ICSI PROCEDURE

Day 0
1. Prepare culture dishes
   - G-TL™ or G-1™ PLUS
   - OVOIL™
   Prepare dishes with G-TL or G-1 PLUS and equilibrate at
   37°C 6 % CO₂ 6 h

2. Prepare denudation dishes
   - HYASE™-10x
   - G-MOPS PLUS
   - OVOIL
   Dilute HYASE-10x with G-MOPS PLUS
   Prepare rinsed 5-well denudation dishes with HYASE-10x diluted in G-MOPS PLUS and with G-MOPS PLUS for wash, cover with OVOIL and warm at 37°C 15 min
   Use within one hour.

3. Prepare ICSI dishes
   - ICSI™
   - G-MOPS PLUS
   - OVOIL
   Prepare ICSI dishes with ICSI and G-MOPS PLUS droplets covered with OVOIL and warm in a warming incubator without CO₂ at 37°C 15 min
   Ensure that the denudation and washing procedures are performed at 37°C

4. Denudation
   Denude the oocytes in HYASE-10x and wash three times in warmed G-MOPS PLUS
   Exposure to hyaluronidase for longer than 30 seconds may damage the oocytes

5. ICSI
   Add sperm to the ICSI droplet.
   Transfer the denuded oocytes to G-MOPS PLUS droplets. Place one oocyte per droplet and maximum four oocytes at a time, and inject the oocytes.
   Adjust temperature of the heated stage used for ICSI to maintain 37°C in the droplets

6. Wash and culture
   Wash the injected oocytes in several droplets of G-TL or G-1 PLUS and transfer to fresh G-TL or G-1 PLUS droplets for culture at 37°C 6 % CO₂ overnight
   For fertilisation assessment and culture, see separate Embryo culture protocol.