

Blastocyst Embryo Harvesting

1.0 Equipment

- 1.1.** Dissection stereo microscope
- 1.2.** Incubator (37°C, 5% CO₂)
- 1.3.** Dissecting instruments (fine-pointed scissors, fine forceps)
- 1.4.** Positive displacement pipette
- 1.5.** Cell Tram
- 1.6.** Laminar Air Flow Recycling (LAF) Cabinet
- 1.7.** P1000 Gilson pipette
- 1.8.** Flushing Needle 34G x ½ in.

2.0 Supplies

- 2.1.** 70% Alcohol
- 2.2.** 60mm embryo culture dishes (35:3004)
- 2.3.** M2 medium (Filtered through 0.2µm)
- 2.4.** KSOM medium (QC tested)
- 2.5.** 25G needles
- 2.6.** EZ-Tip 170/200 µm pipette tips
- 2.7.** 1000 pipette tips
- 2.8.** Paper tissues
- 2.9.** 1ml syringe

3.0 Procedure

3.1. Preparation

- 3.1.1. Prepare embryo culture dishes (3004) for the oviducts and/or uteri by pipetting a small drop of M2 media ~100ul. One dish per female to be sacrificed.
- 3.1.2. Prepare a wash dish by pipetting 2x 250µl drops of M2 into an embryo culture dish.
- 3.1.3. Prepare a KSOM culture dish by 100µl of KSOM into an embryo culture dish and placing into the incubator.

3.2. Dissections

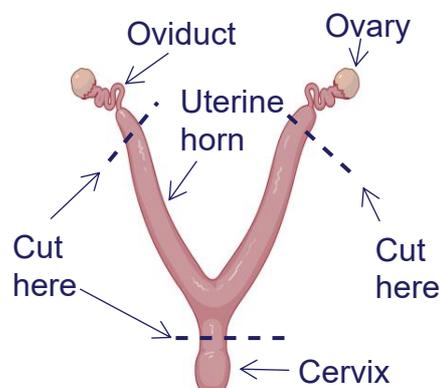
- 3.2.1. Retrieve females and place M2 dish for collection into the LAF cabinet.
- 3.2.2. Within LAF cabinet place the cage containing mice and a paper tissue on the base. Ensure the mice in the cage cannot see the mice being sacrificed.
- 3.2.3. Cull the mouse by cervical dislocation and place onto its back onto the paper towel and wet the abdominal fur with 70% alcohol. Check for secondary confirmation of death before proceeding.
- 3.2.4. Using forceps and scissors pinch the skin and make a small lateral incision at the midline with scissors. Firmly hold the skin above and below the incision, pull the skin in opposite directions towards the head and tail until the abdomen is exposed.
- 3.2.5. Using forceps and scissors make an incision in the peritoneum just below the sternum in the direction of the extremities and down to the tail, to expose the abdominal organs.
- 3.2.6. Use the forceps to gently push the viscera up towards the head, exposing the reproductive organs.
- 3.2.7. Use fine forceps to grab the junction between the oviduct and the uterus (Picture 1).

Picture 1

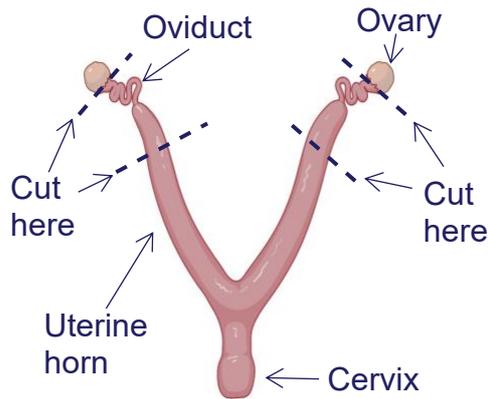
3.2.8. Insert the tip of a closed pair of scissors where the membrane and uterus meet to remove adherent tissue and fat.

3.2.9. **Whole uteri:**

Cut at the ovary-oviduct junction and then just above the cervix to remove the uterus. Repeat on the other side to remove the other uteri (Picture 2).

Picture 2**Oviduct:**

Cut at the ovary-oviduct junction and then ~5mm down the uterus from the oviduct-uteri junction. Repeat on the other side to remove the other oviduct (Picture 3).

Picture 3

3.2.10. Place the oviduct/uteri in the dish containing M2. Transfer the dish with Uteri in to the Stereo microscope.

3.3. Flushing the Uteri

- 3.3.1. If flushing the whole uteri, cut through the uterine horns ~5mm away from the oviduct. Move the oviducts to another prepared dish containing M2 (follow steps 3.4.1 – 3.4.2 to flush the oviducts).
- 3.3.2. To flush the uterine horns, fill a 1ml syringe with room temperature M2 and attach a 25G needle. Expel any air bubbles and check that M2 medium is flowing smoothly from the needle.
- 3.3.3. Using one hand, hold one horn of the uterus with fine forceps and carefully insert the needle into the cervix and slightly down one of the uterine horns.
- 3.3.4. Flush with at least 0.5ml of M2, the uteri will inflate and the media and embryos will flow through the end of the uterus.
- 3.3.5. Repeat step 3.3.3 – 3.3.4 for the other uterine horn.
- 3.3.6. Once flushed discard the uterus.

3.4. Flushing the Oviducts

- 3.4.1. Fill a 1ml syringe with room temperature M2 and attach a flushing needle. Expel any air bubbles and check that M2 medium is flowing smoothly from the needle.

- 3.4.2. Carefully insert the needle into the infundibulum and flush with at least 0.2ml of M2. The oviduct and uteri will inflate and the media and embryos will flow through the end of the uterus.
- 3.4.3. Repeat step 3.4.2 for the other oviduct.
- 3.4.4. Once flushed discard the oviducts.

3.5. Oviducts held in Eppendorf's

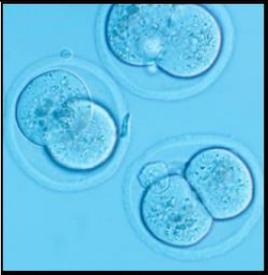
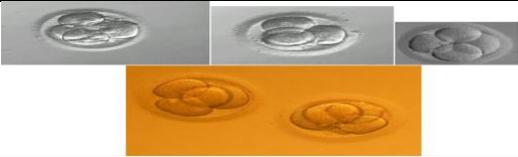
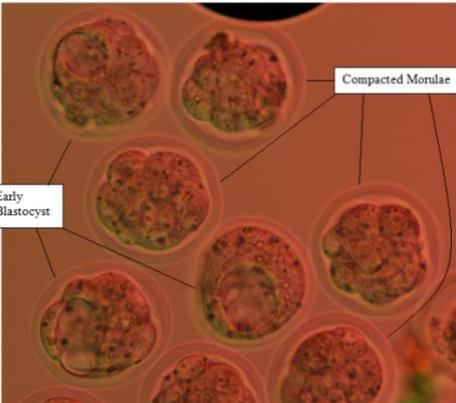
- 3.5.1. If the oviducts/uteri were held in Eppendorf's prior to flushing the Eppendorf will need to be rinsed to check for embryos.
- 3.5.2. After the oviducts/uteri have been removed from the Eppendorf, empty the contents of the Eppendorf into an embryo culture dish.
- 3.5.3. Add 500µl of M2 to the Eppendorf then gently invert the Eppendorf 3-5 times.
- 3.5.4. Tip out the M2 into the dish containing the rest of the Eppendorf contents.
- 3.5.5. Check this dish along with the flushing dishes for any embryos.

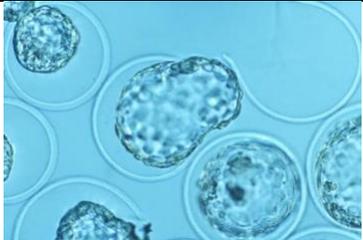
3.6. Washing and culturing embryos

- 3.6.1. Using an embryo handling device and EZ -Tip, filled with clean M2, collect all the embryos and place them in one drop of the dish containing 2 drops of M2 medium. Distribute the embryos as widely as possible throughout the drop to dilute any cells or debris.
- 3.6.2. Gently swirl the "flushing" dish with large, and then small, circular movements to encourage the embryos to collect in the centre. Wait for the embryos to settle. To aid visibility, change the position of the microscope mirror to make the outer membrane, the zona pellucida shine.
- 3.6.3. Remove all visible embryos and swirl again until all are collected. Pay particular attention to the perimeter of the dish where the embryos appear very small, and waft them away from the edge using your EZ-Tip. Scan the whole base of the dish using a grid pattern and check for any embryos in the dish.

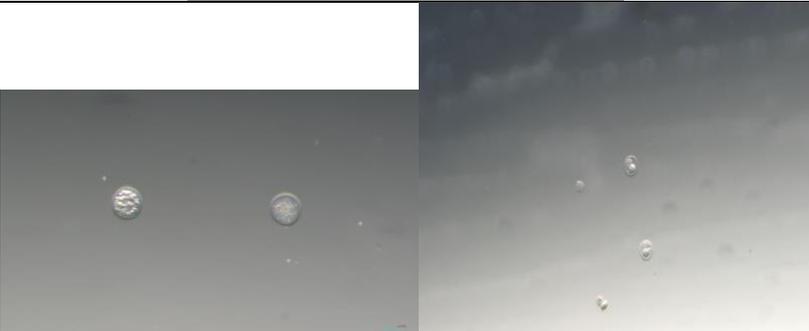
- 3.6.4. Examine all the embryos collected and transfer only healthy embryos (Appendix 1) to the second drop of M2.
- 3.6.5. Transfer the healthy embryos to the drop of the KSOM. Count and record all the healthy embryos.
- 3.6.6. Label the KSOM dish with the embryo Strain, stage and date if not already done. Return the culture dish to the incubator.
- 3.6.7. Count and record the 'abnormal' embryos remaining in the wash drops (Appendix 2), to estimate the rate of superovulation.

4.0 Appendix 1

| Cell Development Stage | |
|------------------------|---|
| 2 cell |  |
| 4 cell |  |
| 8 cell |  |
| Morula |  |

| | |
|----------------------------|--|
| <p>Blastocyst</p> |  |
| <p>Hatching blastocyst</p> |  |

5.0 Appendix 2

| <p>Degeneration State</p> | |
|----------------------------------|--|
| <p>Fragmented</p> |  |
| <p>Dead</p> |  |