

# SPERM PREPARATION

## Swim-up method

### Day -1

#### The day before oocyte pick-up



G-IVF™ PLUS

Pre-equilibrate G-IVF PLUS at

**37°C 6 % CO<sub>2</sub>  
overnight**



G-RINSE™

Pre-rinse all utensils, including tubes and dishes, with G-RINSE.

### Day 0

#### 1. Assess the semen sample

#### 2. Prepare swim-up tubes

Pipette 1.0 mL of semen into a rinsed tube. Make up 2-4 tubes, depending on semen volume. Carefully overlay 2.0 mL of pre-equilibrated G-IVF PLUS. Place the tubes in the incubator at



**37°C 6 % CO<sub>2</sub>  
30-60 min**

#### 3. Wash I

Aspirate the top medium without touching the underlying semen and transfer to a clean tube. Add 5 mL of equilibrated G-IVF PLUS, mix and centrifuge at



**300-600g 10 min**

#### 4. Wash II

Discard the supernatant and re-suspend the pellet in 5 mL of equilibrated G-IVF PLUS and repeat the centrifugation at



**300-600g 10 min**

#### 5. Assess sperm preparation

Discard the supernatants and combine all pellets. Re-suspend the pellets in 0.5-1.0 mL of equilibrated G-IVF PLUS, depending on sample quality.



Determine motility and concentration of spermatozoa in the washed sample.

#### 6. Dilution

Dilute with equilibrated G-IVF PLUS to a final concentration of 75,000–200,000 motile sperms/mL.

#### 7. Preparation of insemination dishes

Prepare rinsed insemination centre well dishes with 0.5-1.0 mL of sperm solution and pre-equilibrate.



**37°C 6 % CO<sub>2</sub> >2 h**

If oil overlay is used, droplets of at least 100 µL volume are recommended. Equilibrate the dishes at

**37°C 6 % CO<sub>2</sub> >6 h**

#### 8. Insemination

Transfer the oocytes to the centre well dishes and leave at

**37°C 6 % CO<sub>2</sub>  
overnight**

**Alternatively:** Add equilibrated sperm suspension to equilibrated centre well dishes with the oocytes already present.