

## VITRIFICATION (FREEZING)

The first human pregnancy from a frozen and subsequently thawed embryo was achieved in Australia in 1984. The method used to preserve that embryo is called 'slow freezing' and it is still the preferred method for preserving embryos throughout the world today. Slow freezing is a reliable and established technique that has served the IVF community well for many years.



Slow-freezing cools the embryos gradually, approximately - 0.3° C /minute until it has been cooled to below -30° C and is therefore fully frozen. Thereafter, storage of frozen cells is in liquid Nitrogen (-196° C). During the freezing of any cell, the water must be removed from the cells without damaging the cell or embryo. This is required as water expands in volume as it freezes, ice formation inside a cell causes the cell to rupture. There is also an associated rate of cell loss during thawing, with approximately 15-20% of frozen embryos not surviving the thawing process. This is particularly an issue with blastocyst stage embryos where slow freezing has not been as successful compared to day one, two or three embryos.

An alternative procedure called vitrification has been developed internationally and adopted by City Fertility Centre to enhance the survival capability of oocytes and blastocysts during and after the cooling process.

### WHAT IS VITRIFICATION?

The idea of vitrification was first described in 1860, and was successfully used on red blood cells in 1937 by Swiss-American Basile J. Luyet. However, it wasn't for another 50 years that Rall and Fahy described vitrification as a potential alternative to slow-cooling. The word 'vitrum' in Medieval Latin means 'glass'.

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Vitrification of oocytes and blastocysts is the process by which the solution containing the oocyte or embryo is cooled so rapidly that the water molecules do not have the time to form ice crystals and instantly solidify into a 'glass-like' structure.

This concept is based upon the idea that if the cell is dehydrated to a certain degree and then cooled fast enough, everything will 'freeze' in place and damage will not have time to occur; crystals will not be able to organise themselves and a vitrified amorphous, a glass-like solid, will form instead of ice. Vitrification cools the embryos an amazing 7,000 times faster than conventional slow freezing techniques (2,000-2,500 Celsius per minute). Since no ice forms, the risk of rupturing the cell is reduced.

Vitrification involves exposure of the cell to high concentrations of cryoprotectants for brief periods of time, followed by rapid cooling by touching a block which has been cooled in liquid nitrogen. The high osmolarity of the vitrification solution rapidly dehydrates the cell and liquid nitrogen quickly solidifies the cell so that the remaining intracellular water does not have time to form damaging ice crystals and hence, drastically reduces the survival rate for thawed embryos.

### HOW ARE EMBRYOS VITRIFIED

The oocyte or blastocysts are suspended in a drop of fluid on a tool with a tiny hook on the end. The droplet is lowered onto a metal block that has been cooled by liquid nitrogen where it hardens into a glass-like bead.

After investing heavily in vitrification training, our Brisbane Laboratory Supervisor Ms Emmy Hung has established a vitrification program at City Fertility Centre.

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