VITRIFICATION KIT

-Cryotop Method-

Vitrification Protocol for Embryo

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Reference)
Kuwayama, M., All round vitrification of human oocytes and embryos. J. Assist. Reprod. Gentic. 17(8) 477, 2000.

Kuwayama, M. Highly efficient vitrification method for cryopreservation of human oocytes. RBM Online. Vol.11 (3), 300-308 2005.

1. Preparation -Vitrification-

- Vitrification Kit

  Step 1 (ES ;Equilibration Solution)

  Step 2 (VS ;Vitrification Solution)

- Microscope
- Two petri dishes (35mm) ex. FALCON 1008
- Liquid N_2
- Rack cooling
- Stopwatch or timer
- Pipette glass
- Tweezers

2. Vitrification Protocol

1. Warm ES and VS to room temperature (20-27℃)

2. Write “ES” or “VS” on the bottom surface of 35mm-dish. Then turn tubes of ES and VS upside down two times to mix the solutions, and pour full content into each dish.

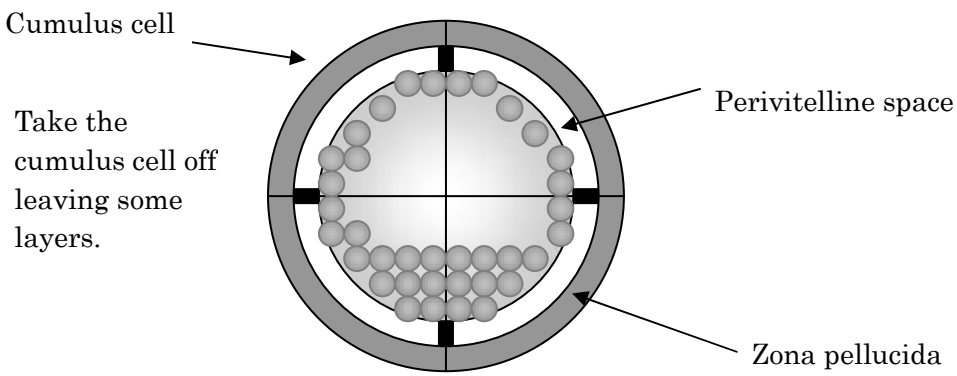
3. Write the necessary information on the bar of Cryotop.

4. Take the culture dish containing an embryo from the incubator.

5. Check the embryo under the microscope morphologically and record the quality and any information.

* Low quality embryos are not appropriate for cryopreservation (vitrification).

6. Observe the embryo rolling it with a pipette.



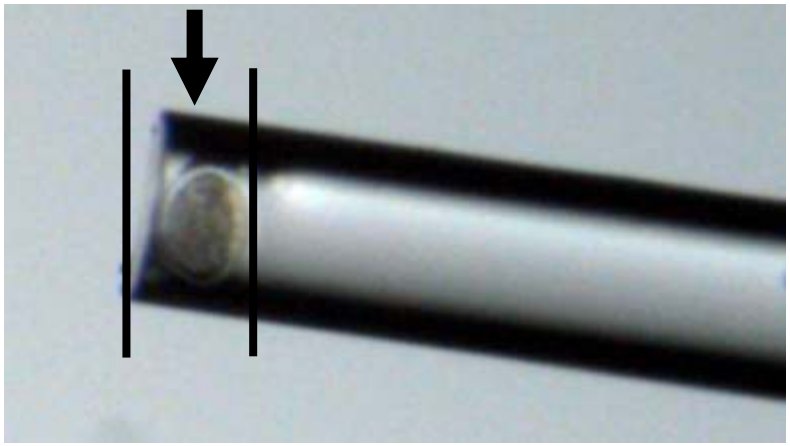
<u>Important</u>

▬ : Compare the width of perivitelline space at these four points with thickness of zona pellucida and keep it in mind (ex. 1:1). It allows seeing the state of dehydration and equilibrium after immersing embryo in ES.

7. Fill the container with fresh liquid nitrogen (～90% full).

8. Aspirate the embryo using a capillary and keep it within the end.

9. Transfer the embryo onto the surface of ES with minimum medium.



The following steps are operated timing with a stop-watch.

10. The embryo is deposited at the bottom and shrinks spontaneously in 30 seconds.
    The embryo volume will recover gradually.
    * For equilibration, the oocyte volume is required to be recovered completely.
    * Immersion time in ES is limited within 15 minutes.

The following steps from 11 to 17 should be completed within 1 minute.

11. Aspirate the embryo with the capillary and keep it within the end.

12. Transfer the embryo onto the surface of VS with minimum amount of ES solution.

13. Expel any remaining ES solution out of the capillary. Then wash inside of the capillary aspirating in VS.

14. Aspirate the embryo with the capillary and pour out into fresh VS. Repeat this operation three times at different position in VS solution.

15. Hold the Cryotop under a microscope and bring in focus by moving it up and down. If it is difficult to focus on, fix the Cryotop sheet on the bottom of an empty dish and put the embryo on it.

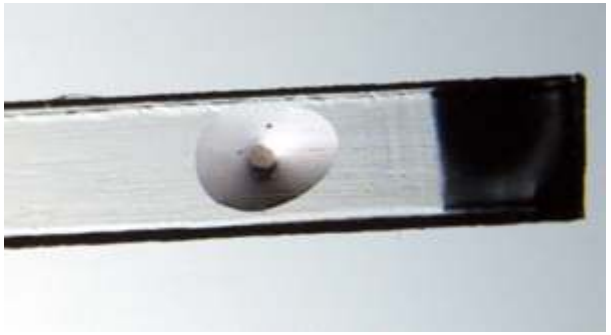
Example

16. Aspirate the embryo within the end of the capillary.

17. Put the embryo on the distal end of Cryotop sheet with minimum volume of VS solution (0.1μl or less) under the microscope.

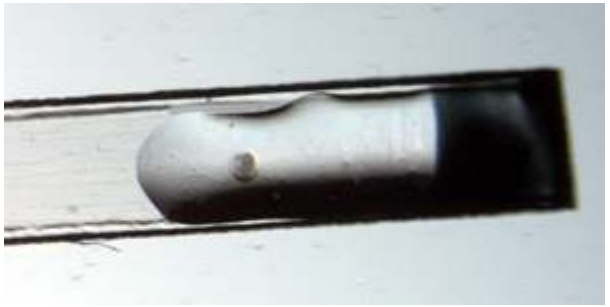
Good example

Droplet is made planar.

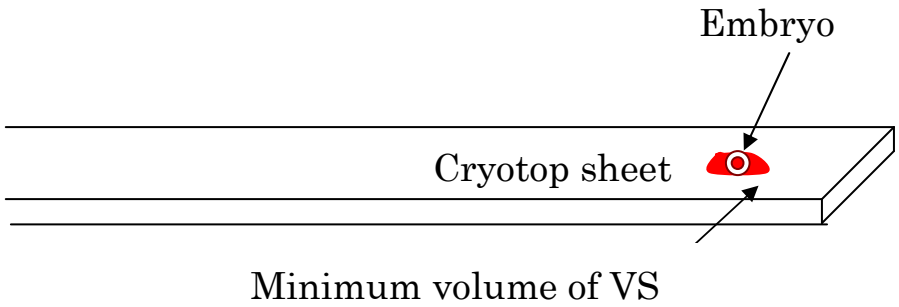


Bad example

Droplet is made steric.



18. Check if the embryo is on the sheet under the microscope.



19. Plunge the Cryotop into liquid nitrogen quickly.

    *Keep the Cryotop sheet in liquid nitrogen after this.

20. Hold the straw cap with tweezers and put the Cryotop in it tightly.

21. Check if the cap is fixed to the Cryotop and put it into the cane. Then transfer it to the storage tank for long time storage.

Straw holder

Code: VT-SH15

3. Preparation -Thawing-

- Vitrification Kit

  Step 1 (TS; Thawing Solution)

  Step 2 (DS; Diluent Solution)

  Step 3 (WS1; Washing Solution 1)

  Step 4 (WS2; Washing Solution 2)

- Microscope
- Four petri dishes (35mm) ex. FALCON 1008
- Liquid N_2
- Rack cooling
- Stopwatch or timer
- Pipette glass
- Tweezers

4. Thawing Protocol

1. Warm TS tubes with caps and dishes to 37℃ in an incubator.

   *Write "TS" on the bottom of the dishes before warming.

2. Transfer the cane into a container filled with fresh liquid nitrogen. Then take out the Cryotop from the cane handling in the container.

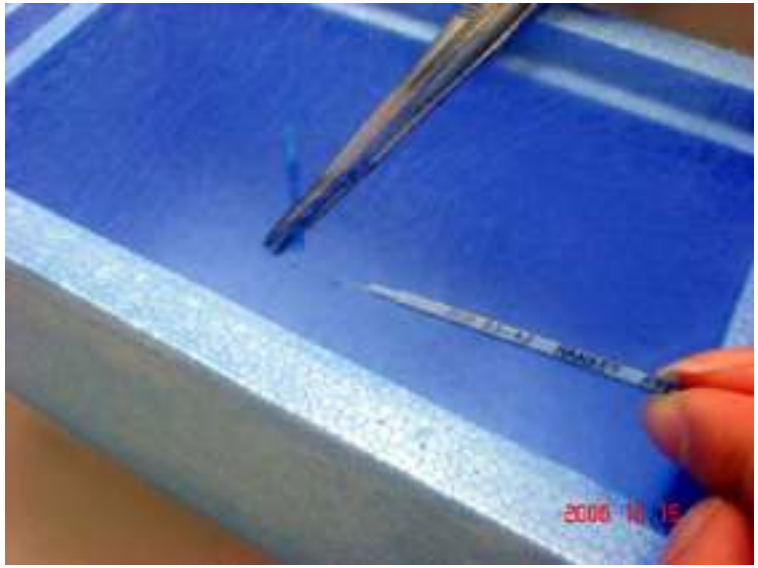
   *Put the container beside a microscope.

3. Write “DS”, “WS1” or “WS2” on the bottom surface of each 35mm-dish. Turn tubes (except for TS) upside down two times to mix the solutions, and pour full content into each dish.

The following steps are operated timing with a stop-watch.

4. Set the TS on a stage of microscope. Then bring the bottom of dish into focus.

5. Retrieve the straw cap from Cryotop with tweezers handling in liquid nitrogen.



6. Quickly immerse the sheet of Cryotop into TS.

7. Aspirate the embryo within the end of the capillary **at 1 minute after putting into TS**.

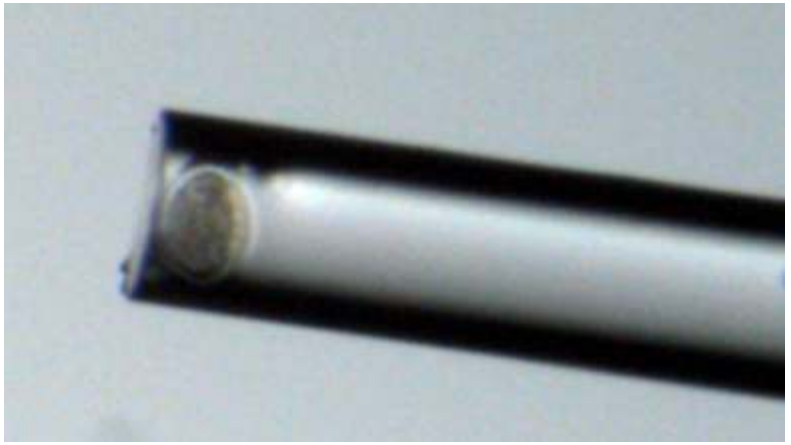
8. Transfer the embryo onto the bottom of DS dish with small amount of TS.

9. In **3 minutes in DS**, the embryo will swell temporally and then begin to shrink.

10. Transfer the embryo onto the bottom of WS1 dish with small amount of DS and keep for **5 minutes**.

11. Transfer the embryo onto the surface of WS2 dish with minimum amount of WS1 and keep for **5 minutes** on a plate warmer (37℃).

Caution: Treat the embryo within the end of the capillary to minimize the volume of WS1.



12. Transfer the embryo into the culture medium.

Culture time for recovery is 2 hours.

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