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Simplified Oocyte Vitrification Protocol for HSV Device

For MII Oocytes – Gradual Exposure to ES

ALL PROCEDURES MUST BE PERFORMED AT ROOM TEMPERATURE (22–27°C)

As referenced in clinical literature, we recommend oocyte vitrification to be performed within 2 hours from time of oocyte retrieval.[1]

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

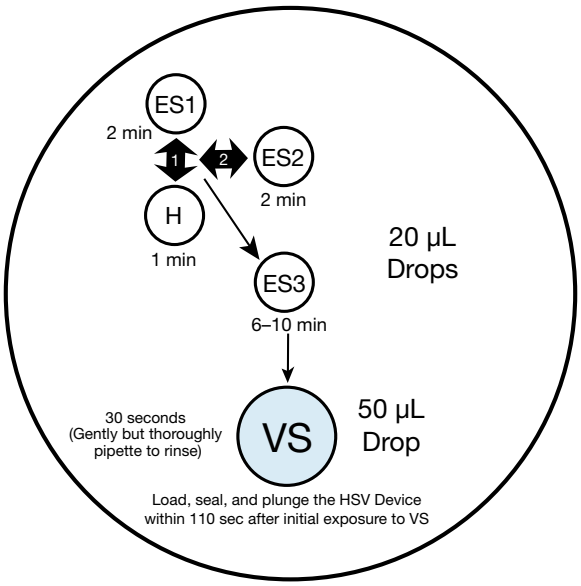
1. Aseptically dispense:
   - one (1) 20 µL drop of H
   - three (3) 20 µL drops of ES (ES1, ES2, and ES3)

   ES1 and ES2 should be in close proximity to H (but not touching).
2. Place MII oocyte(s) (2 maximum), into H and expose undisturbed for 1 minute.
3. Merge ES1 with H. Allow spontaneous mixing for 2 minutes.

   Use tip of transfer pipette to move ES1 towards H until drops merge.
4. Then merge ES2 with H+ES1. Allow spontaneous mixing for 2 minutes.
5. Transfer oocyte(s) from merged drop to ES3, and expose undisturbed for 6–10 minutes.
6. During the 6–10 minute exposure, aseptically dispense one (1) 50 µL drop of VS.
7. Transfer oocyte(s) from ES3 to VS for 30 seconds before loading.
8. Gently but thoroughly pipette oocyte(s) once within VS drop to ensure complete rinse in VS.

   To minimize floating, after 10 seconds pipette the specimen(s) to the bottom center of the VS drop.
9. Load, seal, and plunge HSV Device (per device instructions) within 80 seconds, not to exceed 110 seconds after initial exposure to VS.
10. Refer to HSV Device Loading Protocol, FISI P/N 002120 diagram and product insert for detailed loading instructions.

    See reverse side for tips.



KEY	
H	HEPES buffered medium with protein (eg., mHTF- HEPES with 20% SSS-NX or equivalent)
ES	Equilibration Solution
VS	Vitrification Solution
↔	Merge drops
→	Transfer specimen to next drop

Simplified Oocyte Vitrification Protocol for HSV Device

Tips

- All procedures are to be done at ROOM TEMPERATURE (22–27°C).

  Do not use heated stage.

- Have all necessary materials, tools, and equipment ready and easily accessible before starting procedure.
- HSV Device should be pre-labeled with patient information and the thin end of the blue plastic insertion device should be connected to the colored end of the handling rod.
- Where possible, select only the best quality MII oocytes for vitrification.
- The recommended HSV Device capacity is a MAXIMUM of 2 specimens.
- Process only as many specimen(s) as will be loaded per HSV Device at one time.
- Minimize exposure of specimens to light during equilibration in ES and VS solutions.
- Transfer specimens between drops using a minimal volume of medium.
- The timing for exposure to VS is CRITICAL:
  - Maintain microscopic visualization of specimen(s) by adjusting focus as needed during rapid exposure to VS (specimens will float in the drop).
  - Keep transfer pipette tip close to drop for quick manipulations.
  - Load, seal, and plunge the HSV Device within 80 seconds, not to exceed 110 seconds after initial exposure to VS.

1. Song WY, Sun YP, Jin HX, et al. *Chin J Obstet Gyn.* 2010; 45(8):578-82.

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