

Preparation and Use of Hyaluronidase

1.0 Equipment

- 1.1** Heated stage
- 1.2** Hot block
- 1.3** 10ml Gilson Pipette
- 1.4** P100 Gilson Pipette
- 1.5** P1000 Gilson Pipette
- 1.6** Microcentrifuge tube rack
- 1.7** Permanent marker pen

2.0 Supplies

- 2.1** Safety glasses
- 2.2** Hyaluronidase
- 2.3** M2
- 2.4** 10ml pipette tips
- 2.5** 1.5ml Eppendorf tubes
- 2.6** 100-200 μ l pipette tips
- 2.7** 100-1000 μ l pipette tips
- 2.8** Embryo Culture Dishes (35:3004)
- 2.9** Silicone fluid
- 2.10** hTF (Human Tubal Fluid)

3.0 Procedure

3.1 General information

- 3.1.1 Hyaluronidase is used to denude oocytes of cumulus cells.
- 3.1.2 Hyaluronidase is also used to treat embryos that have cumulus cells attached after overnight incubation, prior to transferring or freezing.
- 3.1.3 It is used to reduce contamination from pathogens.
- 3.1.4 It can also be used as part of assisted reproductive techniques like Acid Tyrode and Laser treatments.
- 3.1.5 Safety glasses must be worn at all times by the person preparing the media, and by those working in the immediate area.

3.2 Preparation of 1% Hyaluronidase Stock Solution (10mg/ml)

- 3.2.1 Remove brown glass bottle of Hyaluronidase powder from the freezer.
- 3.2.2 Remove seal from the bottle and remove bung.
- 3.2.3 Add 3ml of filtered M2 media into the bottle with a 10ml Gilson pipette.
- 3.2.4 Replace bung and invert several times to ensure the powder has dissolved.
- 3.2.5 Once dissolved, dispense 30µl aliquots of 1% concentrated stock of the Hyaluronidase solution into 1.5ml Eppendorfs with a P100 Gilson pipette.
- 3.2.6 Label the Eppendorfs with "HYAL" and the date prepared in the format DD/MM/YYYY.
- 3.2.7 Store at -20°C for 6 months.

3.3 Using Hyaluronidase to remove adherent debris from pre-implantation embryos

- 3.3.1 Take one Eppendorf containing 30µl of 1% concentrated Hyaluronidase out of the freezer for every stock to be treated and place in a hot block set at 37°C.
- 3.3.2 Add 500µl of M2 media to the Eppendorf of Hyaluronidase and leave it in the hot block to warm up for a minimum of 5mins but no more than 30mins.
- 3.3.3 Turn on heated stage; also set to 37°C.
- 3.3.4 When the heated stage is up to temperature and the Hyaluronidase solution has been incubated for 5mins, pipette 500µl of the Hyaluronidase solution into a 60mm embryo culture dish.
- 3.3.5 On a heated stage, wash the required number of embryos through the Hyaluronidase drop using an embryo handling device.
- NOTE:** Embryos should be in the Hyaluronidase drop for approximately 1min depending on how many there are and how much cumulus is attached. It is helpful to aspirate the embryos up and down the pipette to encourage the attached cumulus cells to detach.
- 3.3.6 Once all the adherent debris has been removed, wash the embryos through two drops of M2.
- 3.3.7 Change the tip of the handling device between each drop.