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Laser-Assisted IVF

A. Dish preparation

1. Prepare one 60mm Petri dish (PAA10060X, PAA Laboratories Ltd, UK) for Laser treatment use.

2. Into each dish, carefully pipette 4 drops of high-calcium HTF medium as follows (Fig. 1):

   1 x 150µl for washing

   3 x 50µl for holding medium during laser treatment

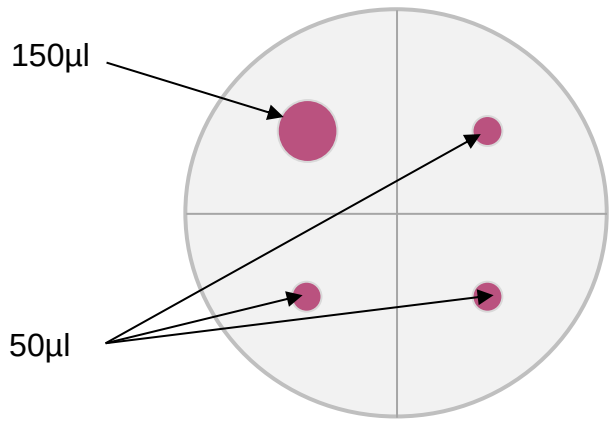


Fig. 1

3. Carefully overlay the drops with mineral oil and equilibrate the dishes overnight at 37^0C, in 5%CO_2 in air.

B. Laser microscope preparation

1. Switch on the microscope and USB laser controller.

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

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

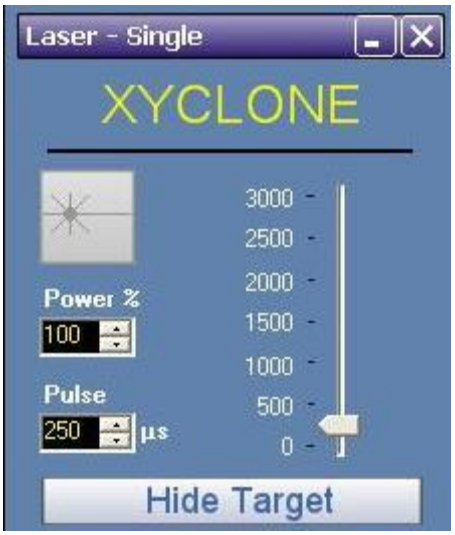


Fig 2.

4. Using a black Marks-a-lot® marker, place an ink mark on a glass microscope coverslip.

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

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.

7. Rotate the XY Clone 20x objective lens into the light path (Fig 3).

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

Fig 3.

C. Preparation of IVF dish

1. Prepare the IVF dishes in the same way as for your standard IVF procedure.

2. Fresh or frozen sperm samples should be dispersed into the fertilization drops **before** the oocytes are harvested.

D. Harvesting oocytes

1. Dissect the oviducts from three superovulated female mice and place into a dish of M2 medium (M-7167, Sigma).

2. Under a dissecting microscope, on a heated stage, hold each oviduct down with forceps and gently tear the swollen ampulla with a second pair of forceps to release the cumulus masses into M2 medium. Remove the oviduct from the dish.

3. When all the cumulus masses have been extracted, using a P1000 Gilson pipette with a standard tip, pick up all the clutches of eggs in a volume of 500µl or less. With the clutches of eggs still in the pipette, aspirate 500µl of Hyaluronidase (H-4272, Sigma) solution that has been held at 37˚C.

4. Dispense the clutches and 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 3-5 minutes.

5. After the hyaluronidase treatment, wash the denuded oocytes in M2 medium 3 times.

6. Transfer all of the denuded oocytes to the 150µl droplet of high-calcium HTF medium in the tissue culture dish, then place 25-30 oocytes into each 50µl droplet.

E. Laser Treatment

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

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

3. Only make one hole in each oocyte.

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

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

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

7. Repeat steps 1-4, above to laser drill the remaining oocytes.

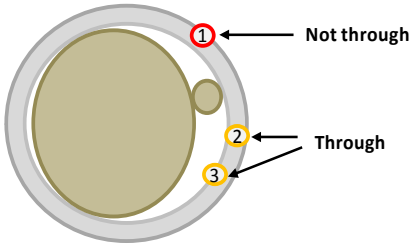
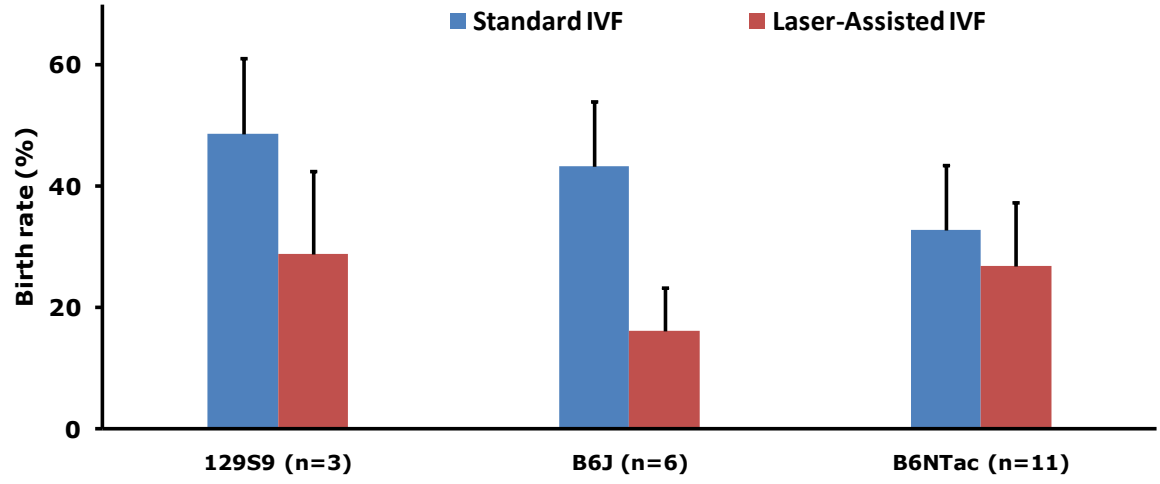
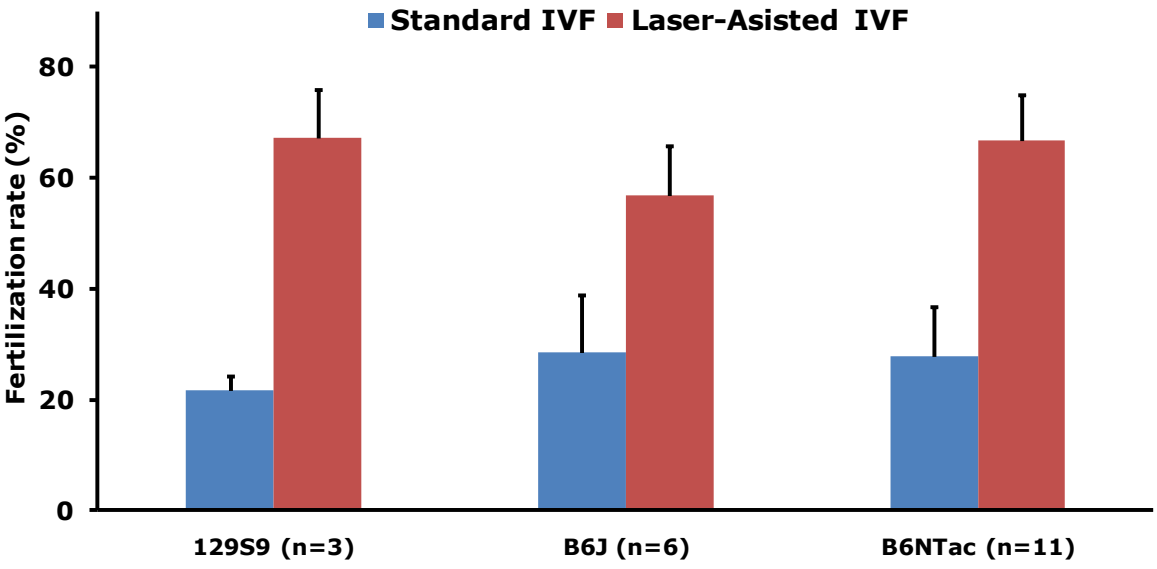


Fig 4.

F. Experimental Results





G. References

Peters DD, Lepikhov K, Rodenacker K, Marschall S, Boersma A, Hutzler P, Scherb H, Walter J, de Angelis MH. Effect of IVF and laser zona dissection on DNA methylation pattern of mouse zygotes. Mamm Genome. 2009 Sep-Oct; 20(9-10):664-73.

Anzai M, Nishiwaki M, Yanagi M, Nakashima T, Kaneko T, Taguchi Y, Tokoro M, Shin SW, Mitani T, Kato H, Matsumoto K, Nakagata N, Iritani A. Application of laser-assisted zona drilling to in vitro fertilization of cryopreserved mouse oocytes with spermatozoa from a subfertile transgenic mouse. J Reprod Dev. 2006 Oct; 52(5):601-6.