Innovative Cryo Enterprises L.L.C.

OOCYTE VITRIFICATION PROTOCOL

Use for vitrification of human oocytes only. Do not use for blastocysts.

There are a variety of different devices/methods you can use for vitrifying oocytes. We suggest the following: "Straw Method (micro-column)". Alternatively we suggest the Micro-secure device by Dr. Mitchell Schiewe, this is available upon request. We provide different methods because some clinics have better success with one or more of these methods. Everything else is the same, but you can choose which device is best for your lab. Furthermore, the media will work with any micro-volume device such as a cryo-top, cryo-loc, cryo-pette, rapid-i, etc. Please contact me for more information on using one of these other devices.

Oocyte Vitrification

1) V1-Oocyte 5 min at 23-26°C
2) V2-Oocyte 2.5 min at 23-26°C
3) V3-Oocyte 2-5 min at 23-26°C

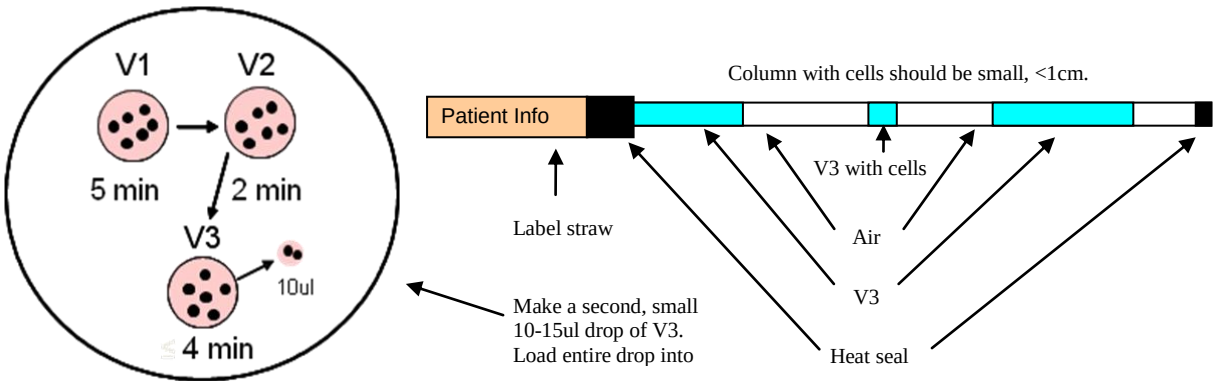
Use a 270-300um pipette to move cells.

Transfer oocyte(s) into V3-Oocyte with minimal carry-over & load into straw or on device.

Be sure to give the oocyte(s) at least 2min in the V3 prior to plunging into LN2.

Straw Method (micro-column):

4) Load oocyte(s) in straw between columns of V3-Oocyte. Be careful to keep the size of the column with the oocytes small. This can be done by placing the eggs into a separate small 10-15ul drop of V3 and then load the entire drop into the straw. Aspirate the first column of V3 to the cotton plug, as this will allow the columns to remain intact.



5) Heat seal both ends of the straw. *You must heat-seal. Periodically check seal under microscope to ensure proper seal.*
6) Plunge and store in LN_2.

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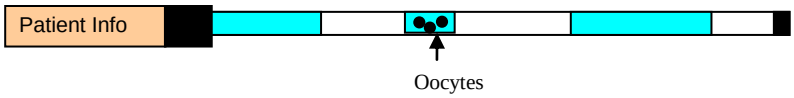
OOCYTE THAWING PROTOCOL:

Please Note: Oocytes are vitrified in a conventional 0.25cc straw or 0.3cc CBS straw, but other devices can be used. The cells are located in the small middle column. See diagram below.



Straw (Micro-column) Method:

1) Thaw straw 3 sec in room temperature air and then 7 sec in 37-40°C water. *Pull straw out of tank slowly and hold in air. Move straw around in the water bath, make sure straw is completely submerged.*
2) Quickly wipe straw and cut off top heat-sealed end of straw, insert syringe and cut off other sealed end.
3) Empty contents of straw into a 60mm dish. Watch through the microscope to see the oocytes come out of the straw. They will appear flat and "ghost" like. You may see what is an empty zona or dead oocyte, but it is not.

Transfer oocyte(s) to (50ul-75ul microdrops under oil):

4) T1-Oocyte	2 min at 23-26°C
5) T2-Oocyte	3 min at 23-26°C
6) T3-Oocyte	3 min at 23-26°C
7) T4-Oocyte	3 min at 23-26°C
8) T5-Oocyte	3 min on 37°C place on warmer
9) Equilibrated, warmed culture medium	37°C incubator

10) Wait 2-3 h before starting ICSI.

For optimal performance:

Wait 1 hr after egg collection, strip most of the cumulus cells off, wait 1 hr then vitrify.

Leaving some cumulus cell on helps with locating the eggs upon thawing.

Label the straw directly or use a flattened 0.5cc straw as a label that can be heat-sealed onto the 0.25cc straw.

Use a 60 mm dish to freeze in. Use large drops (75-100 µl) with no oil overlay.

Because the time allowed in V3 can be up to 5 min, there is time to prepare multiple straws for vitrification.

Use a new dish (new medium) for each set of cells frozen.

Aspirate the first column of V3 to the cotton plug, as this will allow the columns to remain intact.

For best results use a new dish (new medium) for each straw thawed.

Culture thawed cells in media containing 15-20% HSA or a similar protein for the first 24 hours after thawing.

After the first 24 hours, you can switch to your normal protein concentration for culturing.

Store media at 5°C.

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