Laser-Assisted IVF

1.0 Equipment

1.1 Dissecting microscope

1.2 Incubator (5% CO_2, 37°c)

1.3 Computer

1.4 XYCLONE software

1.5 Heated stage

1.6 Dissecting Scissors

1.7 Dissecting forecepts

1.8 Watchmaker forecepts

2.0 Supplies

2.1 Embryo culture dish (35:3004)

2.2 Human Tubal Fluid (hTF)

2.3 Silicone fluid

2.4 Permanent marker pen

2.5 Glass microscope coverslip

2.6 Hyaluronidase

3.0 Procedure

3.1 Dish preparation

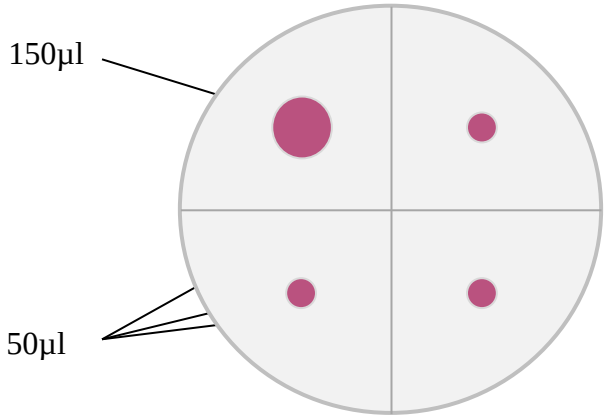
3.1.1 Prepare one embryo culture dish for Laser treatment use.

3.1.2 Into each dish, carefully pipette 4 drops of hTF as follows (Picture 1):

1 x 150µl for washing

3 x 50µl for holding medium during laser treatment

Picture 1



3.1.3 Carefully overlay the drops with silicone fluid and equilibrate the dishes for 10-20mins or overnight in an incubator.

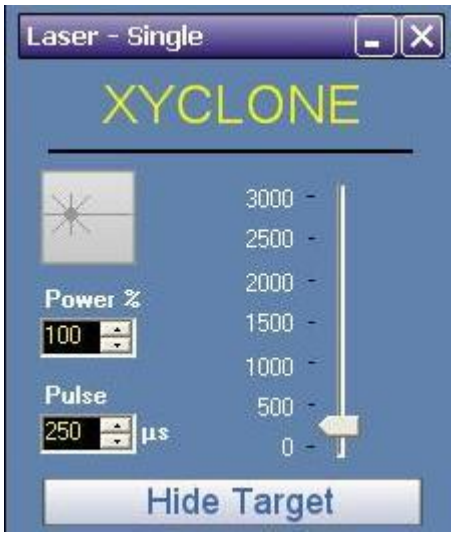
3.2 Laser microscope preparation

3.2.1 Switch on the microscope and USB laser controller.

3.2.2 Switch on the computer and open the XY Clone or similar device's software.

3.2.3 Adjust the power to 100% and Pulse to 250µs (the lowest settings that breach the zona pellucida) (Picture 2).

Picture 2



3.2.4 Using a black permanent marker, place an ink mark on a glass microscope coverslip.

3.2.5 With the inked-side facing upwards, place the coverslip into a 35mm Falcon Petri Dish on the microscope stage.

3.2.6 Switch on the inverted microscope, and with the 4x objective in place, move the stage so that the ink-covered coverslip is in the light path.

3.2.7 Rotate the XY Clone 20x objective lens into the light path (Picture 3).

3.2.8 Fire the laser to burn a hole in the ink.

3.2.9 Align the laser target ("red eye") on the computer screen and click "Alignment OK".

Picture 3

3.3 Preparation of IVF dish

3.3.1 See IVF protocols for dish preparation.

3.4 Harvesting oocytes & cumulus cell removal

3.4.1 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 though a 0.2µM filter.

3.4.2 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.

3.4.3 Under a dissecting microscope release the cumulus masses into the hTF, then remove the oviducts from the dish.

3.4.4 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.

3.4.5 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.

3.4.6 After the hyaluronidase treatment, wash the denuded oocytes through 3 x 150µl drops of hTF in a 60mm embryo culture dish.

3.4.7 Transfer all of the denuded oocytes to the 150µl droplet of hTF medium in the embryo culture dish as prepared in 3.1, then place 20-25 oocytes into each 50µl droplet.

3.4.8 Repeat 3.4.2 – 3.4.7 for all remaining females.

3.5 Laser Treatment

3.5.1 Aim the laser beam at the point on the zona pellucida where the perivitelline space is widest, to avoid laser-induced cytoplasmic damage (Picture 4).

3.5.2 Fire the laser once to drill through the zona pellucida.

3.5.3 Only make one hole in each oocyte.

3.5.4 Work quickly, so that the oocytes are exposed to ambient conditions for the shortest time possible.

3.5.5 Approximately 50 oocytes can be drilled in 5 minutes by an experienced technician.

3.5.6 Transfer the laser treated oocytes to the IVF fertilisation drops, and continue to follow the standard protocol for the remainder of the IVF.

3.5.7 Repeat steps 3.5.1 - 3.5.4 to laser drill the remaining oocytes.

Picture 4