

CRYOPRESERVED OOCYTES

The field of assisted reproductive technology is advancing day by day with newer options and treatments being discovered. The area of Cryopreservation (freezing) is one of them. Till date it has been possible to freeze sperms and fertilized embryos for years. It involves treating these gametes with special cryoprotectants to enable them to withstand the freezing and thawing process. The treated gametes are further cycled through a slow freezing process using a cryofreezer and then plunged into liquid nitrogen at -196C. The world's first baby born from frozen embryos was pioneered by Dr. John Leeton of Queen Victoria hospital, Australia in the year 1984. Since then several births have taken place worldwide.

Cryopreservation (freezing) can provide an increased cumulative pregnancy rate while decreasing the risk of multiple gestations and the risk of ovarian hyperstimulation syndrome. Cryopreservation also provides an additional chance of pregnancy in a treatment cycle which does not involve full ovarian stimulation and oocyte retrieval.

Cryopreservation involves

- Initial exposure to cryoprotectants
- Cooling to sub zero temperature
- Storage and thawing
- Dilution and removal of cryoprotectants

The goal is to maintain the structural integrity of the cell (total protection of the cell) throughout the Cryopreservation procedure



INDICATIONS FOR OOCYTE CRYOPRESERVATION

- Surplus oocytes obtained during retrieval as in cases of Ovarian hyper stimulation syndrome
- Those at risk of losing ovarian function due to pelvic disease like severe endometriosis and infections.
- Those who wish to delay childbearing
- Donor programme where there is a possibility of oocyte banks
- Single mothers/divorcees
- Premature ovarian failure
- Those women who are likely to lose ovarian function following cancer chemotherapy or radiation



ADVANTAGES

The advantages of exclusively freezing oocytes are:

1. No legal issues concerning single gamete freezing. Just like sperms being frozen for a man, a woman may be entitled to freeze her oocytes for later use. This would be of help in the following scenarios:-
 - Women who wish to postpone childbirth, either for educational or career demands or the absence of a stable relationship.
 - An egg bank facility would make donor programmes a cheaper option when there will be no need for synchronizing the treatment cycle. Just like a sperm bank women will get to use oocytes frozen from potential donors.
 - If a couple opt to step out of a relationship they still would have a legal tangle regarding ownership of frozen embryos. Hence freezing individual gametes would save trouble in decision making
2. To assist siblings in familial cases of premature ovarian failure to have a child using the donated oocytes from the same family.
3. Risk of losing ovarian function due to pelvic disease or following surgery
4. Multiple pregnancies can be avoided
5. Useful in cases of OHSS
6. Moral, ethical and legal problems do not exist like how it does for embryos

COMPLEXITY OF OOCYTE CRYOPRESERVATION

- First live birth Chenetal(1986)
- 21 studies in peer-reviewed journals
- Mean survival rate – 47%
- Fertilization rate 52.5%
- Mean pregnancy rate / thawed oocyte – 1.52% (Oktay et al., Lancet 2004)
- 1.8% - (Borini et al, Hum.Reporod 2006)

REASONS

- Metaphase –II oocyte is a highly specialized cell which contains the meiotic spindle with chromosomes which can get disrupted
- Post freeze the outer shell of the oocyte known as the zona may get hardened so the sperms are injected into the oocyte rather than allowed for normal fertilization as in conventional IVF.(Zona hardening).
- Depolymerization of the meiotic spindle may occur when exposed to low temperatures and may be associated with numerical abnormalities of chromosomes such as aneuploidy
- Chilling injury

FUTURE

The future is certainly aimed at 2 prospective outcomes in this field of Cryopreservation

1. The preservation of fertility in young cancer survivors using oocyte harvest and freezing, ovarian tissue freezing and ovarian transposition or transplantation.
2. Creation of oocyte banks for donor programmes or self for the above mention clinical scenarios

The guidelines are yet to be fully outlined for the conventional IVF programmes, surrogacy and egg donation and since this area is still under research, it may take a while for us to offer it to public.

BREAK THROUGH BIRTH OF THE FIRST FROZEN OOCYTE BABY IN INDIA – 27/8/2008

The patient is a 29 year old, with a previous bad obstetric history and an abnormal karyotype which necessitated the use of donor oocytes for the present conception. The reason for use of frozen oocytes was the non availability of compatible fresh donors. The absolutely exclusive oocytes were in store for cases like these where there is an unexpected non availability of compatible donors in the fresh cycles and the couple are keen on continuing the treatment cycle.

The patient was also counseled about the use of frozen thawed oocytes and the expected success rate for the cycle-not a convincing 2%! The pregnancy resulted from thawing of 10 oocytes, of which unusually 8 survived the thawing process, 6 fertilized with ICSI and transfer of 5 reasonable quality embryos(3 and 2 cells, grade II-III). Why 5 and not the recommend 3 embryos? Well, considering our previous experience of inadequate embryos for transfer following thawing and negative result for pregnancy, we decided to transfer at least 5 of this crop which as I mentioned did not have the conventional cleavage or grade as expected (i.e 4 cells grade I-II)

Given the low pregnancy rates, we had to thaw more oocytes in order to enhance the chances of achieving enough good grade embryos for transfer-success having evaded us earlier. Until date, there is only 50% retrieval rate for frozen oocytes and hence to obtain at least 3-4 reasonable quality embryos, thawing of at least 6-10 oocytes are needed. She progressed uneventfully with early fetal screening tests being normal, until the 5th month when she was diagnosed with mild pregnancy induced hypertension and is currently on treatment with antihypertensives.

During her level-2 scan, where the fetal screening was normal, a shortened cervix of 1.5cm was imaged suggestive of cervical incompetence. She has since then been in admission with us since from 26 weeks for careful surveillance of mother and baby. She did moderate physical activities and her BP was well controlled with medications. An elective cesarean was done on 27/8/2008 and she delivered a normal healthy male baby weighing 2.54Kg. Both the mother and the baby were discharged uneventfully.

The hold of this little bundle of joy-signals our another landmark achievement for serving as a ray of hope to many young women in need of oocyte freezing technology.

- Successful birth of the first frozen oocyte baby in India - Volume 2 / Issue 1 / Jan - Jun 2009 (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2700691/>)
- First successful birth of twins in India following the transfer of vitrified oocytes Year : 2010 / Volume : 3 / Issue : 1 / Page : 44-48 (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2890911/>)