## EMBRYO CULTURE

### Day 0
- **G-1™ PLUS**
- **OVOIL™**

Prepare micro-droplet culture dishes with 25 µL droplets of G-1 PLUS for washing and for culture. Cover with OVOIL and pre-equilibrate at 37°C 6% CO₂ overnight.

### Day 1
1. **Fertilisation assessment**
   - For inseminated oocytes, transfer the oocytes to a centre well dish with pre-warmed G-MOPS PLUS. If denudation and fertilisation assessment can be performed within 2 minutes, G-IVF™ PLUS can be used instead of G-MOPS PLUS. Remove cumulus and corona cells from oocytes using a denudation pipette and assess fertilisation at 37°C.

2. **Culture**
   - Wash the zygotes extensively in the G-1 PLUS micro-droplet dishes prepared on Day 0 and transfer the zygotes to 25 µL G-1 PLUS culture droplets covered with OVOIL. Culture at 37°C 6% CO₂ overnight or for 2 days.

* An adequately calibrated warming block can be used for tubes instead of a warming incubator.

### Day 2
- **Assessment**
  - Assess embryo cleavage.

### Day 3
- **Assessment**
  - Assess embryo cleavage.

### Blastocyst culture
1. **Prepare micro-well dishes for Blastocyst culture**
   - **G-2™ PLUS**
   - **OVOIL**

In the morning of day 3, prepare micro droplet culture dishes with 25 µL droplets of G-2 PLUS for washing and for culture. Cover with OVOIL and pre-equilibrate at 37°C 6% CO₂ ≥ 6 h.

2. **Move embryos to G-2™ PLUS**
   - In the afternoon of day 3, wash the embryos extensively in equilibrated G-2 PLUS droplets and transfer the embryos to G-2 PLUS culture droplets, maximum 5 embryos per droplet. Culture at 37°C 6% CO₂ 2 days.

### Day 4
- **Prepare micro-droplet culture dishes**
  - **G-2 PLUS**
  - **OVOIL**

Prepare centre well dishes with fresh G-2 PLUS. Prepare micro-droplet dishes for prolonged culture if needed and pre-equilibrate at 37°C 6% CO₂ overnight.

### Day 5
- **In the morning of day 5**
  - Assess embryo cleavage, and move the blastocysts selected for transfer and cryo preservation to the equilibrated G-2 PLUS centre well dishes and leave at 37°C 6% CO₂ until 10-30 min before transfer.

For blastocyst transfer, see separate Embryo transfer protocol.