SPERM FREEZING
Sperm freezing when using cryovials

**Freezing**

1. **Assess the semen sample**
   - Ensure that both liquified semen and SpermFreeze Solution are at ambient temp.
   - Measure the total volume of semen and carry out semen analysis as required.

2. **Add SpermFreeze Solution**
   - Dilute with equal volume of semen and SpermFreeze Solution. Add SpermFreeze Solution slowly and dropwise to the semen and then carefully tilt after each drop added. Close the lid tightly and turn the tube upside down 20 times, being careful not to create bubbles.

3. **Leave in room temperature**
   - > 10 min

4. **Load vials**
   - Mark cryovials with patient ID and load the semen mixture into cryovials. Do not fill cryovials completely to allow for expansion.

5. **Freezing**
   - Place the cryovials upright on a 1-3 cm styrofoam board in a liquid nitrogen bath. Leave for 30 min.

6. **Store in N₂(l)**
   - Transfer the cryovials quickly into the liquid nitrogen and store at -196 °C.

**Thawing**

The day before thawing:

1. **Remove cryovials from N₂(l)**
   - Remove cryovials from -196 °C and place them in a water bath at 35 ± 2 °C for 10 min.
   - Wipe the cryovials dry with a clean paper towel.

2. **Dilute with G-IVF PLUS**
   - Transfer semen mixture to clean test tubes and dilute with equal amount of equilibrated G-IVF PLUS. G-IVF PLUS should be added dropwise to the semen mixture and the solution carefully mixed after each addition.

**G-IVF™ PLUS**

Pre-equilibrate G-IVF PLUS at 37°C, 6% CO₂ overnight.

**3. Gradient separation**
   - Continue with gradient separation according to the G-series manual.

   | SpermGrad™ | G-IVF PLUS | G-IVF RTU |

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**Vitrolife**

*Freezing program for machine:
   - Start temperature: +20°C
   - -5°C/min to -8°C
   - Hold 1 min
   - -10°C/min to -25°C
   - -25°C/min to -196°C

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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.