Warming

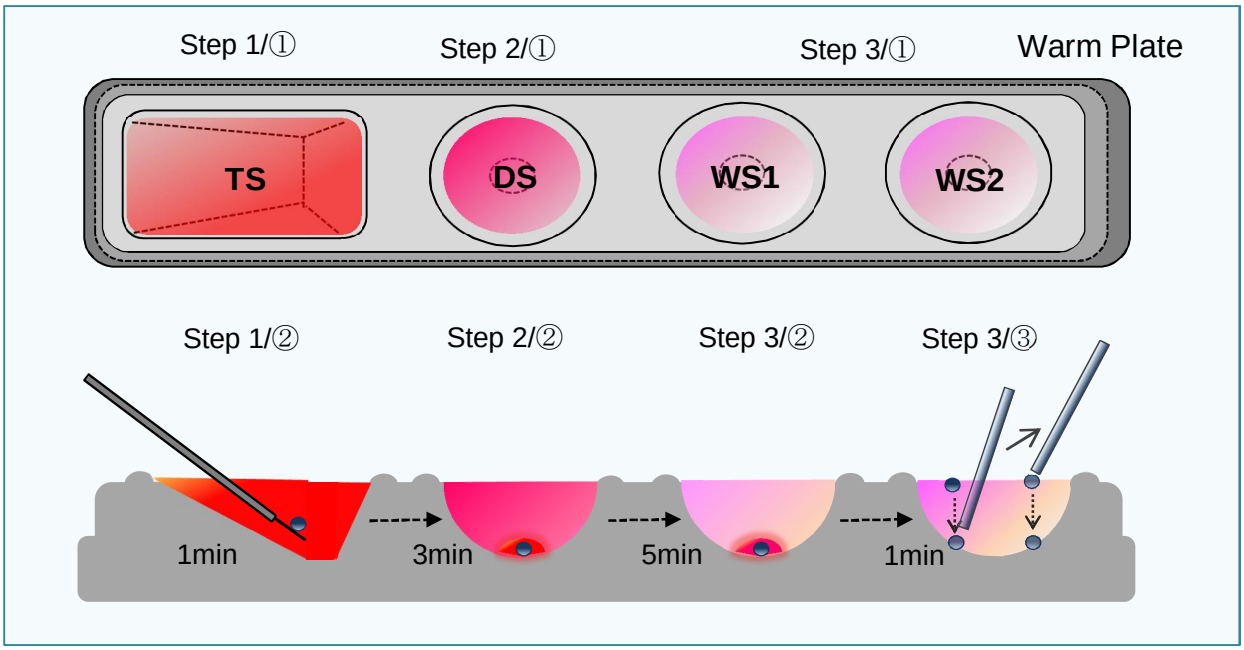
Materials

- **Cryotech Vitrification Kit**
  - -Warming Solution (TS): 1 vial of 1.8 ml
  - -Diluent Solution (DS): 1 vial of 0.5 ml
  - -Washing Solution (WS): 1 vial of 1ml
  - -1 Warm Plate with 4 wells
- Microscope (Turn off the heating plate)
- Stop watch (with count up function)
- Tweezers
- Micro pipette for 300εl

Preparation

1. Place the Warm Plate and TS vial (with rid) in the incubator at 37°C >3 hours before warming (overnight storage is recommended).
2. Bring DS and WS vials to room temperature (25~27°C) at least 1 hour before warming.
3. Prepare fresh liquid nitrogen.
4. Take the patientŝ cane out from a liquid nitrogen tank.
5. Take the Warm Plate and TS out of the incubator, and fill the rectangular well with 1.8 ml of TS (Fig. 7, Step 1/①).

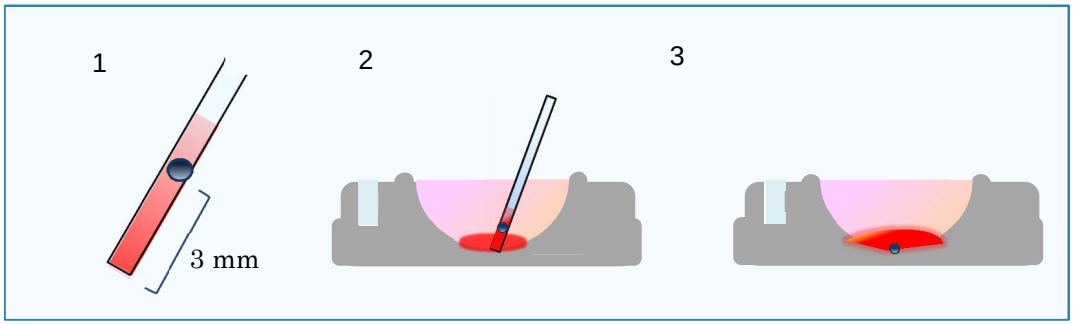
Fig. 7. Procedure for warming (Step 1-3)



Warming (1 min)

1. Quickly (within 1 sec) put the Cryotec into TS well.
   Start the stop watch for 1 min (Fig. 7, Step 1/②).
2. Oocyte/embryo separates from the Cryotec sheet by itself, and begins to float.
3. Confirm the oocyte/embryo existence in TS well.
4. While waiting, fill the DS well with 300 µl of Dilution Solution (Fig.7, Step 2/①).

Fig. 8. Gradual replacement of solutions (TS→DS, DS→WS1)



Dilution (3 min)

1. Aspirate the oocyte/embryo and 3mm long of TS into the pipette (Fig. 8, 1).
2. Blow out TS to the bottom center of DS (Fig. 8, 2), and expel the oocyte/embryo slowly bottom of TS layer in DS well (Fig. 8, 3). This is for most gradual replacement from TS to DS.
   Wait for 3 min (Fig. 7, Step 2/②).
3. While waiting, fill the WS1 and WS2 well with 300 µl of Washing Solution (Fig. 7, Step 3/①).

Washing 1 (5 min)

4. Aspirate the oocyte/embryo and 3 mm long of DS into the pipette (Fig. 8, 1).
5. Blow out DS into the bottom center of WS1 (Fig. 8, 2), and expel the oocyte/embryo slowly bottom of DS layer in WS1 well (Fig. 8, 3). This is for most gradual displacement from DS to WS1.
   Wait for 5 min (Fig. 7, Step3/②).
6. Give a survival judgment at the end of this step if the shrunk oocyte/embryo to be recovered or not.

Washing 2 (1 min)

7. Aspirate the oocyte/embryo with minimal volume of WS1.
8. Put the oocyte/embryo on the surface of the WS2 well (Fig. 7, Step3/③).
9. When oocyte/embryo sinks to bottom, aspirate and put on the surface to wash for 2 times in total.
10. Put the oocyte/embryo in the droplet of the culture media for the recovery for ICSI and ET.
    (After the warming, 2 to 4 hours culture for oocyte, and 3 hours for embryo is recommended)