SPERM PREPARATION

Density gradient centrifugation method

Directions for supplementation of un-supplemented G-Series™ media can be found in the G-Series Manual on www.vitrolife.com. Once supplemented, the media should be used as the G-Series PLUS media described below.

Day -1

The day before oocyte pick-up

G-IVF™ PLUS

Pre-equilibrate G-IVF PLUS at

37°C 6 % CO_2 overnight

G-RINSE™

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

Day 0

1. Assess the semen sample

2. Prepare gradient solutions

SpermGrad™

G-IVF PLUS

or

SpermGrad RTU

If you use SpermGrad RTU go to paragraph 3.

Dilution of SpermGrad

Mix SpermGrad with G-IVF PLUS in separate rinsed tubes to obtain 90 % and 45 % stock solutions. For 90 % stock solution, mix 9.0 mL SpermGrad with 1.0 mL G-IVF PLUS and for 45 % stock solution, mix 4.5 mL SpermGrad with 5.5 mL G-IVF PLUS.

3. Prepare gradients

Pipette 1.5 mL of the 90 % solution into the rinsed tube first and then slowly pipette 1.5 mL of the 45 % solution on top of it. Finally, 1.0 mL of the semen is layered on the top.

Make up 2-4 gradient tubes. Before use, allow the stock solutions to warm to ambient temperature.

4. Centrifuge the gradients at

300-600g 10-20 min

5. Wash I

Remove the two top layers. Transfer the pellets to new rinsed tubes and re-suspend with 5 mL equilibrated G-IVF PLUS and centrifuge again at

300-600g 10 min

6. Wash II

Aspirate and discard the supernatant. Transfer the pellets to new rinsed tubes and re-suspend with 5 mL equilibrated G-IVF PLUS and centrifuge again at

300-600g 10 min

7. Assess sperm preparation

Aspirate and discard the supernatants. Combine all pellets in a new rinsed tube and re-suspend 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.

8. Dilution

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

9. Preparation of insemination dishes

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

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

37°C 6 % CO_2 >2 h

10. Insemination

Transfer the oocytes to the insemination centre well dishes and leave at

37°C 6 % CO_2 overnight

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

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