A CRITICAL ASSESSMENT OF CURRENT VITRIFICATION METHODS

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Disclosure

Scientific Director for The World Egg Bank
A For-Profit Company providing Oocyte Donors and Cryopreserved Donor oocytes to Fertility Clinics and Patients in the US and Worldwide

No financial interests in any products being Marketed to IVF Clinics other than TWEB Products and Services
Goals:

- Learn about the historical developments of Ultra-Rapid Vitrification
- Understand the sensitivities of current vitrification methods
- Explain what we should know, and yet do not
- Interpret the sensitivities and use that information as a guide to day-to-day practices
- Be able to examine a vitrification protocol and determine if the method is likely to be robust/repeatable
History of vitrification by “ultra-rapid” cooling

Basile J. Luyet (1897 – 1974)

“One will obtain rapid elimination of heat by reducing the material to sheets with the smallest possible thickness and the largest possible area”. P. 208

“In order to reduce the heat capacity of the preparation, one must use very thin supports.” P. 218

“Instead of thin supports, we sometimes used, with advantage, a ring of about two millimeters in diameter, made of as thin a metal wire as possible and fastened to a light, rigid rod. One simply dips this loop into the culture and thus obtains, in the thin film within the ring, quite a considerable number of organisms.” P. 218

Text from Luyet BJ, Gehenio PM. Life and death at low temperatures. Normandy, Missouri: Biodynamica, 1940;341.
representing a $^1 \Sigma - ^1 \Sigma$ transition, gave $B_1' = 0.5747$ and $B_2' = 0.5707$ cm$^{-1}$. The agreement is good.

Our analysis shows that the red system represents a $^1 \Sigma - ^1 \Pi$ transition. A more detailed paper on the red bands will appear in Arkiv för Fysik.

Albin Lagerqvist
Ulla Ulfber

Physics Department,
University of Stockholm.
April 23.


Revival of Spermatozoa after Vitrification and Dehydration at Low Temperatures

The effect on spermatozoa of vitrification at temperatures of −78°C and below has been studied by several authors. Human spermatozoa appear to be the most resistant; a substantial proportion may show good motility on thawing after even prolonged vitrification. Revival is far better when semen is frozen in bulk than when minimal amounts in capillary tubes are used. No explanation of this result is yet forthcoming, but it would appear that rapidity of freezing is less important than the avoidance of surface effects.

Positive results have also been obtained with frog and fowl spermatozoa, though in both these cases...

Human Oocyte Cryopreservation Outcomes

Figure from Steven Mullen, The World Egg Bank, Phoenix AZ USA. See Reference List

Image from Irvine Scientific, Santa Ana, CA USA

Image from Kitazato, Shizuoka Japan
CURRENT VITRIFICATION METHODS

The Good, The Bad, and The Ugly
Technically Demanding

For skill embryologists/technicians:

1. Observation during the first week of training

2. Start training with spare material (IVM or unfertilized oocytes), under the guidance of the supervisor. Signed informed consent (the material will be used for teaching purposes).

3. Daily training during approximately 2 months (Estimated, depending on the skills and material available).

4. Start loading 1 oocyte per device and increase the number sequentially to be able to load 4 oocytes per device, in strict compliance with the protocol directions.

5. Over 80% SV in approximately 200-250 oocytes.

6. Beginners are not allowed to perform entire cases.
Are We Really Vitrifying??

“…survival alone is not unequivocal proof of vitrification.”

Effect of common cryoprotectants on critical warming rates and ice formation in aqueous solutions

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Abstract
Ice formation on warming is of considerable greater importance to ice formation on cooling in determining survival of cryopreserved samples. Critical warming rates required for ice formation of vitrified aqueous solutions of glycerol, dimethylsulfoxide, ethylene glycol, polyethylene glycol 200 and water have been measured for warming rates of order 10^11°C/s. Critical warming rates are typically


Image from Kitazato, Shizuoka Japan

Warming Rate 96,000 K/min
(= 1.6 x 10^3 K/sec)
What about 15% DMSO
15% EG 0.5 mol/L Sucrose, Etc ????
Current Warming for Cryotop
about $10^3$ K/s

“Our preliminary attempts to measure critical cooling and warming rates in aqueous solutions containing up to 50% w/w lysozyme suggest that soluble proteins are very poor cryoprotectants, as has been found in studies of ice formation in hydrated protein powders.”


Image from Kitazato, Shizuoka Japan
Big Unknown

Image from Kitazato, Shizuoka Japan

What is the vitrification solution composition in this scenario?

Shouldn’t we Know??
Moving On...

Photo by S. F. Mullen
Thermal Sensitivity...

<table>
<thead>
<tr>
<th>Seconds of Exposure to Room Temperature</th>
<th>Percent of Viable Embryos 5 hours post-warming</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
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<td>10</td>
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<tr>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
</tr>
</tbody>
</table>

Image from Kitazato, Shizuoka Japan
To $T_g$ in about 1 second

To $T_g$ in about 15 seconds

Data from Steven Mullen, The World Egg Bank, Phoenix AZ USA
So...what does this mean in practice?

Sample reception at the destination

1. Fill the Dry Shipper with LN
2. Bring a recipient with LN
3. Take the gobelet to the container quickly
4. Storage

Temperature

The challenge of vitrifying oocytes
Moving On...

Mechanical Sensitivity...
Massive Fracture Event in Vitrified Solution in a Straw

Figure from Steven Mullen, The World Egg Bank, Phoenix AZ USA
Movie from Rebecca De La Cruz and Steven Mullen, The World Egg Bank, Phoenix AZ USA
What to Take Away…

• Vitrification research has a long history, and we are probably re-visiting many issues already investigated

• Vitrification, as is currently practiced, is technically demanding and requires a lot of practice and training to become proficient

• There are a lot of details that are simply unknown, and can lead to increased variability and difficulties in troubleshooting problems

These include:
• Whether vitrification is actually occurring
• To what solution composition the cells are being exposed
• How sensitive the samples are to accidental warming
• How sensitive the samples are to thermomechanical stress

• Do we really need to go to these extremes?
References


[20] Bianchi V, Coticchio G, Distratis V, Di Giusto N, Flamigni C, Borini A. Differential sucrose concentration during dehydration (0.2 mol/l) and rehydration (0.3 mol/l) increases the implantation rate of frozen human oocytes. Reprod Biomed Online 2007;14:64-71.


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THANK YOU FOR
YOUR ATTENTION!