Freezing Embryos and the Replacement of Frozen Embryos

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Why Freeze Embryos?

The vast majority of invitro fertilisation cycles (including ICSI) use fertility drugs to stimulate oocyte (egg) production. This usually means there are more embryos available than can be replaced in that cycle. Providing those 'spare' embryos have developed to the appropriate stage within a certain time they can be preserved by freezing and replaced at a later date, without the need for further ovarian stimulation. This increases the overall chance of pregnancy from each oocyte pick-up. Embryo freezing is also an important way to preserve embryos in cycles where fresh replacement is inadvisable, for example, where the woman is considered at risk of developing Ovarian Hyperstimulation Syndrome (OHSS).

At What Stage Can Embryos be Frozen?

Embryos are most often frozen from the one-cell to eight-cell stages (day one to day three after pick-up) and also at blastocyst stage. The stage at which freezing takes place depends on the number of embryos created, the day of the embryo replacement and, of course, the wishes of the couple. It is not unusual for some couples to have embryos frozen at two or three different stages.

Should a cycle of IVF and ICSI result in a large number of embryos (nine or more), it is normal practice to culture seven or eight embryos until the time of replacement (though some clinics may culture all embryos to the time of replacement regardless of number). Excess embryos would be frozen at the single cell (or pro-nuclear) stage of development. Many (but not all) studies have shown that embryos frozen at this stage have the highest chance of surviving freezing and thawing. In cases where all embryos are frozen where there is no intention to replace fresh embryos, it would normally be done at the pro-nuclear stage.

The next stage at which embryos are frozen will depend on the day of replacement, normally day two or day three after pick-up. At the time of fresh replacement, the two (or exceptionally three) embryos showing the fastest and most even development will be selected for replacement. The 'spares' will also have to have reached the appropriate cell stage (four cell on day two and eight cell on three) and show reasonable evenness of development to be considered for freezing. Embryos with less than eight cells or a high degree of fragmentation are regarded as good candidates for freezing due to their poor chance of survival and low pregnancy potential, but could cultivate further.

Occasionally embryos not regarded as suitable for freezing on day three grow on to become blastocysts by day five. While (presently) not as successful as earlier stages, clastocysts can also be frozen. The technique is similar, but due to the high complexity of a blastocyst compared to, for instance, a one-cell pronuclear embryo, a different cryoprotectant media has to be used which is more gradually introduced before cooling.

How are Embryos Frozen?

Embryos to be frozen are placed in a solution of cryoprotectant (anti-freeze) medium which allows the cells to survive freezing and thawing. The cryoprotectant draws water out of the embryo, therefore reducing the chance of lethal ice crystal formation within the cells upon cooling. Embryos are then loaded into plastic straws labelled with patient identification information. Once loaded, the straws are placed within a special freezing machine which cools them very slowly to - 35°C. Once the freezing machine has completed its programme the straws ar immediately plunged in liquid nitrogen (at a temperature of - 196°C). Straws are then placed within plastic goblets, also labelled with patient identification details, then those goblets are placed in a liquid nitrogen bank for storage.

Thawing Embryos for Use

For women with reasonably regular cycles frozen/thawed embryos can be successfully replaced in a natural cycle, preventing the need for any drug administration. The woman would normally inform the clinic within a day or two of the start of her period and the clinic would arrange a plan to track the cycle to pinpoint ovulation. Tracking is normally by blood tests, 'QuickPeak™' urinary ovulation predictor kits, ultrasound scans or a combination. Tracking establishes the day of ovulation during that cycle and that becomes day zero. The embryos are then thawed in synchrony with the cycle. For instance, embryos frozen on day one following pick-up will be thawed on day one post ovulation of the natural cycle. This synchronisation ensures that the developing embryo is ready to implant when the uterine lining is at its most receptive.

Women who do not cycle or have very irregular cycles can have their embryos replaced in an artificial cycle. Different clinics have different strategies for mimicking a natural cycle with medication, but all have two essential steps. Firstly the uterine lining is stimulated to grow (usually with oestrogen tablets or patches). Secondly, once it has reached a certain thickness, it is primed to become receptive to an implanting embryo (usually with progesterone supplementation.

The process of thawing embryos normally takes around one hour. Straws containing the embryos are removed from the liquid nitrogen bank and allowed to warm rapidly. The straw seals are then cut off and the embryo's placed in a petri dish. The embryos are then passed through a series of solutions which slowly draws the cryoprotectant out of the cells. It is only when the embryos have come out of the final thawing solution and been placed in culture medium that survival can be fully assessed. At this stage we can evaluate how many cells have survived. If 50% or more cells have survived the embryo is regarded as viable and can be considered for replace-

ment. The chance of an embryo surviving completely intact is 50-70% but can vary between individual couples and even between different cycles for the same couple. What is unknown only a few hours after thawing is the potential for that embryo to continue development. If embryos have been frozen at the day one (pronuclear), or day two (four-cell), they would often be cultivated overnight before replacement. During that time the thawed embryo would be expected to continue cleaving. With embryos frozen on day three (eight-cell), resumption of development is not usually seen as embryos are usually replaced a few hours after thawing.

Embryo Replacement.

You would normally have been asked to call the clinic beforehand to check embryo survival. Frozen/thawed embryos would usually be replaced on the equivalent of day two or day three post ovulation. The embryo replacement procedure is the same as that used for replacing fresh IVF or ICSI embryos. Women having their embryos replaced in an artificial cycle will need to carry on progesterone support until the time of a pregnancy test (and beyond if they do become pregnant) while those using their own, natural cycle need no supplementary drugs. Pregnancy testing is around fourteen days after 'ovulation' for both types of cycle.

The Chance of Success

As with all fertility treatments the chance of success depends on several factors, such as the woman's age, but it is fair to say that the chance of pregnancy following the replacement of frozen/thawed embryos tends to be lower than that achieved replacing the same number of fresh embryos. There are several reasons why this should be.

• During a cycle of IVF or ICSI, several embryos are cultured to the day of replacement. The fastest and most evenly developing embryos are selected for fresh replacement, leaving the 'second best' for freezing.

• During most frozen/thawed replacement cycles, only as many embryos are thawed as are intended to replace. There is, therefore, no choice of embryos as in a fresh replacement. If however, there are several embryos in storage (particularly if they have been frozen at the pronuclear stage), extra embryos could be thawed and cultured, therefore allowing a choice. If, after replacement, the 'spare' embryos are still of a freezable quality, they can be frozen again.

• Frozen/thawed embryos can lose up to 50% of their cells and still be regarded as viable and therefore can be replaced. Fresh embryos are almost always totally intact.

Further Considerations

Immersed in liquid nitrogen at -196°C, all biological activity ceases within stored embryos. This means that, in theory, they can be stored for hundreds if not thousands of years without degrading. Quite a lot can happen to a couple over that time! Some clinics will impose a time limit for storage (normally 5-10 years) and almost all will want to know the wishes of the couple on the fates of stored embryos should the couple separate or should one partner die. These difficult decisions have to be made before any embryos are stored.

As clinics continually improve chances of success with all treatments, it is likely that many couples may be in a position where they have decided that their families are complete, but, embryos remain in storage. The options open to the couple at this stage are to remove the embryos from storage and allow them to perish or to donate the embryos to a research project. While there is the option to donate embryos to another infertile couple, the issues to consider are complex. While common in many other countries, embryo donation has not yet happened in New Zealand.

Perhaps the most important point to ponder is that you, the couple, are responsible for letting the clinic at which your embryos are stored, know if you move or your circumstances change. It may come as a surprise to know that embryo storage banks all over the world, including New Zealand, contain embryos belonging to untraceable couples. Eventually, at the end of the agreed storage period, these embryos are removed from the banks and disposed of, without the couples ever being able to be informed. If you move, let the clinic know! If you move to a long way from the clinic, or even overseas, it is possible to ship frozen embryos to another more convenient clinic.

Whether or not you decide to have embryos frozen following an IVF or ICSI cycle will depend on a number of variables: how many excess embryos are available, the relative success rates between fresh and frozen/thawed embryo replacement or even your own feeling about placing your own embryos in deep freeze. Before starting a cycle of IVF or ICSI, your clinic should discuss all matters relating to embryo freezing with you. You will then be in a position to make your own informed choices.

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