

# Embryo Cryopreservation (freezing)

## Introduction

Most people call it embryo freezing, but more correctly it is “embryo cryopreservation” because the ultimate goal is to store the embryo cryogenically and to preserve its viability for future initiation of pregnancy.

Before good embryo cryopreservation protocols were available, most IVF clinics had to destroy any excess embryos left over after embryo transfer. Early embryo cryopreservation programs usually had limited success and usually resulted in the destruction of most of the embryos for which it was attempted. Today, many commercially prepared solutions are available and more successful protocols have been introduced that have made embryo cryopreservation much more successful. Many IVF children now have younger siblings that were conceived at exactly the same time, but born years apart due to the increased success of embryo cryopreservation!

## Basic Principles of Embryo Cryopreservation and Thawing

Most living organisms ( eggs and embryos included) contain a significant percentage of water. We should be reminded that water covers more than 70 % of the earth's surface and makes up more than 65 % of the average human being.

**There are now 2 types of Freezing technologies that are used in IVF laboratories.**

The older technology is called **SLOW FREEZING** and until recently has been the gold standard.

The second and newer technology is called **VITRIFICATION**.

Both of these will be discussed below.

## Slow freezing

The failure or success of embryo cryopreservation is dependent upon how successful or unsuccessful the removal of water has been from the individual cells of the embryo. If water is left in the cells, it forms crystals when frozen. These crystals act like knives and disrupt the inside of the cells of the embryo or “cut” through the outer layer or “membrane” of the cells. If this cutting or disruption has occurred, the embryo will not survive. In order to avoid the formation of the water crystals, a “cryoprotectant” is added which replaces most of the water inside the embryo. Under the proper conditions, the cryoprotectant will not form crystals and the embryo can safely withstand the drastic reduction in temperature required for cryogenic storage.

When the embryo is removed from cryogenic storage, for use, the reverse process must occur. The cryoprotectant is removed from the cells of the embryo and replaced back with water.

Even though an embryo may look strong and healthy, it may not be able to move water and cryoprotectant in and out of its cells efficiently. If this occurs parts of the embryo or all of the embryo will not survive the cryopreservation and thawing process.

## **Vitrification**

This is a process which allows "glass like" solidification of a solution without ice crystal formation in the living cells. Normally when a solution is cooled, ice crystals will form - right up until minus 130 degrees Centigrade !

Vitrification is like "flash Freezing" The embryos are plunged into liquid nitrogen at around minus 200 degrees. The liquid therefore does not have time to form crystals and assumes a "glass like" state. Therefore there is no damage caused by ice crystals.

Up until recently there have been concerns about the safety of vitrification, however years of research and study have now reassured us that it is a process as safe as slow freezing.

At VFC we will be introducing routine Vitrification in 2010.

## **Stage of Embryo Development and Cryopreservation**

Different IVF programs cryopreserve embryos on different days of development and all are reportedly successful. The more common stages chosen for embryo cryopreservation are Day 1, Day 2, Day 3 and blastocyst (Day 5-7). The stage of development and day of cryopreservation is usually dependent upon the routine and preferences of the individual laboratories.

We usually freeze embryos on either day 3, or when they reach a blastocyst stage on day 5 or 6

## **Embryo Suitability for Cryopreservation**

The embryos most suitable for and most successful for cryopreservation are those that have reached the proper cleavage stage on the day of cryopreservation, have minimal fragmentation and are not undergoing active cleavage. At VFC, if we do a Day 3 embryo transfer, any excess good quality embryos may be considered for cryopreservation. For cryopreservation to occur on Day 3, the embryo should preferably be between a 6 and 8 -cell stage, and have less than 25% fragmentation. If the embryo is very advanced on Day 3 (starting to form a morula stage embryo), it may be cultured to Day 5 for cryopreservation. Embryos that are growing slowly or that are of questionable quality on Day 3 may be cultured until Day 5 and have the suitability for cryopreservation reassessed at that time. Any embryos that are of poor quality or are in cleavage arrest on Day 3 will not be cryopreserved.

If we do an elective day 5 transfer, then excess suitable blastocysts will be cryopreserved. If some of the embryos have not reached a blastocyst stage, they may be cultured an extra day ( to day 6 ) before being frozen.

## **Success of Embryo Cryopreservation and Thawing**

The chances for an embryo surviving the freeze/thaw process is improved by using the newer "Vitrification" technology.

Although we have traditionally used 'Slow freezing" and have enjoyed good pregnancy rates, at VFC we are switching to Vitrification in 2010 - having had our embryologists trained in this technology by some of the leading U.S. scientists in this field.

With slow freezing our embryo survival rate was approximately 70 % for day 3 embryos and 50 - 60 % for blastocysts. With vitrification we expect this to be much improved based on provisional studies to date. With slow freezing our pregnancy rate per transfer was approximately 30 % - which we expect will also be improved by the introduction of Vitrification.

## **Length of Cryostorage**

Recent reports have described pregnancies that have occurred from embryos cryopreserved and stored for over ten years. The only current limiting factor is for these older embryos is the quality of the cryopreservation protocol in place at the time of storage.