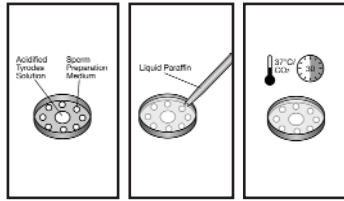
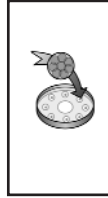


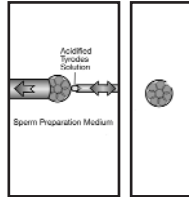
## Procedure (assisted hatching):



1. Prepare a petri dish with one microdrop of Acidified Tyrodes Solution and several droplets of pre-equilibrated Sperm Preparation Medium (Cat.No. 1069/1070). Cover the dish with pre-equilibrated Liquid Paraffin (Cat.No. 1010) and place the dish in a CO<sub>2</sub> environment at 37°C for 30 minutes prior to use.



2. Place each embryo in a microdroplet of Sperm Preparation Medium.

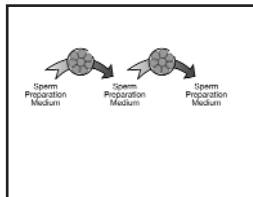


3. Load the zona-drilling pipette with Acidified Tyrodes Solution and stabilise the fluid movement.

4. Clamp the embryo onto the holding pipette (syringe suction system) so that the micro needle filled with Acidified Tyrodes Solution is exposed at the 3 o'clock area. With the pipette just touching the zona, move the pipette back and forth slightly whilst slowly ejecting an extremely small amount of Acidified Tyrodes Solution. Suction is recommended at this point, to aspirate all of the expelled acid solution.

*Note! The total time to breach the zona should be in the order of 5-7 second.*

5. It is crucial to watch this procedure carefully in order not to hurt the blastomeres. The outer layer of the zona dissolves first and then the inner. In approximately half the cases the zona pops open, whereas in the other half the zona may be widened mechanically by moving the microneedle through the opening in a tearing motion to achieve an even size hole (15-20 μm).



6. Immediately wash the embryo(s) thoroughly by transferring them between several droplets of Sperm Preparation Medium and finally place them in medium for embryo transfer. Subsequent handling of embryos should be gentle so as to reduce the risk of blastomere loss.