



# THE VITAVITRO OPS METHOD

## USER GUIDE

### CHECKLIST

- VitaVitro® Vitrification Kit (HHM, HV1, HV2)
- VitaVitro® Warming Kit (HHM, HW1, HW2)
- VitaVitro® Straw Set (OPS & container straw)
- Stereomicroscope
- Heated stage on microscope
- Heated pad close to the microscope
- 1 x four-well dish (4WD)
- 1 x 60 mm Petri Dish
- 1 x 35 mm Petri Dish
- 10, 100 and 1000 µl automatic pipettes with the appropriate sterile tips
- Forceps/tweezers
- Stopwatch
- Liquid nitrogen and box
- Sealer

## STAGE I PREPARATION

- 1 All procedures should be performed at room temperature (25-27°C).
- 2 All dishes should be warmed to 25-27°C.
- 3 HHM, HV1, HV2 and HW2 should be warmed to 25-27°C; HW1 should be warmed to 37°C.

## STAGE II EQUILIBRATION

- For oocyte vitrification, follow steps for **HUMAN MII-PHASE OOCYTES**.
- For vitrification of zygotes/embryos, follow steps for **HUMAN ZYGOTES AND EMBRYOS**.

### HUMAN MII-PHASE OOCYTES

NOTE: Oocytes can be vitrified between 2-6 h after ovum pick-up (preferably 2 h) and shortly after oocyte denudation.

- 1 In the lid of a 60 mm Petri dish, pipette a 50 µl drop of HHM and a 50 µl drop of HV1 close to each other (Fig. 1).
- 2 Transfer the denuded MII oocytes to the HHM drop, wait for several seconds, then bridge both drops.  
➔ BRIDGING I
- Wait for 3 min.
- 3 Pipette a new 50 µl drop of HV1 close to the bridged drops. Move the oocytes into the bridge.
- 4 Bridge the new HV1 drop and the existing bridge.  
➔ BRIDGING II
- Wait for 3 min.
- 5 Pipette a new 100 µl drop of HV1 far from the existing drops. Transfer the oocytes to the top of the new drop, let them sink, and wait for approx. 9 min. **▲ DO NOT MIX, MOVE, OR DISTURB THE DROP.** Carefully cover the lid with the inverted bottom of the dish.
- Wait for 9 min.
- 6 While waiting, place a container straw into the rack of the liquid nitrogen box.
- 7 After 9 min (the total equilibration time so far is 15 min), check the oocytes under a stereomicroscope to see if they are fully recovered (i.e. the perivitelline space is as narrow as before the start of equilibration).

- If fully recovered, proceed to step 8.
- If not fully recovered, wait for an additional 3-6 min until they are recovered, then go to step 8.

- 8 Pipette two new 100 µl HV2 drops far from the existing drops and each other.
  - a) Transfer the oocytes into one of the new drops and mix by pipette for approx. 20 s.
  - b) Transfer them to the other new drop and mix for another 20 s.
- 9 After mixing, remove oocytes or embryos with 1 µl of solution and go to **LOADING AND COOLING**.

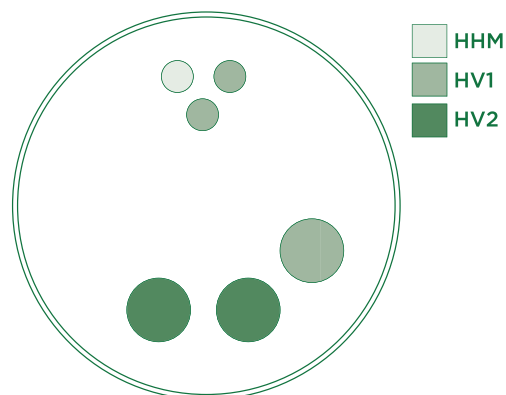


Figure 1. Arrangement of drops in the lid of a 60 mm Petri dish for oocyte vitrification

### HUMAN ZYGOTES AND EMBRYOS

NOTE: Zygotes and embryos are less sensitive than oocytes; therefore, no stepwise equilibration in HV1 is needed.

- 1 Pipette a 50 µl drop of HHM in the lid of the 60 mm Petri dish (Fig. 2), and transfer embryos there for 2 minutes.
- Wait for 2 min.
- 2 Pipette a 100 µl drop of HV1 in the lid of the 60 mm Petri dish and transfer the embryos there. Carefully cover the lid with the inverted bottom of the dish
- 3 Follow steps 6-9 from EQUILIBRATION OF HUMAN MII-PHASE OOCYTES

- 12-15 min

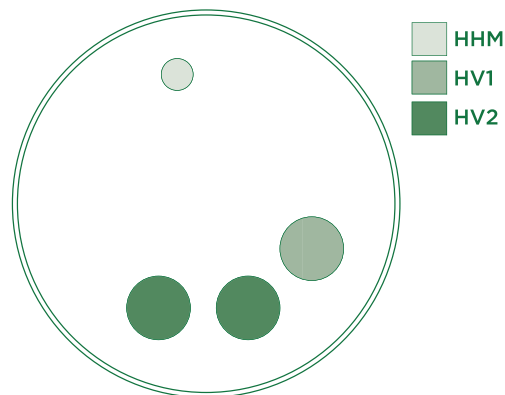


Figure 2. Arrangement of drops in the lid of a 60 mm Petri dish for vitrification of human zygotes and embryos

## STAGE III LOADING AND COOLING

- 1 Pipette a small droplet containing the oocytes or embryos in the lid of the Petri dish at the 9 o'clock position.
- 2 Immediately touch the droplet with the narrow end of the OPS (Fig. 3). The solution should enter the OPS and form a square-shape cylinder containing the sample.  
➔ **LOADING**

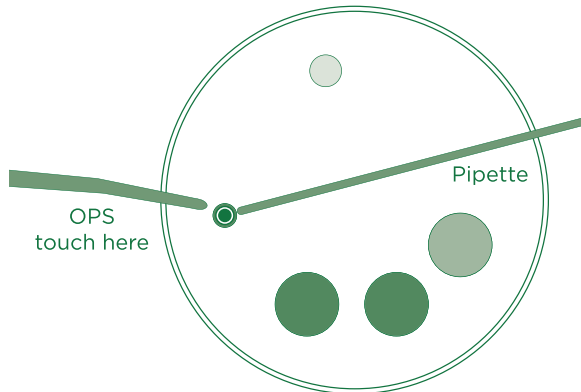


Figure 3. Loading oocytes into the OPS

- 3 Immerse the OPS in the LN<sub>2</sub> in the rectangular compartment of the liquid nitrogen box with a single, continuous, rapid movement (Fig. 4).
- 4 Quickly transfer the OPS into the container straw. Insert the narrow end of the OPS first. Seal the open end of the container straw using the sealer.

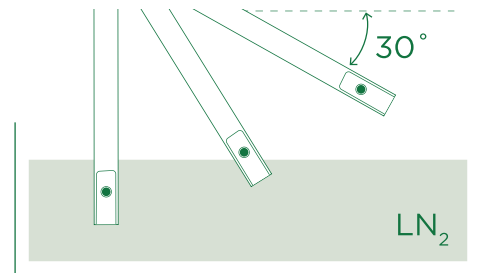


Figure 4. Immersing the OPS in LN<sub>2</sub>

## STAGE IV WARMING AND EXPELLING

- 1 Prepare the warming dish (35 mm Petri dish) 1 hour in advance. Pipette 3,000  $\mu$ l of 25–27 °C HW1 and warm it to 37 °C.
- 2 Transfer the container straw containing the OPS to the rack of the liquid nitrogen box.
- 3 Just before warming, prepare the Nunc four-well dish:
  - a) Pipette 900  $\mu$ l of 25–27 °C HW2 solution into well 2
  - b) Put 900  $\mu$ l HHM into well 3
  - c) Put 900  $\mu$ l HHM into well 4
- 4 Lift the upper end of the container straw slightly above the LN<sub>2</sub> vapor level and cut it with scissors.
- 5 Using small tweezers, remove the wide end of the OPS from the container straw and hold it between your thumb and middle finger. Make sure you will be able to close the

end with your index finger. **▲ THE NARROW END OF THE OPS MUST REMAIN WELL BELOW THE LIQUID NITROGEN.**

- 6 Remove the OPS, and within 3 s immerse the vitrified liquid column into the 35 mm Petri dish. Observe the immersion process under the stereomicroscope until the vitrified column melts and HW1 enters the OPS.
  - 7 Immediately cover the open end of the OPS with your index finger. ➔ **IMMERSE & EXPEL**
- NOTE: If the solution remains in the OPS, use a 10 or 20  $\mu$ l micropipette to expel air into the wide end of the OPS to eject the liquid.
- 8 After 1 min, remove oocytes or embryos with approx. 10–20  $\mu$ l of solution in a pipette and go to **DILUTION AFTER WARMING** (see below).

## STAGE V DILUTION AFTER WARMING

- 1 Transfer the oocytes or embryos to well 2 using the pillow and sheet method (Fig. 5):
  - a. Make a “pillow” with the transferred HW1 (1M sucrose) solution on the bottom of well 2 filled with the less concentrated HW2 (0.5M sucrose).
  - b. Put the oocytes or embryos on the pillow.
  - c. Cover them with a “sheet” of HW1 solution.
- 2 After 3 min, transfer oocytes or embryos to well 3 using the pillow and sheet method. Wait 5 min.

● **Wait for 5 min**

- 3 Transfer oocytes or embryos to well 4. There is no need to use the pillow and sheet method. Wait 5 min.

● **Wait for 5 min**

- 4 Before performing ICSI or embryo transfer, transfer oocytes to fertilization media (or transfer embryos to culture media) for 2–4 hours.

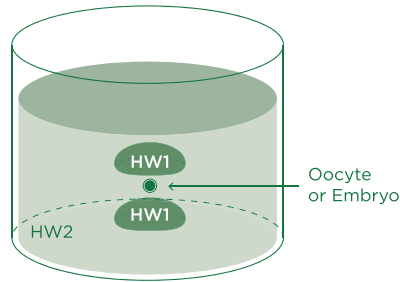
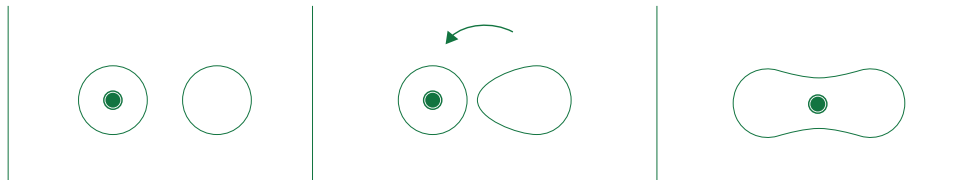


Figure 5. Arrangement of HW1 and HW2 in well 2

## TECHNIQUES

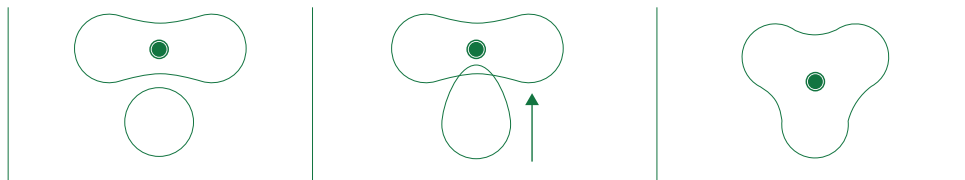
### BRIDGING I

Using a pipette, touch the drop on the right and gently drag left until it combines with the drop on the left.



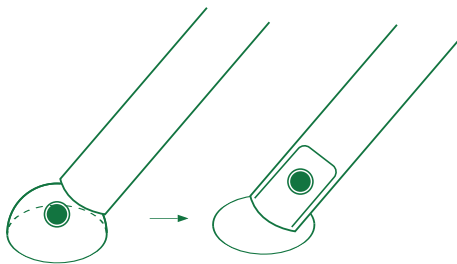
### BRIDGING II

Using a pipette, touch the drop on the bottom and gently drag up until it combines with the existing bridged drops.



### LOADING

Touch the droplet with the narrow end of the OPS. The capillary effect will cause the solution to enter the OPS.



### IMMERSE & EXPEL

Tightly cover the wide end of the OPS with your index finger, making sure no air can escape. The solution will flow out from the OPS as a result of the increased pressure of the warming air inside the OPS.

