Non- Surgical Embryo Transfer.

This protocol is based on the **N**on-**S**urgical **E**mbryo **T**ransfer (NSET) protocol, developed by ParaTechs, USA and **T**rans- **C**ervical **E**mbryo **T**ransfer (TCET) protocol, developed by Prof Xiangyun Li, Hebei University, China. In this procedure the TCET device is used and the procedure is performed under general anaesthesia.

A. General Information

1. Recipient females should be at 2.5d of pseudopregnancy.

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

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

B. Loading the TCET device

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

2. Remove a TCET device from its sterile packaging and attach to a 2.5µl Eppendorf pipette that is set to 1.8µl (Fig. 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.

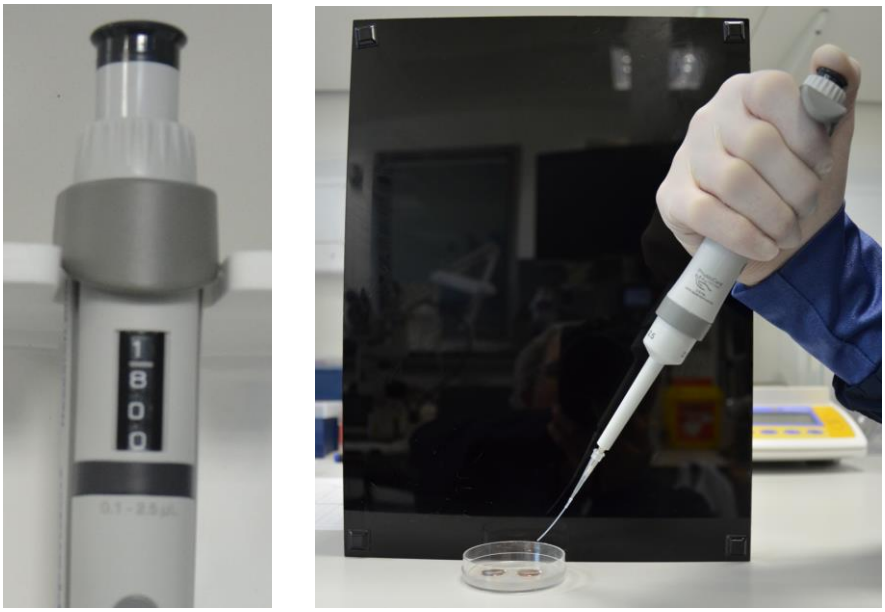

Fig 1.

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

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

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

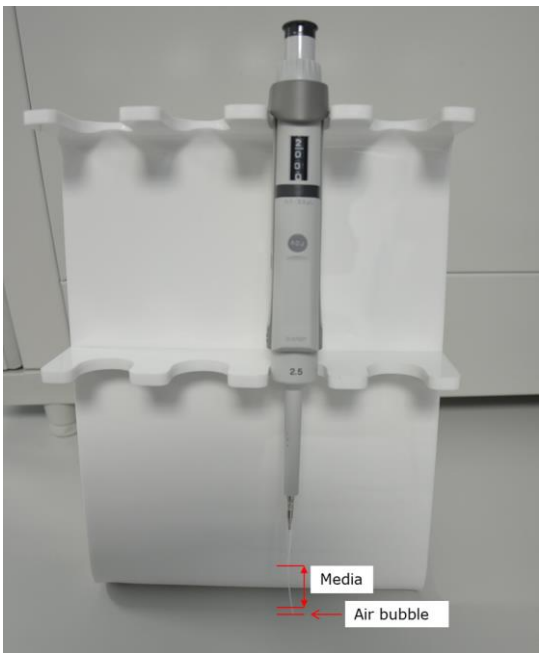

Fig. 2

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

C. Procedure

1. Wearing a surgeon's 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.

2. Anaesthetise the mouse, lay the mouse on her front, on the surgical stage. The surgical stage should be in the horizontal position at this point.

3. Check the mouse is fully anaesthetised, by checking the pedal reflex, before continuing with the procedure.

4. Use an alcohol wipe to clean the vaginal area of the mouse.

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

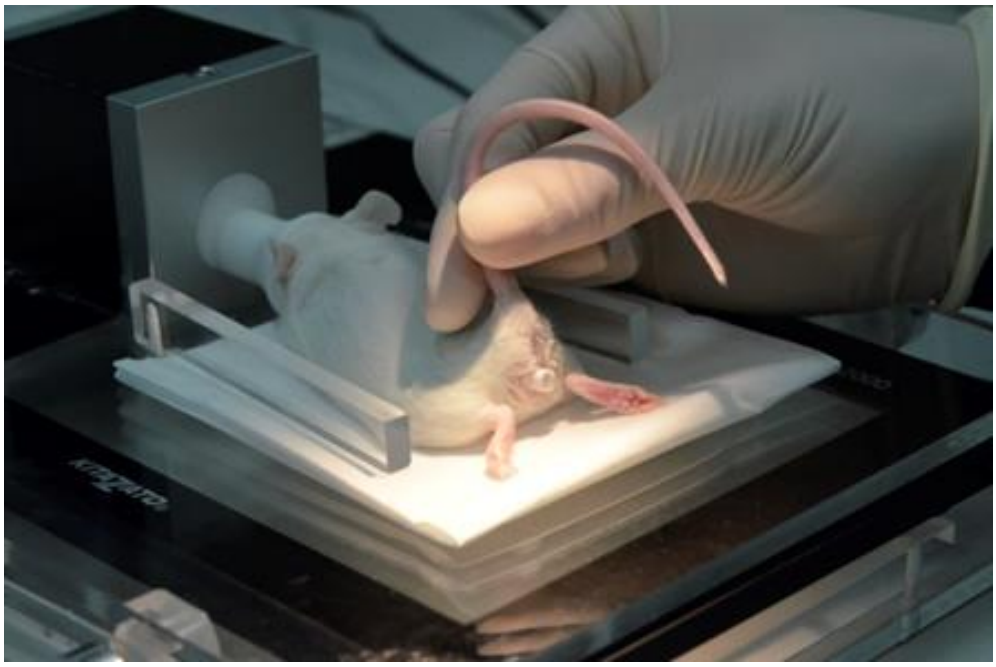

Fig. 3

6. 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 (Fig. 4).

Fig. 4

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

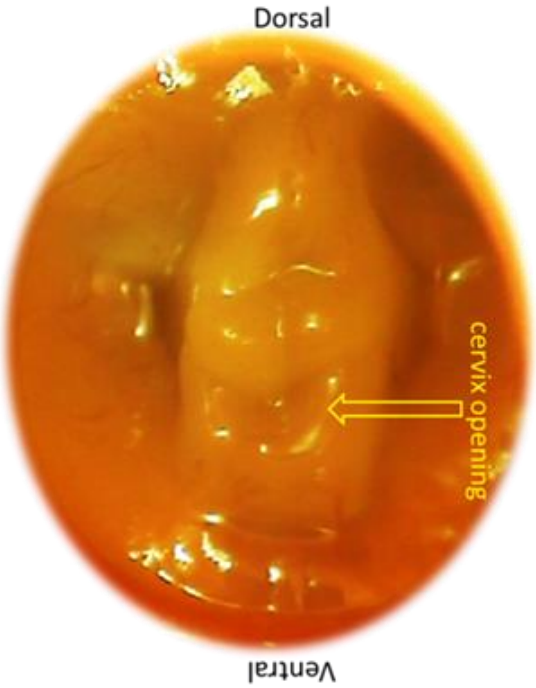

Fig. 5

7. 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.

8. Gently guide the tip of the device towards the opening of the cervix (Fig. 6).

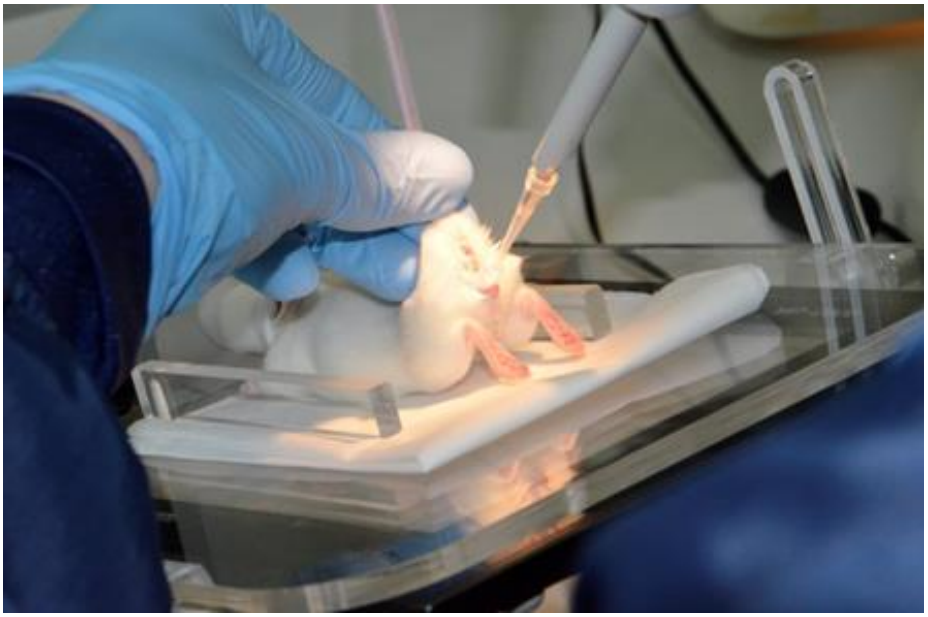

Fig. 6

9. 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 (Fig. 7).

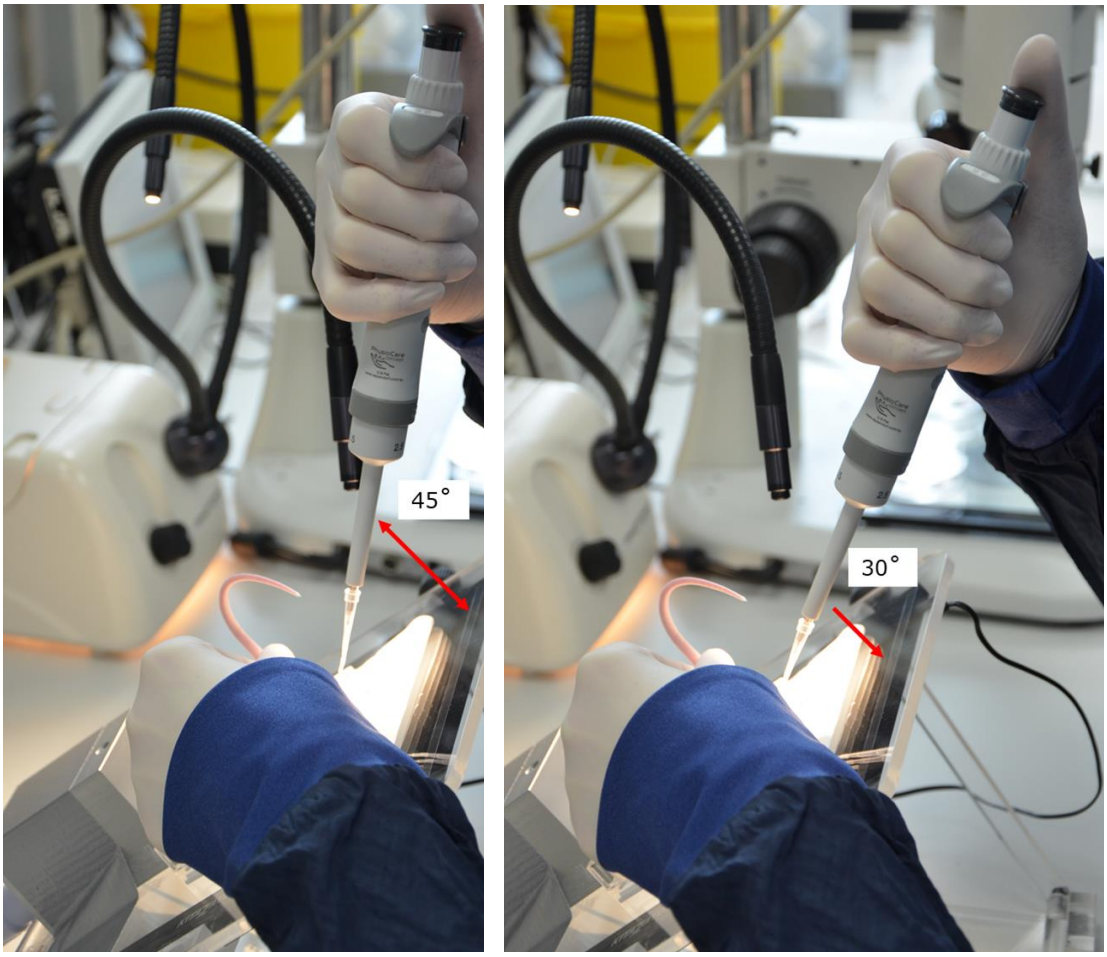

Fig. 7

10. While the device is being inserted into the uterine horn, rotate the pipette by 90° so that the comfort grip is now facing upwards. This will help the curved tip to follow the natural shape of the uterine horn (Fig. 8).

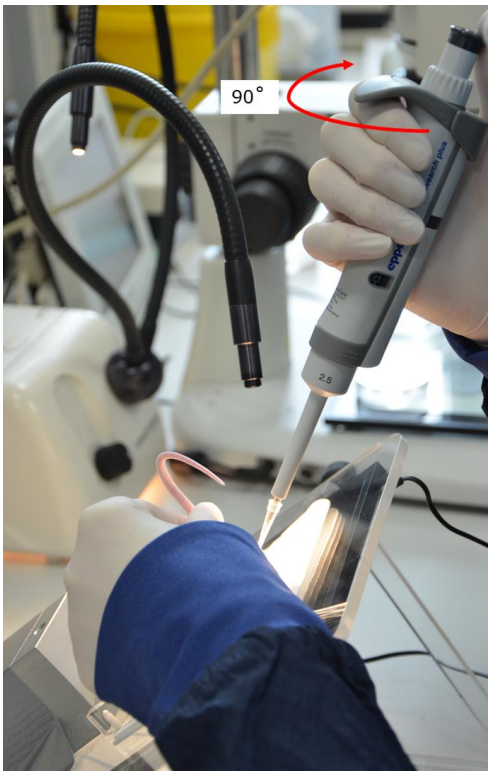

Fig. 8

11. 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.

12. Hold the device in position with the plunger depressed for 5 seconds.

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

14. Place the pipette plus device onto the pipette holder.

15. 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.

16. Remove the speculum from the vagina of the mouse.

17. 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.

18. Return recipient female to cage and allow to recover in recovery rack or on a heat pad.