RAPID-i™ VITRIFICATION OF OOCYTES

Vitrification may only be performed by staff trained in vitrification procedures.

Incorrect handling or sealing of the RapidStraw™ can cause a pressure build up inside that may result in damage or even explosion of the straw during the warming procedure


Rapid-i™ kit


SmartBox™


Rapid-i™ Sealer


Rapid-i™ Cryocane with goblet


RapidVit™ Oocyte kit

All procedures should be performed at +37°C and ambient atmosphere. Deviations from 37°C will alter the permeability of cryoprotectants, which may compromise oocyte survival

1. Fill the SmartBox with liquid nitrogen up to 1 cm from the box's rim and place the lid on top of the box.

Always maintain a sufficient level of liquid nitrogen in the SmartBox

2. Place 1 ml of each of the following solutions into separate wells of a 5-well plate and warm to

37°C

Vitri 1™ Oocyte
Vitri 2™ Oocyte
Vitri 3™ Oocyte

The recommended volumes should not be changed. Failure to use the correct volume of media may result in osmolality changes, which could cause suboptimal oocyte survival

3. Label the exact number of RapidStraws needed with the patient's identification. Place the label below the top, black mark of the straw.

4. Transfer the oocytes into Vitri 1 Oocyte. The oocytes should remain in the solution for

Vitri 1 Oocyte 5-20 min

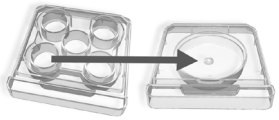
5. Move an appropriate number of oocytes into Vitri 2 Oocyte. The oocytes remain in this solution for

Vitri 2 Oocyte 2-5 min

The oocytes should have re-expanded to their original volume within five minutes.

6. Place the RapidStraw in liquid nitrogen. Make sure that the RapidStraw is securely attached to the magnet in the SmartBox.

7. When the oocytes have fully re-expanded, make a droplet of Vitri 3 Oocyte on a non-toxic surface, preferably a 40 mm culture dish.

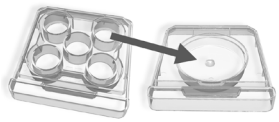


The 20 µl droplet can only be used once

8. Transfer the oocytes into the 20 µl droplet of Vitri 3 Oocyte and let them remain in this solution for 25-35 sec, including the time it takes to load the Rapid-i and vitrify.

Remove the stainless steel rod from the RapidStraw after 15 seconds have elapsed and discard.

Vitri 3 Oocyte 25-35 sec

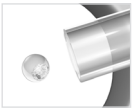


9. Place Rapid-i on the microscope stage with the flat side down. Locate the correct plane of focus so that the hole of Rapid-i is in view, for easy loading.

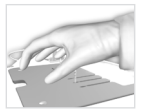
10. Collect the oocytes with a Vitrolife micropipette. Keep the oocytes close together at the end of the pipette.

Avoid overfilling the hole or else the oocytes may float out

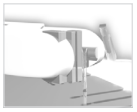
11. Slide the hole of Rapid-i into view, in the microscope. Move the tip of the pipette close to the wall of the hole in Rapid-i and expel the oocytes into the hole.



12. Quickly place Rapid-i vertically into the pre-cooled straw sitting in the Smartbox. Cover the hole immediately after insertion for a few seconds to prevent that Rapid-i accidentally pops out.



13. Immediately seal the top of the RapidStraw using the Rapid-i Sealer. Inspect the seal to ensure that sealing was correctly performed. Place the storage vessel (e.g. cryocane with attached goblet) into the SmartBox.



14. Move the sealed RapidStraw from the lid and into the goblet, so that the RapidStraw with the oocytes does not leave the liquid nitrogen.

15. Transfer the cryocane with goblet to long term storage.

Vitrolife

RAPID-i™ WARMING OF OOCYTES

SmartBox™

Rapid-i™ forceps

Rapid-i™ cutter

RapidWarm™ Oocyte kit

1. Place the Smartbox on the lab bench close to the microscope and fill it with liquid nitrogen up to 1 cm from the box's rim and place the lid on top of the box.

2. Place 1 ml of each of the following media into separate wells of a 5-well plate and warm to

37°C

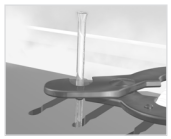
Warm 1™ Oocyte
Warm 2™ Oocyte
Warm 3™ Oocyte
Warm 4™ Oocyte

The recommended volumes should not be changed. Volume changes will affect temperature control in the first warming solution as well as osmolality, which may result in suboptimal oocyte survival

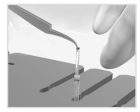
3. Move the cryocane and goblet containing the RapidStraws™ into the liquid nitrogen in the SmartBox.

4. Without leaving the liquid nitrogen, remove one RapidStraw from the goblet and place it in a slit of the lid.

5. Warm the RapidStraw with your fingers around the black mark to get a better view of the black tab on Rapid-i. Hold the RapidStraw well above the black mark and use the Rapid-i cutter to cut the RapidStraw 3 mm above the back end of Rapid-i. Do not lift the RapidStraw from the lid and make sure it stays up-right in the liquid nitrogen.



6. Lift Rapid-i (using the Rapid-i forceps) out of the RapidStraw just enough to enable you to grasp the end with your finger tips. Then quickly (preferably less than 2 seconds), but carefully, remove Rapid-i from the RapidStraw and plunge the tip and hole of Rapid-i into the Warm1 Oocyte solution.



7. Allow the oocytes to fall from the device and sink to the bottom. Leave for

Warm 1 Oocyte 1 min

8. Transfer the oocytes into Warm 2 Oocyte and let the oocytes remain in the solution for

Warm 2 Oocyte 3 min

9. Transfer the oocytes into Warm 3 Oocyte and let the oocytes remain in the solution for

Warm 3 Oocyte 5 min

10. Transfer the oocytes to Warm 4 Oocyte and remain in the solution for

Warm 4 Oocyte 5-10 min

11. Rinse the oocytes in culture media several times and continue culture according to laboratory practice.

12. Discard the used Rapid-i.

If more than one Rapid-i is to be warmed in the same dish, make sure that the temperature reaches 37°C after each warming procedure

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