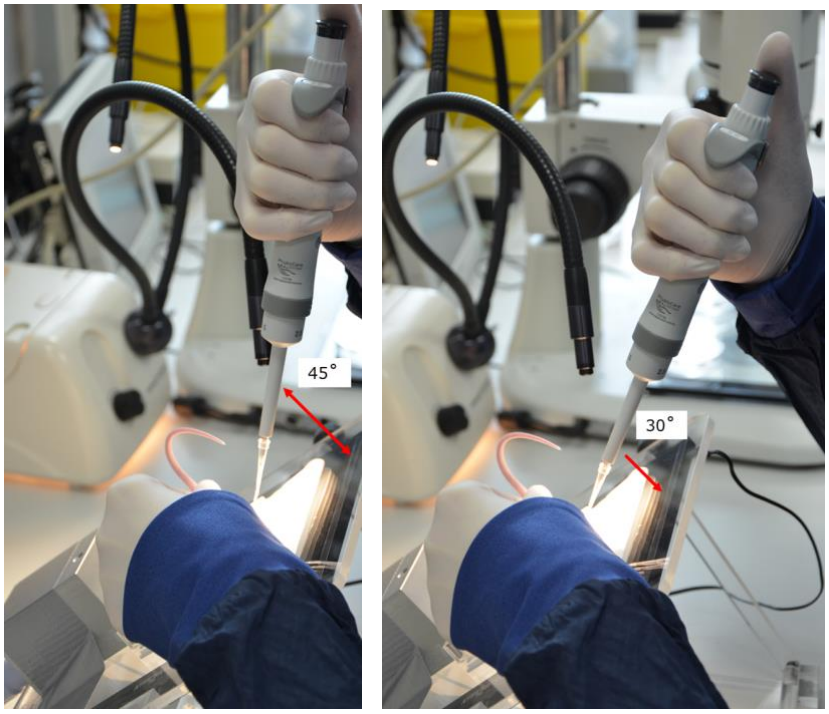
3.4.13 Gently guide the tip of the device towards the opening of the cervix (Picture 6).

Picture 6



3.4.14 Reduce the vertical angle that the device is being inserted to approximately 30°. This will ease the insertion of the device into the uterine horn (Picture 7).

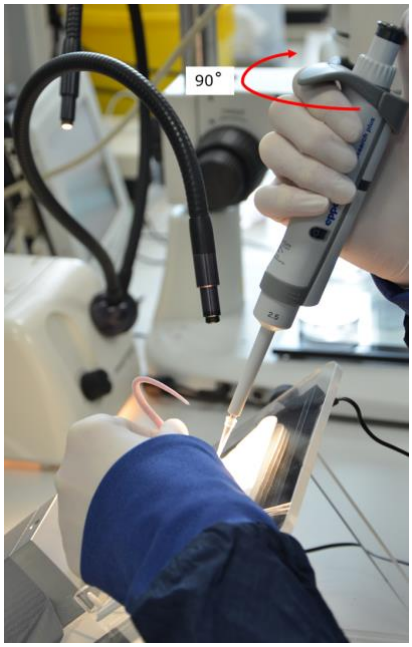
Picture 7



3.4.15 While the device is being inserted into the uterine horn, rotate the pipette by 90° so that the comfort grip is now

facing forwards. This will help the curved tip to follow the natural shape of the uterine horn (Picture 8.).

Picture 8



3.4.16 Once the device is in position, draw the device back slightly, then expel the embryos into the uterine horn by depressing the pipette plunger to the first stop.

3.4.17 Hold the device in position with the plunger depressed for 3 seconds.

3.4.18 Slowly remove the device from the uterine horn, using a circular motion.

3.4.19 Place the pipette plus device onto the pipette holder.

3.4.20 Lower the surgical stage back to the horizontal position. While lowering the stage, the mouse should be supported by pressing the tail of the mouse against the surface of the stage.

3.4.21 Remove the speculum from the vagina of the mouse.

3.4.22 Check that all of the embryos have been expelled from the TCET device by aspirating a small amount of M2 media into the TCET device and expelling 2-3 times.

NOTE: If any embryos have been left behind, these can be transferred into a second recipient female.

If less than 5 embryos have been transferred, cull the recipient female under anaesthesia as the number of embryos that have been transferred are unlikely to lead to pregnancy.

3.4.23 Turn off the isoflurane so the recipient female can receive pure oxygen for a few seconds, to aid recovery.

3.4.24 Switch off the Oxygen supply.

3.4.25 Administer one drop of Hypromellose to each eye.

3.4.26 Wrap the mouse in a tissue and place back into the home cage. Place the cage into the IVC recovery rack.

3.4.27 The mouse should be allowed to recover for approximately 10 minutes.

3.4.28 The total time the mouse is inside the IVC recovery rack should not exceed 30mins.

3.4.29 Never leave the mice in the recovery rack unsupervised for more than 30mins, and monitor for signs of surgical complications.

3.4.30 The temperature and air flow within the recovery rack should be monitored, and mice removed if any deviation from the unit's standard operating conditions is detected.

3.4.31 A new, sterile TCET device should be used for each recipient female.

3.4.32 Following the procedure, the entire work area should be cleaned with 2% Distel wipes. Follow this by wiping with 70% ethanol, or purified water for Perspex surfaces.

3.4.33 Ensure that the heat pad and oxygen are switched off after surgery.