

Acid Tyrode's Treatment of Oocytes

1.0 Equipment

- 1.1** Dissecting microscope
- 1.2** pH meter
- 1.3** Universal tube
- 1.4** P20 Gilson pipette
- 1.5** P200 Gilson pipette
- 1.6** P1000 Gilson pipette
- 1.7** Incubator (5% CO₂, 37°C)
- 1.8** Embryo handling device

2.0 Supplies

- 2.1** Acid Tyrode's Solution
- 2.2** Sodium hydroxide
- 2.3** Embryo water
- 2.4** Human Tubal Fluid (hTF)
- 2.5** 200µl tips
- 2.6** 0.2µm syringe filters
- 2.7** Luer Lock 20ml syringe
- 2.8** Eppendorfs
- 2.9** 1000µl tips
- 2.10** Hyaluronidase
- 2.11** PBS+BSA
- 2.12** 60mm embryo culture dishes (35:3004)

2.13 Wide bore pipette tips

2.14 EZ-Tip 135/170µm pipette tips

3.0 Procedure

3.1 Preparation of Acid Tyrode's Solution (pH 3.0, 3.25 and 3.5)

- 3.1.1 Calibrate the pH meter.
- 3.1.2 Place the probe into the Acid Tyrode's solution (supplied at pH 2.5) and wait for the reading to stabilize.
- 3.1.3 Prepare a 0.5M sodium hydroxide solution by adding 5ml embryo tested water to 5ml 1M sodium hydroxide in a universal tube and mix gently.
- 3.1.4 Add the sodium hydroxide solution drop by drop to the Acid Tyrode's solution, swirling the solution between each drop and letting the reading stabilise.
- 3.1.5 Once the Acid Tyrode's solution reaches the required pH (3.0, 3.25 or 3.5, as appropriate) filter the solution and dispense into 1ml aliquots.
- 3.1.6 Store the aliquots at -20°C until required.

Refer to Superovulation and IVF protocols 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.

3.2 Harvesting oocytes & cumulus cell removal

- 3.2.1 Thaw a 30µl aliquot of Hyaluronidase stock solution (10mg/ml) and dilute with 970µl PBS+BSA to prepare a 30µg/ml working solution. Mix gently and warm to 37°C in an incubator.

NOTE: We have found 56µg/ml (made by diluting 30µl of 10mg/ml hyaluronidase in 500µl PBS+BSA is also satisfactory).

PBS+BSA can be prepared by pipetting 20ml PBS into a 50ml Corning tube and adding a full spatula of BSA to the solution, mix and filter through a 0.2µM filter.

- 3.2.2 Dissect the oviducts from three superovulated female mice and place into a 35mm dish containing 2ml of hTF that has been warmed to 37°C in an incubator for 10mins.
- 3.2.3 Under a dissecting microscope release the cumulus masses into the hTF, then remove the oviducts from the dish.
- 3.2.4 Using a P1000 Gilson pipette with a standard tip, pick up all the clutches of eggs in 500µl or less of hTF. With the clutches of eggs still in the pipette, aspirate 500µl of Hyaluronidase solution that has been held at 37°C.
- 3.2.5 Dispense the clutches and the Hyaluronidase solution into a 60mm embryo culture dish (35:3004). Gently aspirate and dispense the clutches in the Hyaluronidase solution (2-3 times) to help break down the clutches. Hold at 37°C for up to 1min.
- 3.2.6 Transfer the weakened clutches using an embryo handling pipette to a 35mm dish containing 2ml PBS+BSA.
- 3.2.7 Place in an incubator for approximately 10 minutes while preparing the rest of the equipment, or while the same procedure is performed for the next three 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.
- 3.2.8 Using an embryo handling pipette, wash the healthy, Hyaluronidase-treated oocytes from the first dish through a 500µl drop of PBS+BSA in a 60mm embryo culture dish.

3.3 Acid Tyrode's Zona Thinning

- 3.3.1 Using a P20 Gilson pipette with a standard tip, aspirate all of the oocytes from the 500µl drop of PBS+BSA into 20µl drop of PBS+BSA in a 60mm embryo culture dish.
- 3.3.2 Transfer the oocytes into a 400µl drop of Acid Tyrode's solution (pH3.5) which has been kept at 37°C, in a 60mm embryo culture dish.
- 3.3.3 Immediately start the timer.
- 3.3.4 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.
- 3.3.5 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.
- 3.3.6 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 pellucida. It may be necessary to alter the time the oocytes are exposed to Acid Tyrode's for subsequent treatments.
- 3.3.7 Using an embryo handling pipette, collect the treated oocytes in as small a volume as possible and add them to the fertilisation drop in the IVF dish.
- 3.3.8 Repeat the procedure for each subsequent batch of females.
- 3.3.9 Continue as described in the IVF protocol, taking extra care as the zona pellucida will be more fragile than usual.

NOTE: 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.