Ovarian Tissue Vitrification Protocol

Products -Ova Cryo Kit
QR code

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Vitrification protocol

Ovarian tissue cryopreservation flow

Ovarian Tissue Processing → Equilibration of Vitrification Solution → Cooling (Vitrification) → Cryopreservation

PART 1 Materials Required

1. Ova Cryo Kit (Code. VT301S)
   Cryo1：20 mL×1
   Cryo2：20 mL×1
   Cryo3：20 mL×1
2. Ova Rinse (Code. OVR-100)
3. Square Measure (Code: Square Measure)*1
4. Ova Cryo Device Type M (Code：ODT×10)*2
5. Ova Cryo Closed Device CryoSheet (Code：OCS×10)*3
6. CryoSheet Cane (Code：CryoSheet Cane×10)*3
7. 1~10mL syringe ×1
8. 18~21G needle ×1
9. Spitz or centrifuge tube for follicular fluid collection
10. Dish for oocyte inspection
11. Microscope for oocyte inspection
12. Dish (OD 60mm) ×3 : for Cryo1, 2, 3
13. Dish (OD 60mm - 100mm) ×2 : for immersing the ovary in Ova Rinse
14. Scissors Note : 2 types of scissors ; straight-end and curved-end facilitate the preparation of ovarian tissue.
15. Curved tweezers (about 12cm)
16. Tweezers (about 20cm) ×2
17. Surgical knife (Blade No. 10)
18. Sterilized gauze
19. Count-up Timer
20. Liquid nitrogen
21. Liquid nitrogen container (Code: Cooling Rack)
22. Microtome blade with handle*1
23. Microtome blade*1
24. Surgical knife (Blade No.11)*1
25. Cane (C-2 Cane)*2
26. Sealer*3 Note : Tabletop and temperature adjustable sealer, capable of sealing dimensions over 5cm.
    For example, Fujiimpulse P-200.

*1 For the processing of ovarian tissue (Method 2)
*2 For Open System
*3 For Closed System

Ova Cryo Kit
（Ref. 82212　Code：VT301S）

Cryo1　Cryo2　Cryo3

Ova Rinse
（Ref. 82215　Code：OVR-100）

Square Measure
（Ref. 81212　Code：Square Measure）

Ova Cryo Device Type M
（Ref. 81213　Code：ODT×10）

Ova Cryo Closed Device CryoSheet
（Ref. 81214　Code：OCS×10）

Ova CryoSheet Cane
（Ref. 81215　Code：KOCC10）

PART2 Preparation

Preparation

Allow Cryo1, Cryo2, and Cryo3 to reach room temperature (25 to 27°C).
Label the dishes with "Cryo1," "Cryo2," and "Cryo3" using a labo marker.
Pour the entire contents of each corresponding liquid into the labeled dish.

STEP1

Wash the extracted ovarian tissue with Ova Rinse to remove excess blood.

STEP2

Pierce the follicles with an injection needle and aspirate the fluid with a syringe (Figure 1).

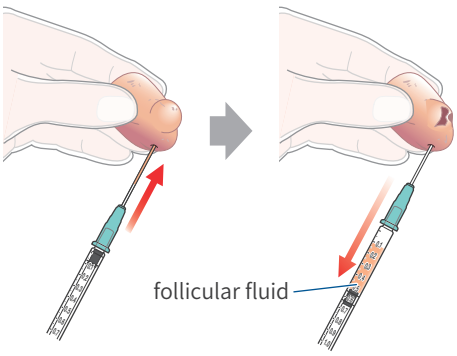


Fig.1

TIP

The immature oocytes retrieved are vitrified after in vitro maturation (IVM). Mature oocytes are either vitrified for preservation or used for in vitro fertilization (IVF).

PART3 Dissection of Ovarian Tissue

Method 1

Carefully proceed with the following steps using Ova Rinse, ensuring the ovarian tissue does not dry out.

STEP1

Using straight scissors, cut the ovaries in half along the equatorial plane (Figure 1).

Fig.1

STEP2

Divide the ovary in half, open it with the medullary side facing upward to prevent drying, immerse it in Ova Rinse, and start fine sectioning (Figure 2).

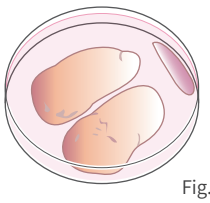


Fig.2

STEP3

Using curved scissors, remove the medulla from the opened ovary, leaving only the cortex. Gently hold the ovary with tweezers, and remove the medulla with curved scissors, repeating until the cortex is 1mm thick (Figure 3). Inserting the curved scissors in a scooping motion facilitates easy removal of the tissue.

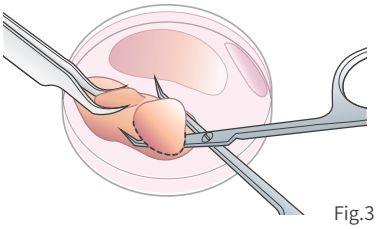
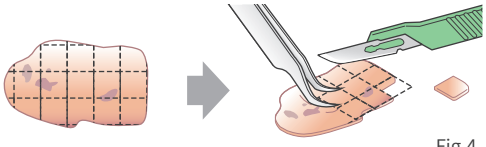


Fig.3

STEP4

Once you have cortex without medulla, use a surgical knife and cut it into 1cm x 1cm size (Figure 4). Removing the medulla after slicing makes the process easier.



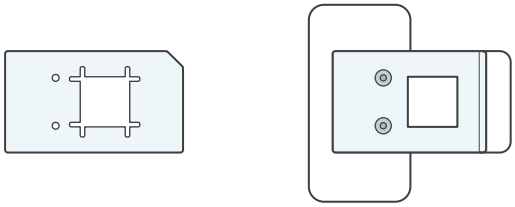
1cm square with a thickness of 1mm

Fig.4

Vitrification protocol

Method 2

Carefully proceed with the following steps using Ova Rinse, ensuring the ovarian tissue does not dry out.
If processing the ovarian cortex using Method 1 is challenging, please use the Square Measure.
The Square Measure comprises parts A and B (Figure 1).



Square Measure A　　Square Measure B

Fig.1

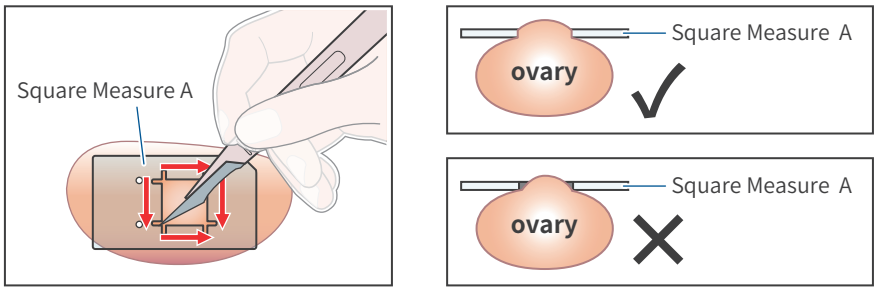
Preparation

Wipe off any excess wetness from the ovarian surface with sterile gauze to prevent the Square Measure from slipping.

STEP 1

Place Square Measure A on the area of ovarian surface where you plan to slice.
With a blade (blade No. 11), create incisions in a pattern along the inner frame of Square Measure A, ensuring the cuts intersect at the corners and penetrate to a depth of at least 1 mm.

TIP



Be sure to cut ovary from inner edge of Square Measure A.
(cut by drawing Sharp "#" line)

STEP 2

Attach Square Measure B to Square Measure A and place it on the ovary surface (Figure 2).

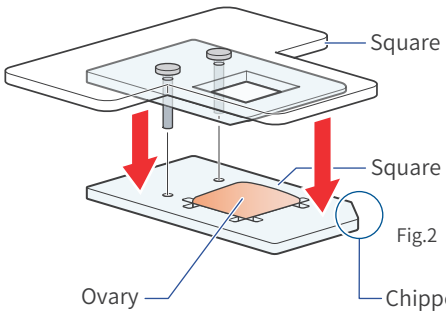


Fig.2

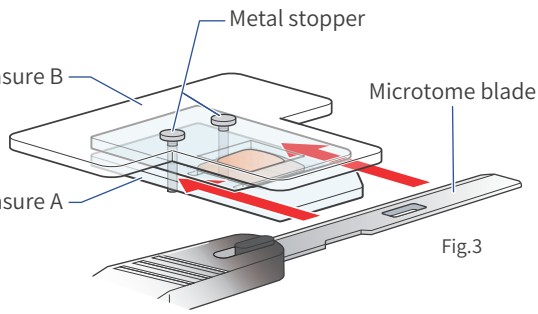
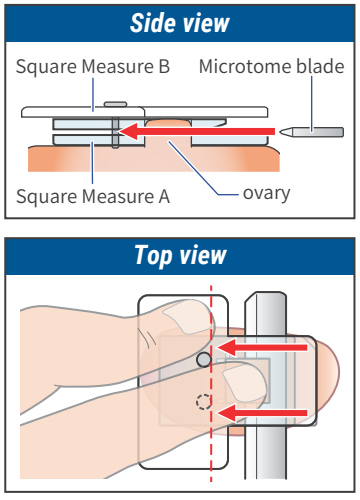


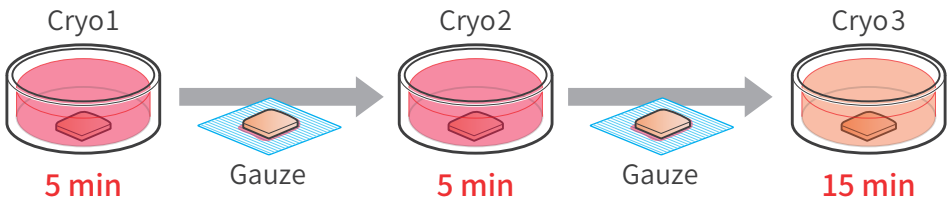
Fig.3



STEP 3

While holding down Square Measure from above, insert the microtome blade between Square Measure A and B, and smoothly slice the tissue until it reaches the metal stopper (Figure 3).

PART4 Equilibration



Multiple ovarian sections can be processed simultaneously with Cryo1, Cryo2, and Cryo3.

STEP1 Wipe off excess wetness from the ovarian sections with gauze and immerse them in Cryo1 for 5 minutes.

STEP2 After equilibrating in Cryo1, gently wipe off excess Cryo1 solution from the ovarian sections with gauze, then transfer them to Cryo2 and allow them to equilibrate for 5 minutes.

STEP3 After equilibrating in Cryo2, gently wipe off excess Cryo2 solution from the ovarian sections with gauze, then transfer them to Cryo3 and allow them to equilibrate for 15 minutes.

TIP

Prepare the Device (ODT or OCS) and liquid nitrogen while the ovarian tissue is equilibrating.

PART5 Vitrification for Cryopreservation

Open System

Preparation

Prepare the same number of Ova Cryo Devices (ODTs) as the number of prepared ovarian tissues. Ensure all ODT vials are labeled with patient IDs and necessary information.

STEP1

Wipe the ovarian sections with gauze. Spread them out to maximize their surface area and place them on the metal part of the device. Position the ovarian sections near the tip of the metal part, avoiding the base where the metal is embedded in the cap, and leave approximately 5 mm of space to place the ovaries (Figure 1).

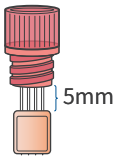


Fig.1

STEP2

Using tweezers, hold the device cap and quickly immerse it in liquid nitrogen (Figure 2).

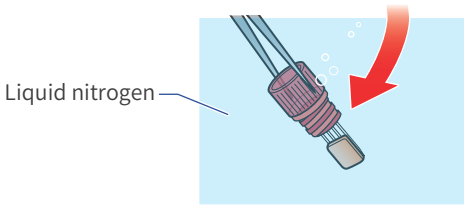


Fig.2

STEP3

After the ovarian sections have cooled, attach the vial to the cap and seal it while immersed in liquid nitrogen (Figure 3).

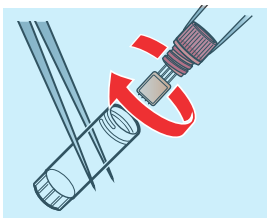


Fig.3

STEP4

Place the device into a cane and store it in the liquid nitrogen tank (Figure 4).

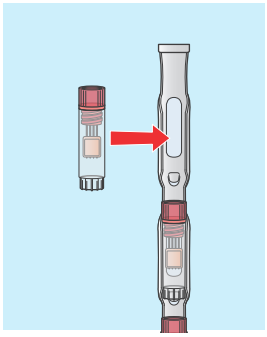


Fig.4

TIP

- Immerse the device into liquid nitrogen with the ovarian sections placed facing downward to prevent them from detaching.
- If ovarian sections detach from the device in liquid nitrogen, place the sections directly into the vial and cap it.

Closed System

Preparation

Label the patient information on the Ova Cryo Sheets for the number of ovarian sections, as well as on the corresponding Ova Cryo Sheet pouches (Figure 1).

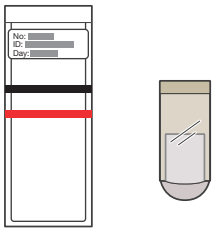


Fig.1

STEP1

Unroll the film portion. Use gauze to wipe the ovarian sections and place them within the frame of the device to maximize their surface area (Figure 2).

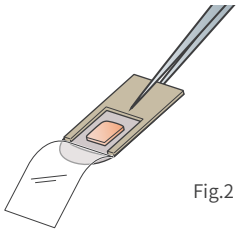


Fig.2

STEP2

Place the film over the ovarian sections, ensuring they are fully covered, and carefully seal it to remove any excess air.

STEP3

Place the device into the pouch designed for Ova Cryo Sheets. Remove air, lightly press the pouch with your fingers, and seal it using a heat sealer along the black line (Figure 3). Please ensure the pouch is sealed at the correct temperature, as excessive heat from the sealer could potentially damage it.
It is recommended that the temperature is checked in advance with a spare pouch.

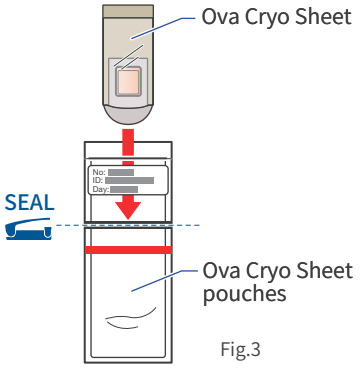


Fig.3

STEP4

Using tweezers, swiftly immerse the device in liquid nitrogen.
After immersing, gently move around the device in liquid nitrogen to ensure rapid cooling (Figure 4).

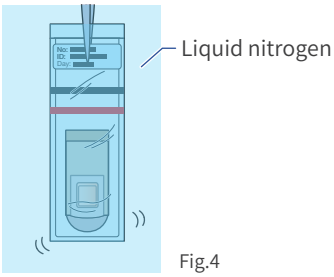


Fig.4

STEP5

Place the device into a cane and store it in the liquid nitrogen tank (Figure 5).

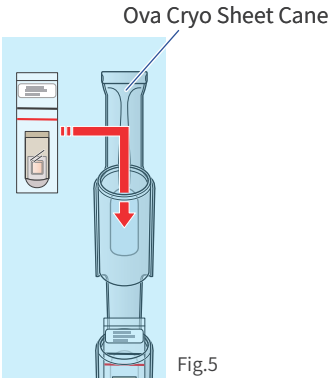


Fig.5

Ovarian Tissue Warming flow

Ovarian Tissue Section Thawing → Dilution and Washing → Transplantation/Culturing

PART 1 Materials Required

1. Ova Thawing Kit (Code. VT302S)
   Thaw1：100mL ×1
   Thaw2：20mL ×1
   Thaw3：20mL ×1
2. Ova Culture (Code. OVCL-100)
   Or Ova Culture with HEPES (Code. OVCM-100) for additional culture
3. Liquid nitrogen
4. Liquid nitrogen with its container (Code. Cooling Rack)
5. Tweezers (about 20cm)：for thawing procedure
6. Water bath
7. Dish (OD 60mm - 100mm) ×2
8. Container (110mL / 4.5 oz.)
9. Tweezers (about 12cm)：for dilution and washing of ovarian tissue
10. Count-up Timer
11. Scissors[*1]：for cutting the pouch

[*1] For Closed System

Thaw1 Thaw2 Thaw3

Ova Thawing Kit
(Ref.82222 Code：VT302S)

Ova Culture
(Ref.82216 Code：OVCL-100)

Ova Culture with HEPES
(Ref.82217 Code：OVCM-100)

PART2 Preparation

STEP1

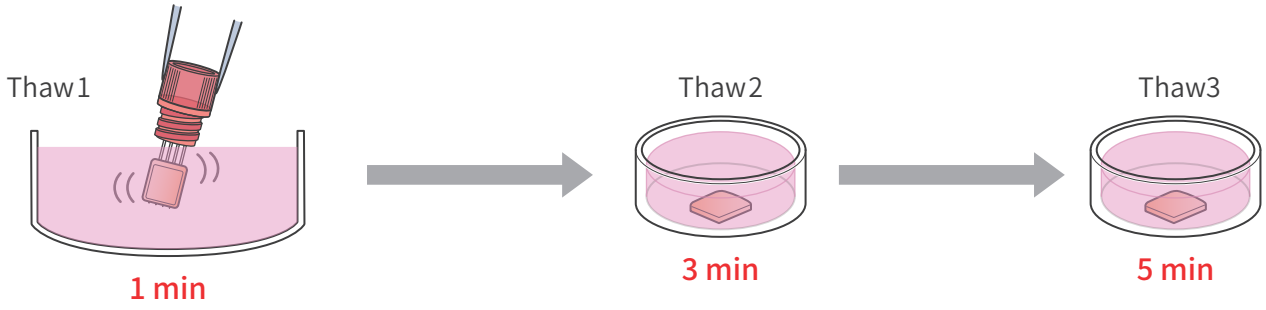
Warm Thaw1 bottle to 37°C in water bath and pour the entire volume of Thaw1 into a sterilized container.
Allow Thaw2 and Thaw3 to reach to room temperature (25-27°C).
Label each container or dish with "Thaw1," "Thaw2," and "Thaw3" using a labo marker, and pour the corresponding liquid into each dish.

TIP

To facilitate rapid transfer of the freezing device from liquid nitrogen to Thaw1 during warming, place the liquid nitrogen container right next to the water bath.

PART3 Warming

Open System



STEP1 Remove the device from the tank and transfer it to a liquid nitrogen container. Using tweezers, carefully remove the cap of the device in the liquid nitrogen. Confirm the presence of ovarian sections.

STEP2 Quickly transfer the device to Thaw1 and gently move around within Thaw1 then equilibrate for 1 minute. Remove the device promptly upon detachment of ovarian sections.

TIP

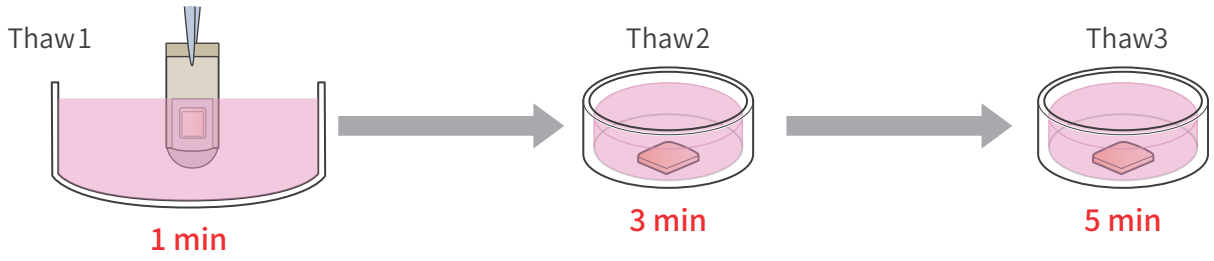
To maintain the temperature of Thaw1, immerse only the metal part of the device in it.

STEP3 After equilibrating in Thaw1, transfer the ovarian sections to Thaw2 and immerse them for 3 minutes.

STEP4 After equilibrating in Thaw2, transfer the ovarian sections to Thaw3 and immerse them for 5 minutes. Once warming is completed with Thaw3, proceed to the next procedures such as transplantation.

Following warming, perform an additional 30-minute culture using Ova Culture with HEPES before proceeding with transplantation. If additional culture exceeding 30 minutes is necessary, utilize Ova Culture and conduct it in the incubator.

Closed System



STEP1

Take out the frozen device from the storage tank and put it into the liquid nitrogen container. While immersed in liquid nitrogen, cut along the red line of the pouch and open it.
Make sure the top of the pouch is positioned above the liquid nitrogen level to avoid any liquid nitrogen from getting inside (Figure 1).

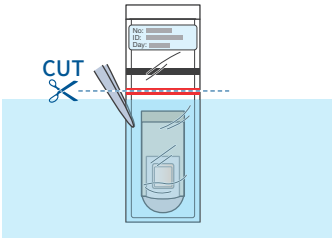


Fig.1

STEP2

Take the frozen device out of the pouch and quickly move it to Thaw1, allowing it to equilibrate for 1 minute. To maintain the temperature of Thaw1, immerse only the ovarian section of the frozen device in it. Once the ovarian section is detached, take out the device from Thaw1.

STEP3

After equilibration with Thaw1, transfer the ovarian sections to Thaw2 and immerse for 3 minutes.

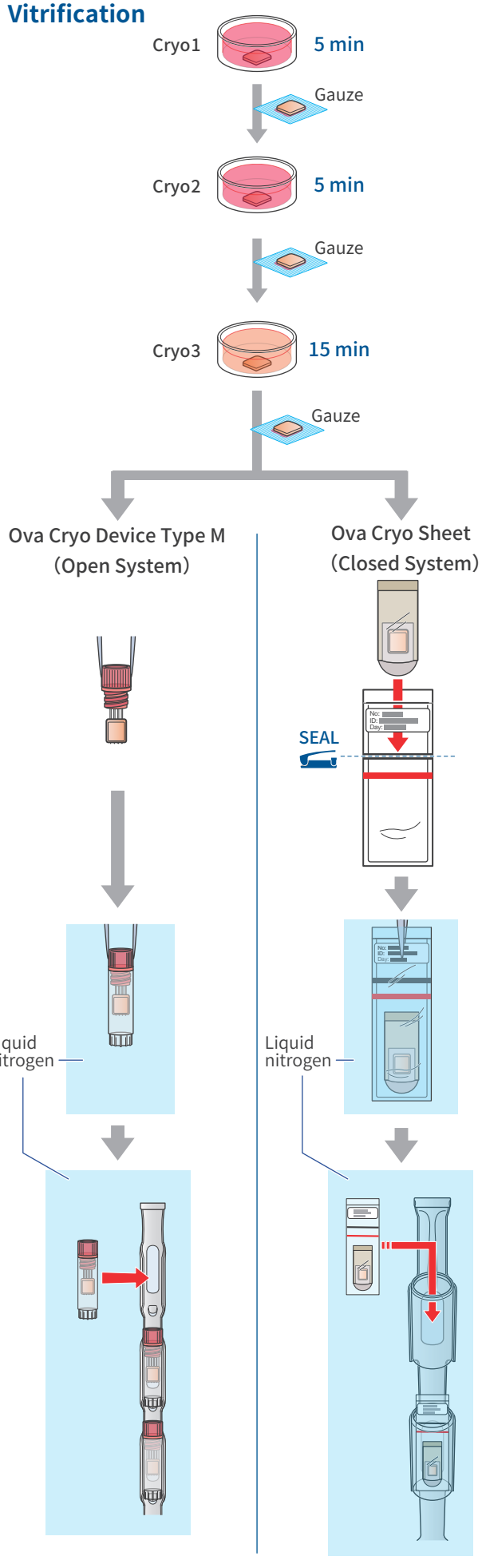
STEP4

After equilibration with Thaw2, transfer the ovarian sections to Thaw3 and immerse for 5 minutes. Once warming is completed by Thaw3, proceed to the next procedures such as transplantation.

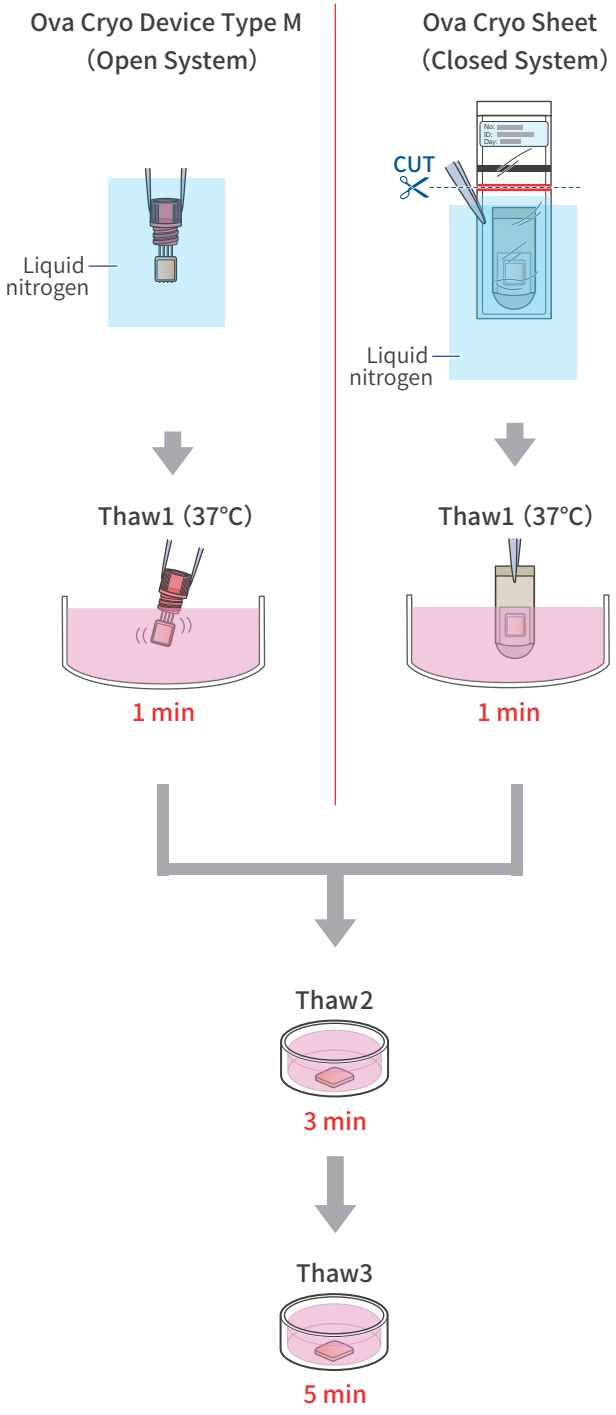
Following warming, perform an additional 30-minute culture using Ova Culture with HEPES before proceeding with transplantation. If additional culture exceeding 30 minutes is necessary, utilize Ova Culture and conduct it within the incubator.

Ovarian Tissue Protocol

Vitrification



Warming

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