

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 % CO₂
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



Semen

**37°C 6 % CO₂
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 % CO₂ >2 h

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

37°C 6 % CO₂ >6 h

8. Insemination

Transfer the oocytes to the centre well dishes and leave at

**37°C 6 % CO₂
overnight**

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