

# 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 % CO<sub>2</sub> 6 h**



G-RINSE™

When applicable, pre-rinse all utensils, including dishes and pipettes with G-RINSE.

**Never place any G-MOPS™ product in a CO<sub>2</sub> incubator**

**Do not use OVOIL equilibrated in a CO<sub>2</sub> environment when covering any G-MOPS product**

### 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 CO<sub>2</sub> at

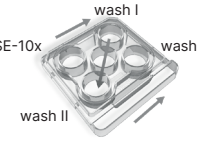
**37°C 15 min**

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 % CO<sub>2</sub> overnight**

**For fertilisation assessment and culture, see separate Embryo culture protocol.**