Acid Tyrode's Treatment of Oocytes

Refer to IVF procedures for details of how to superovulate female oocyte donors and prepare fertilisation dishes and sperm samples. If sperm quality is anticipated to be poor, most of the dishes prepared for a particular IVF are Acid Tyrode's treated. Otherwise, one to two fertilisation dishes may be used for Acid Tyrode's treated oocytes, and the remainder go through the normal IVF procedure.

A. Cumulus Cell Removal

1. Thaw a 30µl aliquot of Hyaluronidase and dilute with 500µl PBS+BSA. Mix gently and warm to 37°C.

2. Dissect the oviducts from six superovulated female mice and place into a preincubated dish of IVF medium. We typically use high-calcium HTF prior to the addition of GSH.

3. Under a dissecting microscope release the cumulus masses into the IVF medium, then remove the oviducts from the dish, as for a conventional IVF.

4. Using a P1000 Gilson pipette with a standard tip, pick up all the clutches of eggs in 500µl or less of IVF medium. With the clutches of eggs still in the pipette, aspirate 500µl of Hyaluronidase solution that has been held at 37°C.

5. Dispense the clutches and the Hyaluronidase solution into a 60mm (PAA10060X) petri dish. Gently aspirate and dispense the clutches in the Hyaluronidase solution (2-3 times) to help break down the clutches. Hold at 37°C for 2-5 mins.

6. Transfer the weakened clutches using a capillary transfer mouth pipette to a 351008 falcon dish containing 2.5ml PBS+BSA.

7. Hold at 37°C for approximately 10 minutes while preparing the rest of the equipment, or while the same procedure is performed for the next six females (if applicable). This allows further cumulus cells to detach from the oocytes. The oocytes from subsequent batches of females should be placed in separate dishes.

8. Using a capillary transfer mouth pipette, wash half of the healthy, Hyaluronidase-treated oocytes from the first dish through a 500µl drop of PBS+BSA in a 351008 falcon dish. Place the remaining healthy Hyaluronidase-treated oocytes in a second dish of PBS+BSA.

B. Acid Tyrode's Zona Thinning

1. Using a P20 Gilson pipette with a standard tip, aspirate all of the oocytes in the first dish in 20µl PBS+BSA and disperse into a 400µl drop of Acid Tyrode's solution (pH3.5) which has been kept at 37°C.

2. Immediately start the timer.

3. Using a P200 Gilson pipette and a wide bore tip, gently aspirate and dispense the oocytes in Acid Tyrode's to ensure an even distribution.

4. As soon as the timer reaches 60 seconds [the optimum time will vary between batches of Acid Tyrode's – see note below], flood the dish with 4ml PBS+BSA.

5. Observe the oocytes to assess the success of the Acid Tyrode's treatment. The thinned oocytes should take on an irregular-looking appearance, but more than 90% of the oocytes should still possess a zona. It may be necessary to alter the time the oocytes are exposed to Acid Tyrode's for subsequent treatments.

6. Using a glass capillary transfer pipette, collect the treated oocytes in as small a volume as possible and add them to the fertilization drop in the IVF dish. Then add the appropriate volume of sperm to the drop, depending on whether freshly harvested or frozen/thawed sperm is being used, and the freezing protocol used to store the sperm.

7. Repeat the procedure for the second group of oocytes, and for each subsequent batch of females.

8. Continue as described in the IVF protocol, taking extra care as the zona pellucida will be more fragile than usual.

NB. The activity of the Acid Tyrode's solution varies with each batch and is also strain specific. If Acid Tyrode's is used at a lower pH (e.g. pH3.25) a shorter treatment time is required. Each batch of Acid Tyrode's solution requires separate testing.