

## DEBATE

# Vitrification versus conventional cryopreservation technique

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During in vitro fertilization treatment (IVF), a surplus of oocytes is produced which results in a surplus of embryos which must be preserved. Due to the recent tendency in ART treatment to transfer fewer embryos in order to avoid multiple pregnancies, partly a result of new laws and guidelines aimed at controlling the number of embryos to be transferred and the number of oocytes to be fertilized, the need for simpler, more effective methods for the cryopreservation of embryos and oocytes is increasing. Currently, zygotes and embryos are typically cryopreserved by means of the traditional slow-rate freezing protocol, while oocyte freezing is still in the experimental phase.

However, during the slow freezing method, ice crystals are formed or produced, which has a deleterious effect on the cells. Intracellular ice crystals can damage the cell wall and structure, while the extracellular precipitation of water as ice crystals increases the salt concentration to levels that cause damage to the cell. A delicate balance between these potentially harmful factors must be maintained throughout the slow freezing process to ensure survival of the cells.

In 1985, the ultra-fast freezing method through vitrification was introduced based on inducing solidification of the cells and the surrounding vitrification solution (glass-like or vitreous) without formation of any ice crystallization. This can be achieved through the combination of the

following factors:

1- Increasing the concentration of cryoprotectant: The cryoprotectant is usually used in much higher concentration than in the slow freezing method. To avoid the toxic effect of the cryoprotectant, cells are equilibrated in a low concentration solution before immersion in the high concentration solution (vitrification solution). The equilibration solution contains between 10 to 15% of the cryoprotectant while the vitrification solution contains between 35 and 40% (around 5.5 Molar) of the cryoprotectant. Selecting the optimal cryoprotectant is critical. It should be of high permeability (low molecular weight) and as non-toxic to the cells as possible. Ethylene Glycol (EG) has these characteristics and, to lesser extent, also DMSO.

2- Dehydration of the cells: This is achieved by using a high concentration of nonpermeating cryoprotectant of a large molecular weight such as disaccharide sugars: sucrose or galactose at a concentration up to 1 Molar. The end concentration of the nonpermeating cryoprotectant is added to the vitrification solution. We cannot overemphasize the importance of minimizing the cells' exposure time in the highly concentrated vitrification solution; successful vitrification procedures must limit such exposure to a few seconds.

3- Very fast freezing rate: The vitrification device containing the cells is directly plunged into liquid nitrogen which can achieve a cooling rate of more than 20,000°C/ minute. The rate depends on the vitrification device used and on the freezing volume. No special equipment is needed to achieve such a freezing rate. When the vitrification device enters the liquid nitrogen, a

coat of liquid nitrogen vapor is generated around it, creating an insulation layer that can lower the cooling rate. Moving the device continuously upon its entry into the liquid nitrogen until complete solidification occurs easily eliminates this effect.

4- Increasing the viscosity of the vitrification solution: The viscosity of the vitrification solution increases during the cooling process until the solution solidifies. The increased viscosity of the vitrification solution is automatically achieved through the rapid cooling process. The viscosity can also be increased through the use of high concentrations of both the permeating and nonpermeating cryoprotectants and the addition of macromolecules such as PVP or Ficoll.

5- Minimizing the vitrification volume: Several devices have been developed and used successfully for the vitrification of oocytes and embryos. Using a "plastic loop" reduces the vitrification volume to a liquid film within the loop and the "open pulled straws" allow for a very small vitrification volume which does not exceed 2 $\mu$ l. In this context, the loading of the cells in the vitrification device and the handling of the device are critical since this is done during the cells' exposure to the highly concentrated vitrification solution. The choice of the device is a personal decision depending on the skill and preference of the individual operator. However, speed and dexterity are crucial as the goal is to minimize the cells' exposure to the high concentration solution prior to solidification.

6- Rapid warming rate: The vitrified cells should be warmed rapidly to avoid crystallization as in the fast cooling process. The vitrified material is immersed directly in the warmed thawing solution and the rehydration of the cells is usually performed in two or three steps.

### **Advantages of vitrification**

1- The vitrification process is both simple and fast; it can be completed in less than 10 minutes.

2- The prevention of ice crystal formation during the vitrification process successfully eliminates the major factors that can cause cell damage during the slow freezing method.

3- The process is easy to master: Vitrification involves simply moving the cells between two

dilutions, loading them into the vitrification device, and plunging them into liquid nitrogen.

4- One of the advantages of vitrification is that it allows the operator to observe the cells during the vitrification process. None viable zygotes and blastomeres can be recognized since they do not contract upon placement into the first or the second vitrification solution. The presence of contracted cells during the warming process is a useful marker for cell survival with the exception of oocytes which might survive the vitrification and warming processes, but show degenerative signs approximately 10 to 15 minutes after the warming is completed.

5- The fact that no special equipment or investments are needed for implementation of the vitrification procedure makes it extremely cost-effective, and offers a tremendous economic advantage over traditional methods.

Vitrification is a very simple effective process and the skill needed to perform it can be acquired in a short time through training on materials to be discarded. Several IVF programs have adopted the vitrification method as the sole procedure for day-3 human embryos and for human blastocysts, with excellent survival and pregnancy rates (2, 3). The challenge now is to find a protocol to successfully vitrify human oocytes, for which the slow freezing method has yet to produce acceptable survival and development rates.

### **REFERENCE**

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