Manufacturer: MFC Global Ltd. 17 Sacharov st. Rishon Lezion 7570719, Israel

Sperm vitrification procedure:

1. Collect the individual sperm cells into one or more micro-drops of sperm handling medium covered with light paraffin oil in a 50-100 mm tissue culture dish.
2. In sterile conditions, remove the SpermVD from the pouch with sterile forceps and put the SpermVD on a sterile surface (sterile petri dish or petri dish cover can be used). (Fig.1)
3. Prepare sperm cryopreservation solution by mixing 1:1 Quinns Advantage™ Sperm Freezing Medium (Origio, Cat.#10670010) and sperm washing medium. Place three 0.5-1 µL drops of this solution in the center of designated areas and immediately transfer the SpermVD into the tissue culture dish with the sperm, prepared in the paragraph 1. Make sure that the drops of cryoprotectant solution are covered with oil. (Fig.2)
4. Using micromanipulator with injection pipette under ×20 magnification, transfer individual sperm cells to one or more drops of cryoprotectant solution on the SpermVD. (Fig.3)
5. Leave the SpermVD with the sperm cells for 15 min at room temperature.
6. Using the sterile forceps, gently take the SpermVD with the individual sperm cell on it out from the tissue culture plate, wait for the excess of oil to drop down and insert it into an empty 1.8-2ml cryovial, properly marked with LN2-resistant label or a cryomarker pen, and close it with the plug. (Fig.4) *Recommendation: do not close the plug tightly, so that the liquid nitrogen will gradually enter and fill the cryovial soon after immersion into the liquid nitrogen and thus allow safe handling of the cryovials with SpermVD.*
7. Put the cryovial with the SpermVD onto storage holder and gently immerse into liquid nitrogen for continuous storage. (Fig.5)

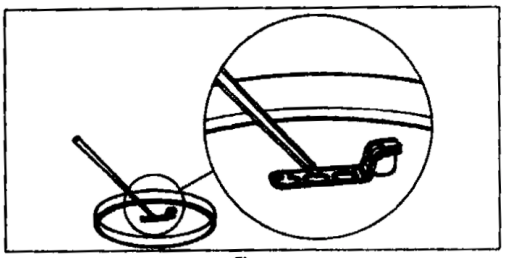
Fig.1

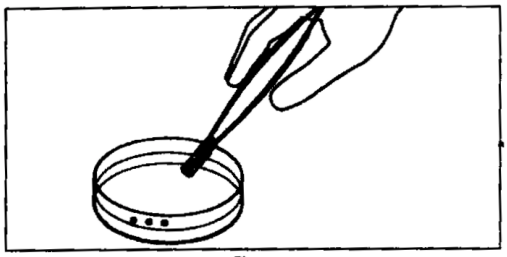
Fig.2

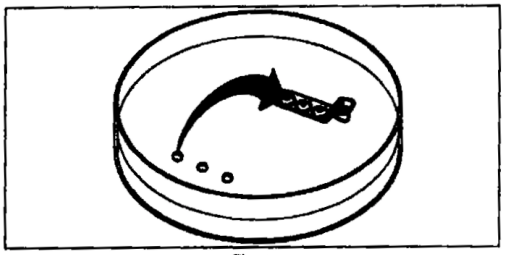
Fig.3

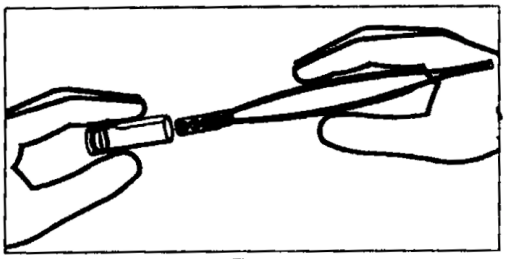
Fig.4

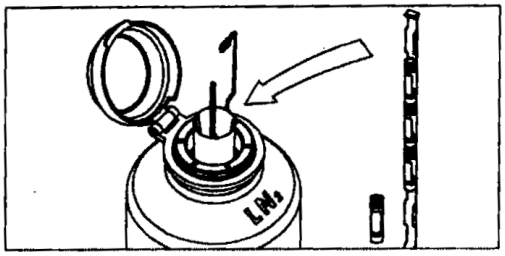

Fig.5

Warming procedure:

1. Prepare the tissue culture plate (50-10mm) with drops of PVP and sperm handling medium covered with oil (transfer dish).
2. Take the cryovial with the SpermVD out of the liquid nitrogen directly into room temperature and leave the cryovial unopened for 5 min at room temperature.
3. With sterile forceps transfer the SpermVD form the cryovial and immerse it into a transfer dish. Make sure that the drops with the sperm are covered with oil.
4. Using micromanipulator with injection pipette under ×20 magnification, transfer the sperm cells from the SpermVD to drops with sperm handling medium. (Fig.6)

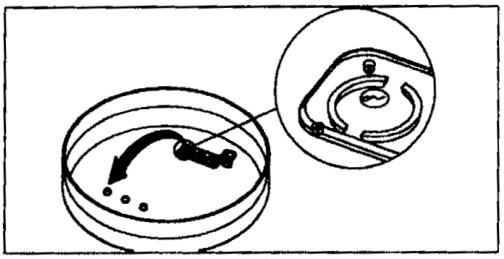
Fig.6