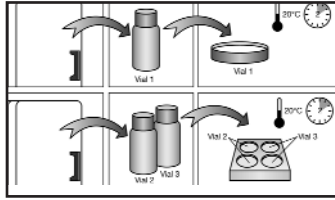


Procedure (OocyteFreeze™)

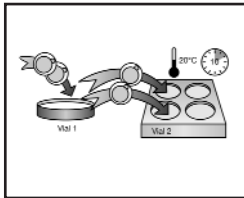


1. Label dishes for freezing solutions:

- A petri dish (Dish 1) is prepared using 3 ml of Vial 1 in which the oocytes are washed.
- Prepare a 4-well dish (Dish 2), with the two wells to the left containing 0.5 ml of Vial 2 and the two wells to the right containing 0.5 ml of Vial 3.

Equilibrate to room temperature.

- ### 2. Label the cryo straws with unique information. Do not write directly on straws as the solvents can penetrate the straw and harm the oocytes, but use special tape to protect the straw. If plugs are used, patient information can be directly transferred onto the plugs prior to insertion into the straw.

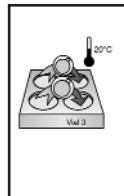


- ### 3. Aspirate some of the freezing solution Vial 2 followed by aspiration of the oocytes from dish 1. 1-2 oocytes per well are transferred from dish 1 to dish 2 where they are placed in the wells to the left and maintained for 10 minutes.

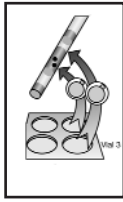
NOTE! The oocytes will float to the surface of the media and slowly sink to the bottom of the dish. It is therefore an advantage if you carefully "catch" the oocytes and place them at the bottom of the dish.

- ### 4. Just before the oocytes are transferred to the Vial 3 solution the insides of the cryo straws are cleaned with the same solution but from another dish.

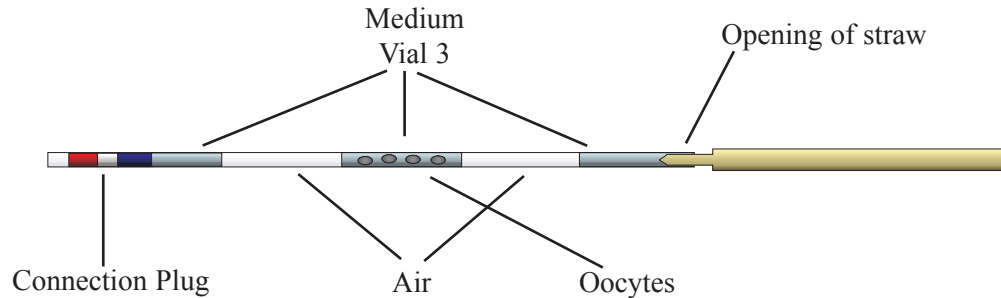
NOTE! Take up some air in the syringe, which facilitates removing the surplus Vial 3 solution. Do not allow this solution to come in contact with the plug.



- ### 5. Take up Vial 3 solution in the pipette. When the timer alarms, carefully take up the oocytes and transfer to the wells on the right side of the dish.

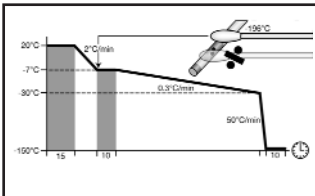


6. Using this medium as a transfer medium, rapidly load the oocytes in the right straw in the same manner as when you perform ET. When the first column of medium touches the plug it will seal, stopping all further movement in the straw (see diagram below).



7. Seal the opening of the straw with a plug, so that liquid nitrogen will not leak into the straw. Repeat for all oocytes.

Note! Heat sealing is not recommended as it causes cracking of the straws and the heat releases toxic material from the straws.

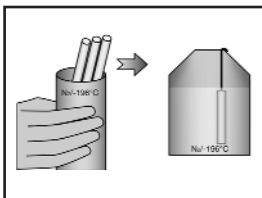


8. The straws are placed in the freezing machine and maintained for 15 minutes at room temperature (20°C) before the cooling program starts:

- Cool from room temperature to -7°C in steps of 2°C per minute.
- Seed manually at -7°C, and hold the temperature for 10 minutes.

NOTE! When the solution is white seeding has been initiated. Do not seed the straw close to the oocyte and do not drop or shake it. If there are air bubbles in the straw it may reduce oocyte survival.

- Cool from -7°C to -30°C in steps of 0.3°C per minute.
- Cool from -30°C to -150°C in steps of 50°C per minute.
- After 10-12 minutes of temperature stabilisation, the straws are transferred into the liquid nitrogen tank and stored until thawing.



NOTE! Attention must be paid to the handling of straws at low temperature as they may thaw very quickly.