Safe Preservation

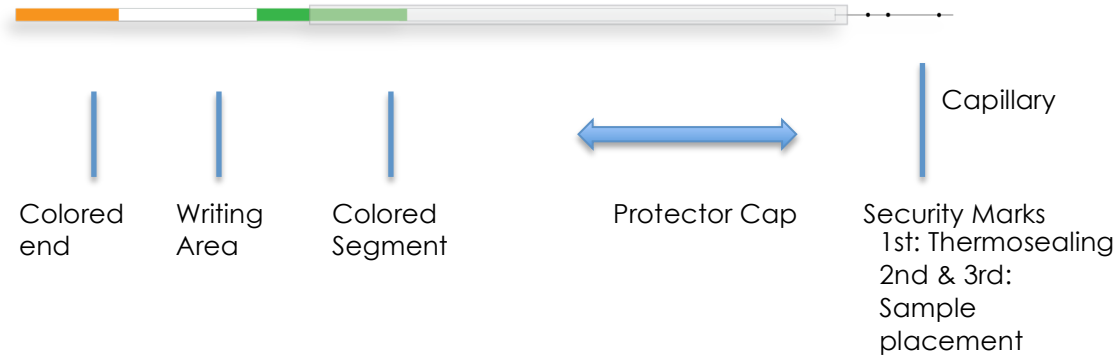
THE SAFESPEED TECHNIQUE

A closed device and serum-free media for ultra rapid and safe vitrification of human oocytes/embryos/blastocysts.

SafeSpeed Device

- The SafeSpeed® is a cryopreservation device which can be sealed as a closed device to hold oocytes and embryos in a specialized medium during cryopreservation procedures and subsequent long-term storage in a liquid nitrogen freezer.

- The SafeSpeed ® consists on a plastic straw container optimized as a closed system for cryopreservation procedures. It is composed of an ultra fine capillary assembled on a plastic straw and a protective cover sleeve.



QUALITY ASSURANCE

- The SafeSpeed ® are manufactured using the highest quality materials available. Each lot is visually inspected to meet optimized dimensional specifications and to assure the integrity of each device.
- The SafeSpeed ® are sterilized by gamma irradiation and validated to meet a sterility assurance level (SAL) of 10-6.
- Each lot of The SafeSpeed ® is tested for: Endotoxin by European Pharmacopeia, Limulus Amebocyte Lysate (LAL) methodology. The Straw Material accomplishes with the current USP. Sterility testing is done in accordance with ANSI/AAMI/ISO 11137. All results of any specific lot are reported Certificate of Analysis (CoA) which is available upon request.

STORAGE INSTRUCTIONS AND STABILITY

- Store in original sterile pack at 15-30 degree Celsius.
- The SafeSpeed ® is stable until the expiration date shown on package label when stored as directed.
- Each SafeSpeed ® is intended for single use.

Vitrification

SafeSpeed cooling technique is designed for rapid and safe vitrification of oocytes, embryos and blastocysts.

SafeSpeed Materials

1 x Vial WS (1 mL): Washing Solution.
1 x Vial ES (1 mL): Equilibration Solution.
1 x Vial VS (1 mL): Vitrification Solution.
1 x SafeSpeed device
1 x SafeSpeed connector.
1 x SafeSealer
1 x SafeBox

Other Materials

1 x Aspiration system (Mouth pipette, micromanipulator)
1 x Visotube/cryocane
2 x Standard embryo-tested Petri dish.
1 x Pasteur or Stripper Pipette

Use a pulled Pasteur pipette or Stripper pipette that has a suitable internal diameter for oocyte (135 µm), embryo and blastocyst (160-200 µm).

Connect the SafeSpeed device to your preferred aspiration system through our dedicated connector to guarantee compatibility.

Preparation

1. Bring the following vials to room temperature for 30 minutes prior to use: Washing Solution (WS), Equilibration Solution (ES) and Vitrification Solution (VS).

2. Fill the Cold Bath of the SafeBox rack with liquid nitrogen and place a visotube/goblet into the handle.

   NOTE: If you do not own SafeBox, skip step 2 and prepare a reservoir (at least 15 cm deep) with enough liquid nitrogen to allow complete submersion of the Safespee device.

3. *For oocytes only*: Pre-equilibration plate: Prepare standard embryotested Petri Dish with the following configuration in a line:

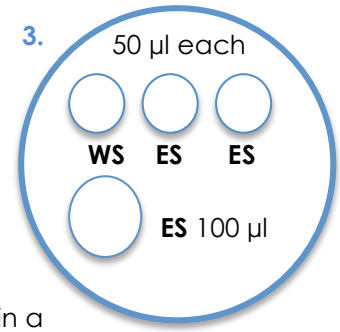
   a. First drop 50 µl Washing Solution (WS)

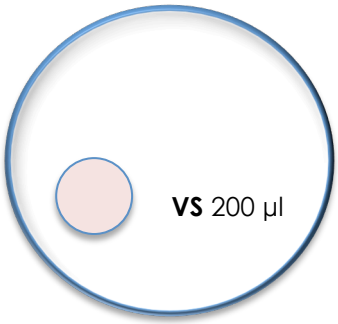
   b. Second drop 50µl Equilibration Solution

   c. Third drop 50µl Equilibration Solution

   d. Add a 100 µl drop of Equilibration Solution

   e. Add a 200 µl drop of Vitrification solution in a separate Petri Dish

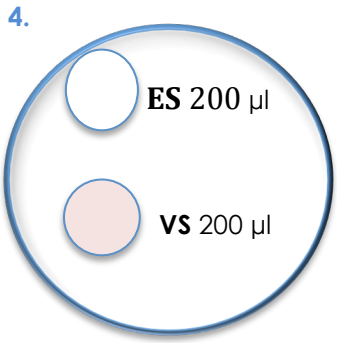




4. For *embryos/blastocysts*: Prepare a Petri Dish with the following configuration:

   a. Place 200 µl Equilibration Solution

   b. Place 200µl Vitrification Solution



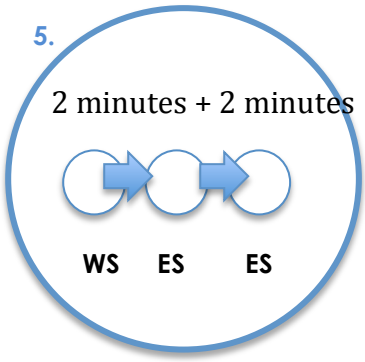
Equilibration

Bring out of the incubator the oocytes/embryos/blastocysts

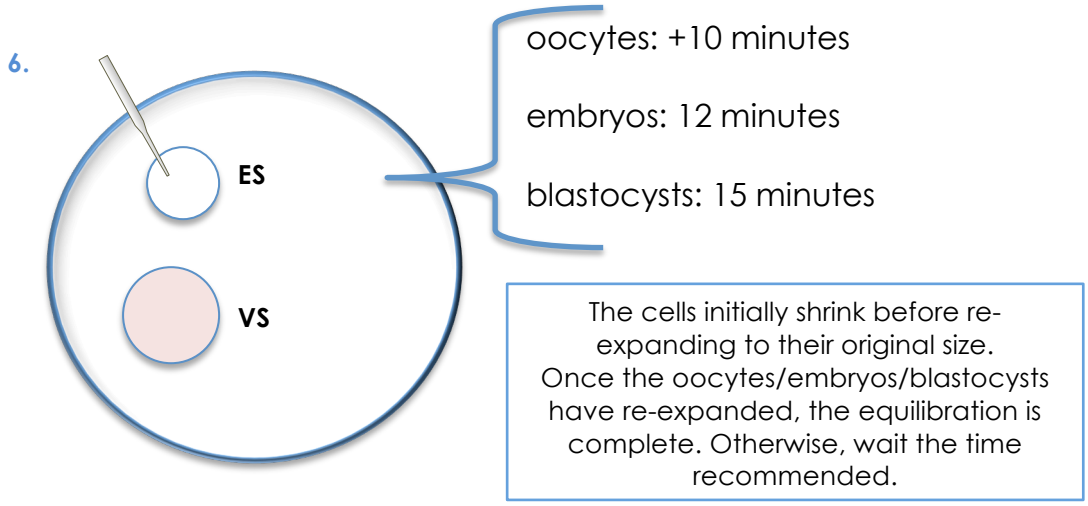
5. *For oocytes only*: Pre-equilibration plate: Using a suitable pipette, transfer a maximum of 4 oocytes in the first drop of Washing Solution (WS) of the lid.

a. Join the first and the second drop and wait 2 minutes.

b. Join the second and third drop and wait another 2 minutes.



6. *For oocytes/embryos/blastocysts*: Using a suitable pipette, transfer the specimens into the surface of the ES drop and let them equilibrate up to 10 additional minutes for oocytes, 12 minutes for embryos and 15 min for blastocysts.



The cells initially shrink before re-expanding to their original size. Once the oocytes/embryos/blastocysts have re-expanded, the equilibration is complete. Otherwise, wait the time recommended.

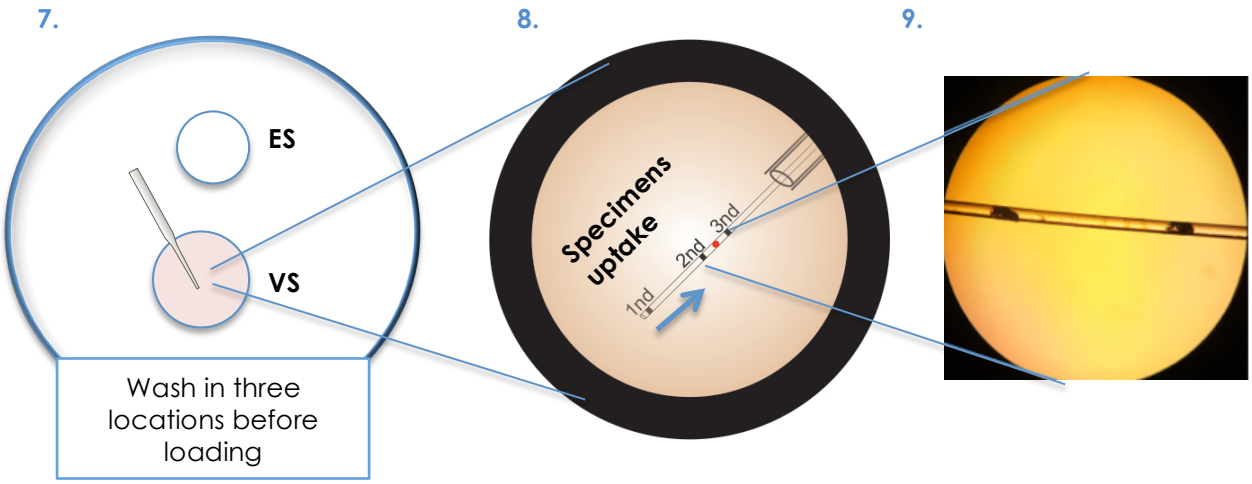
Vitrification

The following steps, from 7 to 12, should be performed in 60-90 seconds.

7. Transfer the oocytes/embryos/blastocysts in a small amount of ES into the VS drop (the cells shrink again), and wash them thoroughly (3 locations) in 30 seconds.

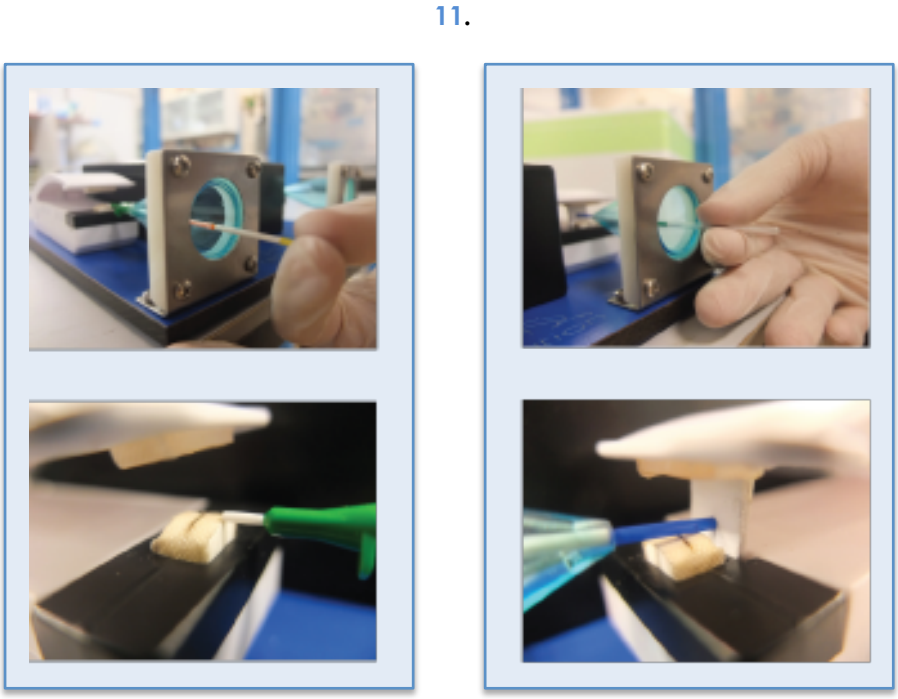
8. Gently load the specimens into the SafeSpeed capillary, placing them in between 2nd and 3rd mark by gentle aspiration. Always perform a controlled uptake of the media and specimens. CAUTION: do not introduce air during the loading process.

9. Confirm that the specimens are located in between the 2nd and 3rd Security mark borders.
CAUTION: Failure to confirm sample location could result in damaged sample.



10. Capillary Sealing: Heat-seal the fine capillary below the 1st mark without removing the connector. Introduce it carefully into the Capillary Slot until it hits the end. The capillary is now on position; firmly press for 1 second.

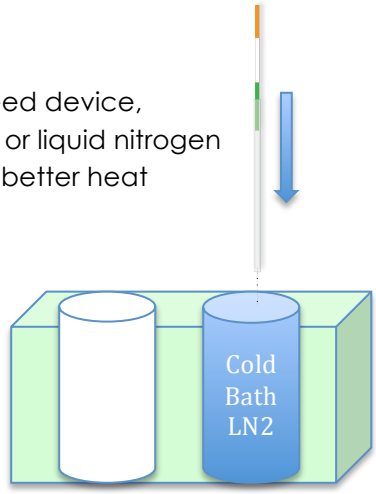
11. Colored end Sealing: Remove the connector and heat-seal the wide end of the SafeSpeed device. To seal, introduce the colored end of the SafeSpeed device into the Wide Slot until it hits the end and press <u>strongly</u> for 3 seconds.



12. Extremely fast, plunge vertically the sealed SafeSpeed device, capillary side down first, into the SafeBox Cold Bath or liquid nitrogen reservoir, shaking by hand during one second for a better heat transfer.

CAUTION: Avoid hitting the walls or bottom of the wells with the capillary.

12.



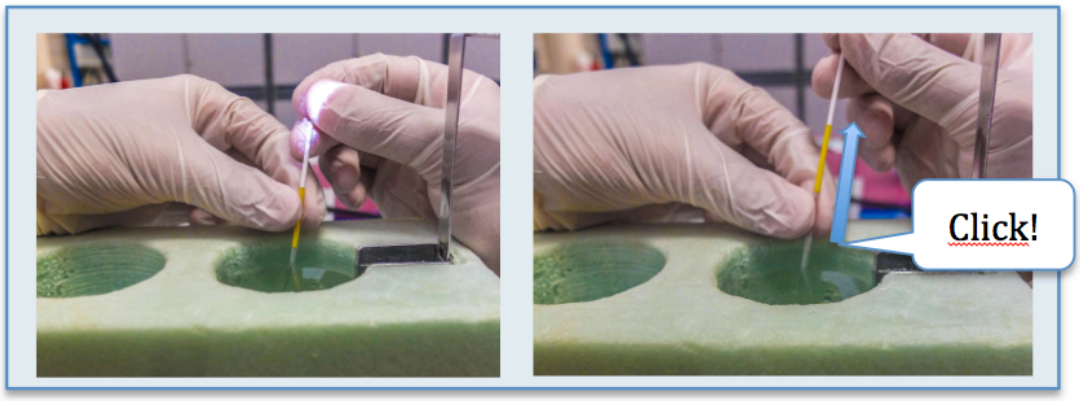
Storage

13. Without releasing the SafeSpeed device, grip the plastic cover sleeve and aseptically pull up the safeSpeed device until the protector clicks, at the end of the second colored area. The capillary is now fully covered. Ensure that during this movement the capillary is completely submerged by liquid nitrogen.

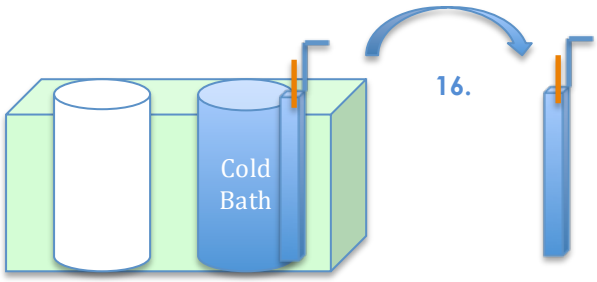
14. Store the sealed SafeSpeed device in a goblet or cryocane in the transfer handle.

15. Following vitrification, quickly transfer the visotube/goblet containing the SafeSpeed device, with the vitrified specimens, to the long-term storage tank. Make sure that the vitrified cells are submerged under liquid nitrogen at all times.

14.



15.



16.

With tweezers, take the visotube/goblet containing the SafeSpeed from the transfer handle to your long storage tank

Warming

SafeSpeed warming technique is designed for ultra rapid and safe thawing of vitrified human oocytes/embryos/blastocysts

Material

1 x Vial TS (5 mL): Thawing Solution.
1 x Vial DS (1 mL): Diluent Solution.
1 x Vial WS (1 mL): Washing Solution.
1 x SafeSpeed connector.
1 x SafeBox

Other Material

1 x Standard embryo-tested Petri dish.
1 x Sharp scissors or cutting instrument.
1 x Aspiration system (Mouth pipette, micromanipulator).

Use a pulled Pasteur pipette or Stripper pipette that has a suitable internal diameter for oocyte (135 µm), embryo and blastocyst (160-200 µm).

Connect the SafeSpeed device to your preferred aspiration system through our dedicated connector to guarantee compatibility.

Preparation

1. Pre-warm the Thawing Solution and a Petri Dish to 37°C in the incubator and expose Diluent Solution and Washing Solution to room temperature for at least 30 minutes.

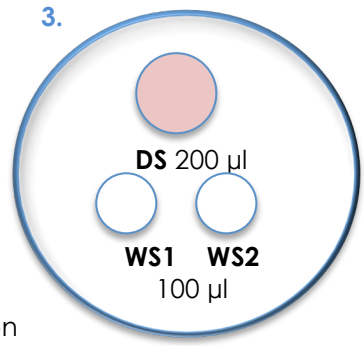
2. Fill the Cold Bath of the SafeBox rack with liquid nitrogen. Otherwise, prepare a reservoir (at least 15 cm deep) with enough liquid nitrogen to allow complete submersion of a visotube/goblet.

3. Prepare a standard embryotested Petri Dish with the following configuration:

    a. Label DS and place 200 µl Diluent Solution.

    b. Label WS1 and place 100 µl Washing solution

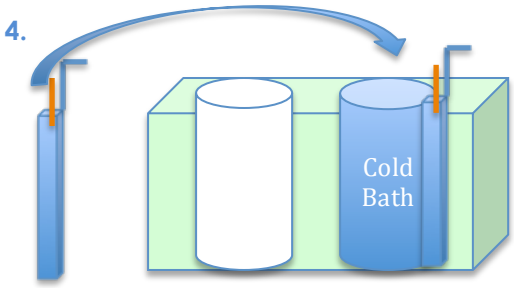
    c. Label WS2 and place 100 µl Washing solution

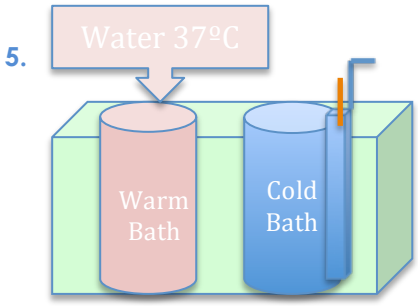


4. Collect the visotube/cryocane containing the SafeSpeed device or with the specimens and transfer it within the Transfer Handle to the Cold Bath of the SafeBox or your liquid nitrogen reservoir. Make sure the SafeSpeed device remains submerged under liquid nitrogen at all times.

5. Fill the Warm Bath of the SafeBox rack with water at 37°C. Otherwise fill a reservoir, at least 15 cm deep, with water at 37°C, and place it close to the LN2 reservoir.

6. Take the vial of Thawing Solution (TS) out of the incubator and place 200 µl into the warm Petri Dish. Place it under microscope view.

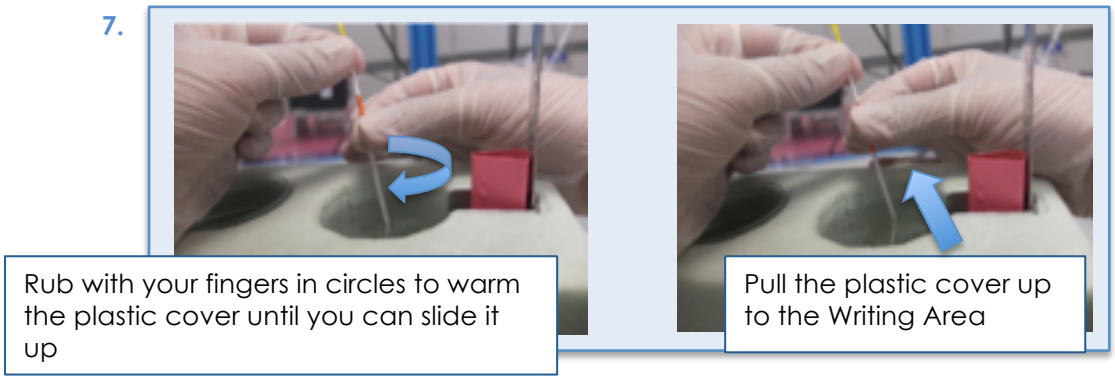




Warming

7. Pick firmly the SafeSpeed device, lift it until you can grip the plastic cover sleeve, and then slide it up carefully along the second colored area to expose the capillary until it reaches the white writing space. The capillary is now exposed. Make sure that the capillary is constantly fully immersed in the liquid nitrogen along the process.

8. Without releasing the device, cut the colored end of the SafeSpeed device. The device is now open, avoid the entrance of LN2.

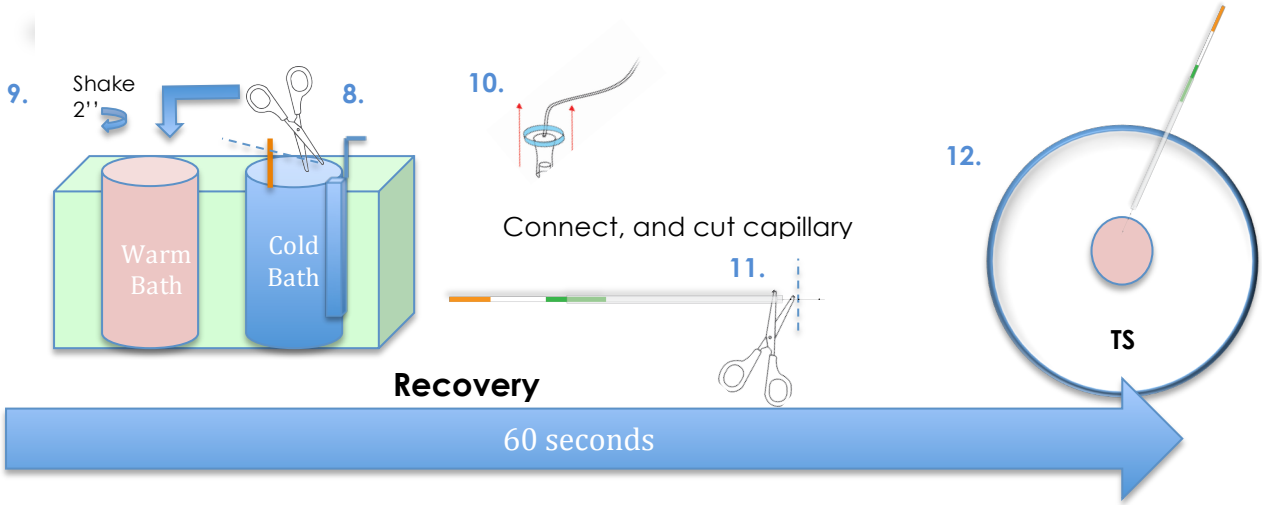


9. Extremely fast, transfer the SafeSpeed device, capillary side down first, into the Warm Bath (water 37°C), shaking by hand during two seconds for a better heat transfer. CAUTION: Avoid hitting the walls or bottom of the water bath in the process.

10. Immediately after, gently wipe off remaining water and connect the aspiration system to the cut colored end of the SafeSpeed device.

11. With sharp scissors, cut the SafeSpeed device just over the 1st Security Mark in the capillary end. Perform the cutting over the drop of Thawing Solution. CAUTION: Use sharp cutting instrument for a clean cut; avoid crushing the capillary.

12. Gently expel the oocytes/embryos/blastocysts into Thawing Solution (TS) at 37°C. Avoid expelling bubbles. Always be aware where they float in this solution. Wait up to 1 minute max before recovering (showing clear membrane boundary).

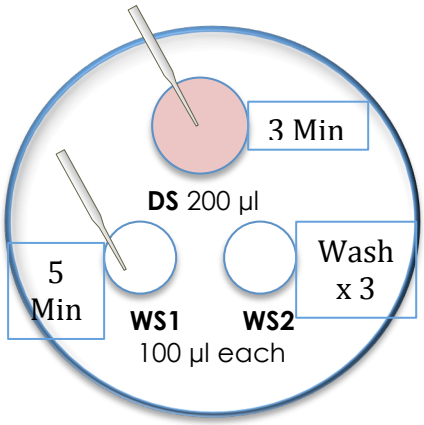


13. Aspirate the oocyte/embryo/blastocyst from TS, taking a small amount of media into the pipette and gently place it on the bottom of DS. For oocytes, gradually push out the oocyte from the pipette to the next solution, by pipetting small volumes in and out. Wait for 3 minutes.

14. Rinse the pipette with clean DS media and aspirate the ocyte/embryo/blastocyst with a small amount of DS media and gently place it on the bottom of WS1. For oocytes gradually push out the oocyte from the pipette to the next solution, by pipetting small volumes in and out. Wait for 5 minutes.

15. Aspirate the oocyte/embryo and gently place it on the top of the WS2 drop. Rinse the pipette with clean WS2 and then recover it from the bottom and wash the oocyte/embryo/blastocyst in three locations.

16. Transfer the oocyte/embryo/blastocyst to a culture dish containing the appropriate culture medium. Incubate the oocyte/embryo/blastocyst at 37°C to complete recovery. Wait for at least two hours before performing IVF/ICSI.



TRICKS AND TIPS

- Before starting vitrification/warming, prepare all the setting carefully. Organize to minimize the time the solutions at 37ºC, TS and water, are exposed to room temperature.

- Pre-load the capillary with VS media carefully, always controlling the amount introduced. If too much media is loaded, it may overflow out the back and block the capillary.

- Always work with the capillary placed horizontally along the Petri dish, this way you will be able to follow the whole loading process without adjusting position and focus.

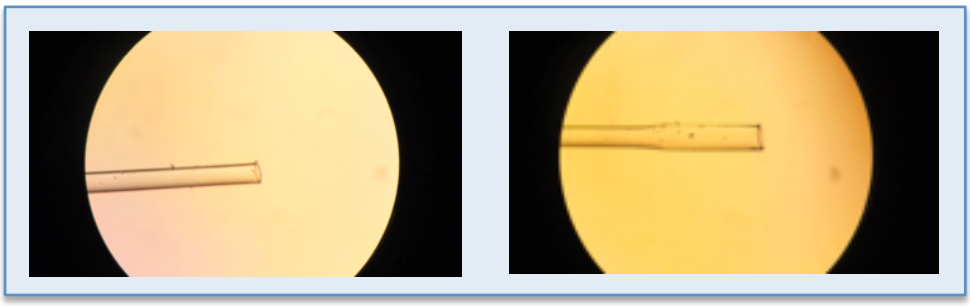
- When loading the oocytes/embryos, set the microscope field to see from the tip of the capillary up to the two security marks, for easier control of the loading process.

- Once the oocytes/embryos are in between the security marks, you can stop aspirating and lift the capillary out of the VS drop. The specimens will be fixed in position.

- The quenching of the SafeSpeed device in liquid nitrogen/37ºC water must always be performed with the cover sleeve pulled up, exposing the capillary, for a better heat transfer.

- Always extreme caution when plunging the SafeSpeed device, to avoid hitting the bottom or walls of the SafeBox baths, capillary could break.

- If you find the tip of the capillary crushed due to an innadequate cut, just cut again over the crushed área before expelling the oocytes/ embryos/ blastocysts.



Clean-cut capillary and crushed capillary