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Simplified Warming Protocol for HSV Device

Oocytes and Embryos

STEPS 9–12 MUST BE PERFORMED AT ROOM TEMPERATURE (22–27°C)

Do not begin warming procedure until you have a pre-equilibrated dish of appropriate culture medium supplemented with SSS or DSS at 20% (v/v), or HSA at 12 mg/mL for final recovery of specimen(s).

Have all necessary materials, tools and equipment ready and easily accessible before starting procedure.

1. Set up thawing dishes (as shown in diagram):
   - **At 37°C:** Aseptically dispense a minimum volume of 250 µL of TS and warm to 37°C (incubator without CO_2) or on a heating stage at least 30 minutes prior to starting warming procedure
   - *For oocytes, dispense a minimum of 1 mL of TS.*
2. Identify HSV Straw(s) to be warmed from LN_2 storage and quickly transfer to a LN_2 filled holding reservoir in preparation for warming procedure.
3. Place LN_2 filled holding reservoir in close proximity to the working area and stage of the microscope in order to achieve subsequent rapid manipulation from reservoir to TS.
4. Remove TS dish from 37°C incubator or heating stage and place it under focus on top of the microscope stage.
5. Lift the straw with forceps enough to expose the colored handling rod. **Make sure the end with the specimen(s) remains immersed in the LN_2.**
6. Use a Knipex (or other wire cutter device) to cut the straw at the height of the colored handling rod. The red cut-length guide on the Knipex should be positioned in maximum length position or removed.
   - Alternatively, use fingers and thumb to spin the straw while making cutting movements with scissors, 10 mm under the top of the colored handling rod.
7. With one swift but controlled motion, quickly grab the handling rod and extract it out of the straw.
8. Immediately plunge the curved spatula (or gutter) of the handling rod into the 37°C TS and gently swirl to detach specimens from device and leave the oocyte or embryo for a total of 1 minute in the TS.

After 30 seconds following the initial plunge, gently pipette the specimen (if floating) and place at the bottom of the TS drop/well.

Steps 9-12 must be performed at room temperature (22-27°C).

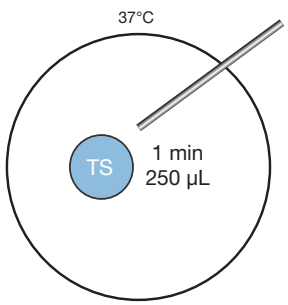
- **At room temperature:** Aseptically dispense one (1) 50 µL drop of DS on a sterile Petri dish

9. Transfer specimen(s) to DS for 4 minutes. Gently pipette specimens once to ensure complete rinse in DS.
10. During the 4 minute exposure in DS, aseptically dispense two (2) 50 µL drops of WS (WS1, WS2) as shown in diagram.
11. Transfer specimen(s) to each WS1 then WS2 for 4 minutes each, undisturbed.
12. Transfer warmed OOCYTE(S) to pre-equilibrated culture medium with 20% (v/v) protein supplement or 12 mg/mL for recovery (2–3 hours to allow time for spindle re-formation) prior to subsequent manipulations.

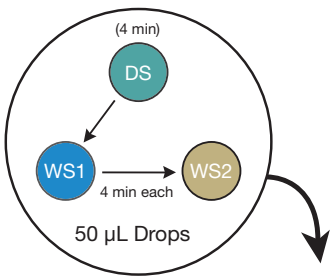
    There are two options for warmed EMBRYO(S):

    a) For immediate transfer to patient: transfer EMBRYO(S) to pre-equilibrated 'transfer' medium containing 20% (v/v) protein supplement or 12 mg/mL.

    b) For further culture: transfer EMBRYO(S) to pre-equilibrated culture medium containing 20% (v/v) protein supplement or 12 mg/mL for a 4-hour recovery period. After recovery period, transfer EMBRYO(S) to culture medium with 10% (v/v) protein and incubate accordingly until desired developmental stage has been reached for transfer to patient.



Minimum 1 mL of TS for oocytes in a 4-well dish



Transfer to culture medium with 20% (v/v) protein for recovery

KEY	
TS	Thawing Solution
DS	Dilution Solution
WS	Washing Solution
→	Transfer specimen to next drop
/	HSV Device

Simplified Warming Protocol for HSV Device

Tips

- Have all necessary materials, tools and equipment ready and easily accessible before starting procedure.
- Refer to Directions for Use or Product Insert that accompany the HSV Device for specific instructions.
- Pre-equilibrate a dish of appropriate culture medium with 20% (v/v), or 12 mg/mL protein for final recovery of specimen(s).
- HSV Device must remain submerged in LN_2 until ready to warm. When transferring HSV Device from LN_2 filled holding reservoir, or between LN_2 storage tanks, vitrification devices should always be submerged in a LN_2 filled goblet to prevent uncontrolled/premature warming in air.
- Set up warming dish with a minimum volume of 250 µL of TS for embryos, or a minimum volume of 1 mL of TS for oocytes, at least 30 minutes prior to beginning procedure.
- Remove the TS dish from 37°C incubator or heating stage right before the warming procedure.
- Immediately immerse the curved spatula (or gutter) into the 37°C TS after removed from LN_2 (within 2 seconds).
- Maintain proper function of your Knipex (or other wire cutter device) with regular monitoring and replacing when needed.
- Gently swirl the gutter in TS to help detach the specimen(s) from the device.
- Keep focusing under the microscope while immersing gutter into TS to locate the specimen(s).
- Limit exposure to light while moving the specimens through the solutions.
- If you choose to, you may overlay DS and WS drops with 8.0 mL to 8.5 mL of equilibrated mineral oil, at least 45 minutes prior to starting warming procedure.

Following complete recovery (2–4 hours post-warming), oocytes must be fertilized by ICSI due to zona hardening during vitrification.

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