Oocyte Harvest for Vitrification

1.0 Equipment

1.1 Dissecting Microscope

1.2 P200 Gilson Pipette

1.3 P20 Gilson Pipette

1.4 P10ml Gilson Pipette

1.5 P1000 Gilson Pipette

1.6 Electronic powerpette

1.7 Cell tram

1.8 Countdown timer

1.9 Vortex

1.10 Incubator (5% CO_2, 37°c)

1.11 Heated stage

1.12 Watchmakers forceps

1.13 Dissection scissors

1.14 Dissection forceps

2.0 Supplies

2.1 60mm embryo culture dishes (35:3004)

2.2 200µl pipette tips

2.3 1000µl pipette tips

2.4 EZ-Tip 135/170µm pipette tips

2.5 1.5ml Eppendorf Tubes

2.6 Silicone Fluid

2.7 10ml Serological pipettes

2.8 FCS (Fetal Calf Serum)

2.9 PB1 solution

2.10 Tissues

2.11 70% alcohol

2.12 Hyaluronidase

2.13 14ml Falcon Tubes

2.14 0.2μm syringe filters

2.15 Luer Lock 20ml syringe

2.16 10ml Diamond Pipette tips

2.17 Permanent marker pen

3.0 Procedure

3.1 General Information

3.1.1 Place the required number of hyaluronidase tubes (one per harvest dish) in the incubator to warm before the females are culled. This will ensure the hyaluronidase is ready when the oocytes have been placed in drop H of the Hyal dish.

3.2 Dish Preparation

3.2.1 **Harvest (Hyal) Dish:**

3.2.1.1 Harvest dishes can be prepared the day before or on the morning of the harvest. One dish is required for every female to be harvested.

3.2.1.2 Pipette ~10ml of PB1 solution into a falcon tube. Then filter the PB1 into a new falcon tube:

7.1.1.1.1 Remove the filter pack lid without touching the filter, leaving the filter inside the plastic casing.

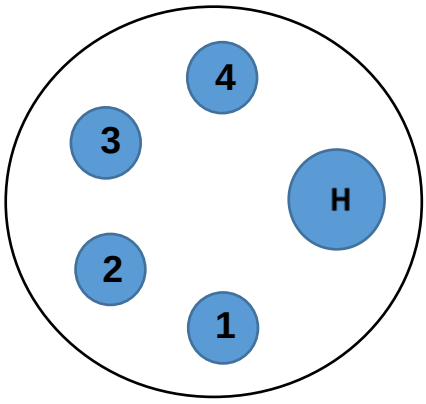
7.1.1.1.2 Pull the plunger out of the syringe, taking care not to let anything come into contact with the end of the plunger that touches the media, and attach the syringe to the filter.

7.1.1.1.3 Pour the PB1 solution into the syringe. Replace the plunger carefully, expelling the first few drops before filtering the rest into a new falcon tube. Make sure to label the falcon tube with 'filtered PB1' and the date.

3.2.1.3 Label an embryo culture dish with 'Hyal' and the dish number on the lid. Write the dish number on the underside of the dish to help orientate the dish once the lid is off.

3.2.1.4 Place 1 x 200µl drop (H) and 4 x 100µl drops (1, 2, 3 & 4) of filtered PB1 solution in the dish as depicted in picture 1. Overlay with silicone fluid. Place dish in an incubator to equilibrate.

Picture 1



3.2.2 **FCS Treatment Dish:**

3.2.2.1 20% FCS and FCS treatment dishes are to be prepared on the morning of the harvest. One dish is required per 3 x females harvested.

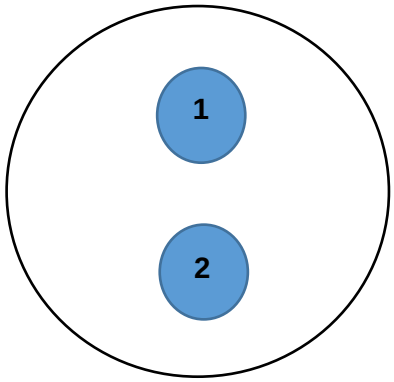
3.2.2.2 To prepare 20% FCS, thaw a vial of FCS stock solution. This can be thawed in the fridge overnight or on the day in a room temperature water bath.

3.2.2.3 Add 200µl of FCS and 800µl of filtered PB1 solution to an Eppendorf then vortex for 5 seconds to mix.

3.2.2.4 Label an embryo culture dish with 'FCS' and the dish number on the lid. Write the dish number on the underside of the dish to help orientate the dish once the lid is off.

3.2.2.5 Place 2 x 150µl drops of 20% FCS in the dish as shown in picture 2 and overlay with silicone fluid. Place dish in an incubator to equilibrate.

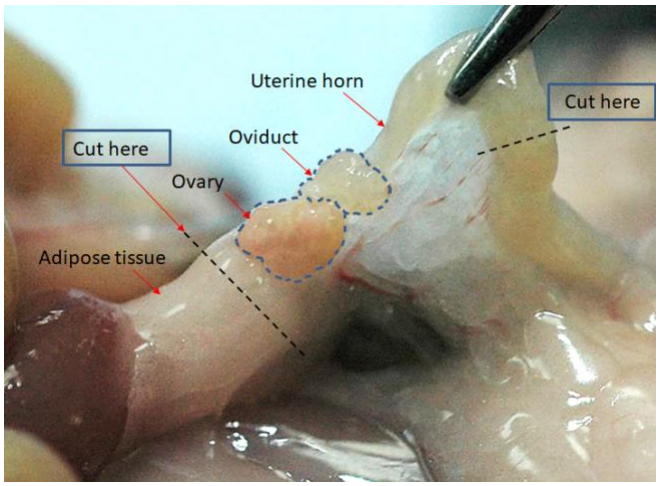
Picture 2



3.3 Oocyte Harvest

3.3.1 Cull superovulated female mice one at a time. Dissect the oviducts (Picture 3) and transfer them into the silicone fluid in the Hyal dish (Picture 4A). Make sure the oviducts do not touch any of the PB1 drops.

Picture 3

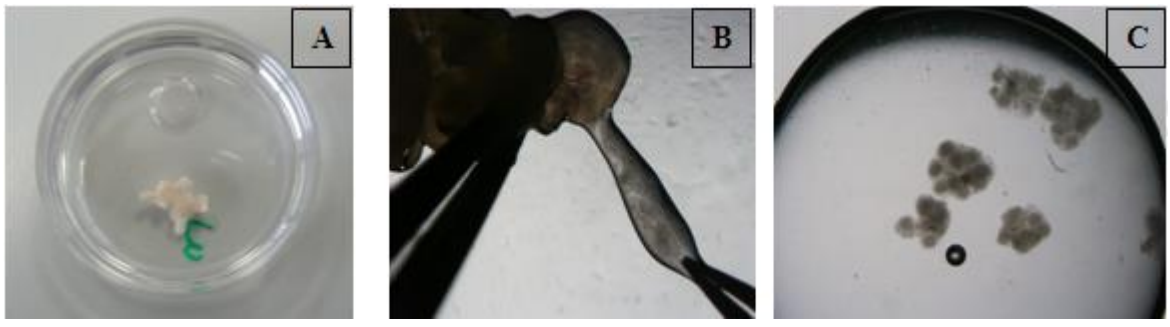


3.3.2 Under the microscope, hold the oviducts down with watchmakers forceps.

3.3.3 Gently tear the swollen ampulla with a second pair of watchmakers forceps to release the cumulus masses into the silicone fluid (Picture 4B).

3.3.4 Using the watchmakers forceps, drag the cumulus masses through the silicone fluid into the H drop of the Hyal dish (Picture 4C). Remove the oviducts from the dish after the cumulus mass has been moved into the drop.

Picture 4



3.4 Denuding Oocytes

3.4.1 After the cumulus masses have been moved into drop H, use a P20 Gilson pipette to add 20µl of hyaluronidase to drop H to denude the oocytes. Place the hyaluronidase around all the clutches.

3.4.2 As the cumulus starts to come off the oocytes, start to place them into wash drop 1 using an embryo handling device. Ensure all the oocytes have been moved to drop 1 within 3 minutes of adding the hyaluronidase.

3.4.3 Wash the oocytes through drops 2, 3 and 4.

3.5 FCS Treatment

3.5.1 When the oocytes from one female have been harvested and treated with hyaluronidase, using an embryo handling device place the oocytes into the first drop in the FCS dish.

3.5.2 Repeat steps 3.3.1 to 3.5.1 until oocytes from 3x females have been harvested and denuded.

3.5.3 Change pipette tip, then move the oocytes to the 2nd FCS drop. Place the FCS dish back in the incubator and start a timer for 15 minutes.

3.5.4 Oocytes should be kept in 20% FCS for a minimum of 15 minutes. Once 15 minutes has passed the oocytes can be vitrified.

3.5.5 Repeat steps 3.3.1 to 3.5.4 until all oocytes have been treated and vitrified.