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A RETROSPECTIVE STUDY COMPARING CONCOMITANT OUTCOMES ACHIEVED WITH FRESH AND CRYOPRESERVED OOCYTES

Gynaecology

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ABSTRACT

Objective- To compare the outcomes with fresh versus cryopreserved oocytes.
Material and Methods-The study was a descriptive retrospective study of 2 years aug 2017 – aug 2019 done at International fertility centre, New Delhi. 475 oocytes from 30 donors were obtained.30 recipients with unexplained infertility were included in the study and divided into vitrified group and fresh group of oocytes which was randomly done.Day 3 embryo transfer was done.
Results Survival rate found was 95.2%. There was no difference in fertilization rates (77.2% / 83.1%), day 3 cleavage (76.5% / 83.5%), and blastocyst formation (47.6% / 46,4%) for vitrified and fresh oocytes, respectively. Embryo quality on day 3 and on day 5 were similar for vitrification and fresh oocyte group (79.8% / 80.1% and 76.1% / 80.1%, respectively).
Conclusion : The present study found potential of vitrified oocytes to fertilize and further development similar to fresh counterparts.

KEYWORDS

cryopreservation, vitrification, embryo development

INTRODUCTION

For a variety of reasons, women, especially in developed countries, wish to delay the age at which they become pregnant.The decrease in fertility is markedly accelerated in women at risk for early menopause, including those treated with gonadotoxic therapy for cancer, multiple sclerosis (MS), systemic lupus erythematosus or other autoimmune disorders, those who have undergone extensive ovarian surgery and women with genetic disorders such as Turner syndrome and carriers of fragile X premutation. The American Society of Clinical Oncology and the American Society of Reproductive Medicine have endorsed ovarian stimulation with gonadotropins followed by IVF and embryo cryopreservation as the only established method of female fertility preservation **(1)**. However, this involves a minimum 2 to 5 week delay to complete treatment, produces relatively high estradiol levels, which may be deleterious in certain malignancies and requires male partner participation . Cryopreservation of an entire ovary potentially allows the greatest number of follicles to be preserved **(2)**, but it has not been successfully performed in humans. Ovarian tissue cryopreservation with orthotopic transplantation is an option but requires two operations, has produced only two published live births and is relatively inefficient **(3)** and in cancer patients carries a small but finite risk of reintroducing malignant cells. In contrast with embryo cryopreservation, oocyte cryopreservation does not require male partner participation and avoids the ethical, religious, and legal concerns of embryo cryopreservation because a woman owns her oocytes. The establishment of banks of donated oocytes had considerably simplified the logistics and means by which oocytes could be donated. These banks had made easier to immediately provide oocytes when required that would be compatible to the couple.Since the very first frozen oocyte pregnancy was achieved in humans, the slow cooling method has been applied with varying success . With the combined use of slow freezing and intracytoplasmic sperm injection (ICSI) some researchers have obtained pregnancy rates (PR) per cycle of approximately 22%–25% **(4).** A very recent meta-analysis revealed that the slow freezing technique to cryopreserve human oocytes remains unsatisfactory. The clinical PR per transfer is only 20.6%, the clinical pregnancy per thawed oocyte is 2.3%, and the implantation rate is only 10.1% **(5).** However, conventional slow-cooling has been associated with oocyte survival rates of only 50 to 65% because of ice crystal formation and has produced inconsistent live-birth rates. Recent advances in oocyte and embryo cryopreservation have been reported by use of vitrification, which was originally applied in embryology by Rall and Fahy with mouse embryos. The physical definition of vitrification is the solidification of a solution at a temperature below the glass transition temperature of the solution, not by ice crystallization, but by extreme elevation in viscosity, using high cooling rates from -15,000 to -30,000_C per minute .This ice-free cryopreservation method has been modified to optimize results in humans. Oocyte vitrification avoids meiotic spindle damage and has resulted in survival rates of over 80% . It is believed that there are two main shortcomings to the vitrification technique: 1. high concentrations of cryoprotectants are required, which may be toxic to oocytes and embryos and 2. if cooling rates applied are insufficient, intracellular ice and chilling may occur. To overcome these problems, several methods have been devised in which very small volumes of sample to be vitrified are used. A variety of these techniques can be found in the literature **(6)** and have been applied with varying degrees of success in several species of mammals, including humans. The present study was done with aim to compare the outcomes with fresh versus cryopreserved oocytes using cryolock vitrification device.

MATERIAL AND METHODS

The study was a descriptive retrospective study of two years (2017 – 2019) done at International fertility centre, New Delhi. The hospital database was reviewed and required data collected. A total of 475 oocytes were obtained from 30 oocyte donors. 30 recipients with unexplained infertility were included in the study , between august 2017 - August 2019.

Protocol for Oocyte Donors

All donors were between the age of 18 and 35 years. Thorough medical history, including current or past exposure to radiation or hazardous chemical substances, IV drug use and reproductive history was taken.There was no family history of hereditary or chromosomal diseases. General and systemic examination including gynecological examination were done for all donors. Apart from routine blood investigations , specific investigations including high performance liquid chromatography , karyotype and screening for sexually

transmitted diseases were done .Donor controlled ovarian hyperstimulation (COH) was started on second day of menses after ascertaining ovarian quiescence by performing transvaginal ultrasound (TVS) on the same day . All donors received GnRH antagonist protocol. Starting dose varied from 150 to 300 IU/day of recombinant FSH or highly purified hMG for the first 5 days, according to age, body mass index (BMI) and response to previous ovarian stimulations. The dose was then adjusted to the ovarian response as determined by TVS, performed at intervals of 2–3 days. Stimulation was carried out untill leading follicles were 18 mm in mean diameter. Recombinant hCG was then administered and ovum pick up was performed 36 hours later. Anonymous donors were matched with their recipients according to phenotype and blood groups.

Protocol for Oocyte Recipients

All recipients included in the study given informed written consent . They were given hormone replacement therapy (HRT). Increasing doses of estradiol valerate were given as follows: 2 mg BD for the first 4 days , followed by 2 mg TDS for the next 4 days .Further increment in dose depends on endometrial thickness assessment by TVS . Once the endometrial thickness reaches more than 7.5 to 8 mm thickness with trilaminar pattern with zone 3 or 4 vascularity ,decision for transfer is taken . Micronized P (800 mg/day, vaginally) was started on the day of oocyte donation . All oocytes retrieved from a single donor were donated to a single compatible recipient. After ovum pick-up, oocytes were maintained in IVF medium for 2 hours in incubator. They were then enzymatically denuded to assess nuclear maturity. Metaphase II (MII) oocytes assigned to a single recipient were randomly allocated to either one of two groups by application of a randomized computer-generated list. Those oocytes to be cryopreserved were allocated to the group "vitrified oocytes," and the remaining ones were maintained in culture and were assigned to the group "fresh oocytes."After being vitrified and stored for at least 1 hour, cryopreserved oocytes were warmed and rehydrated, whereas the fresh oocytes remained in culture for 2 hours. In this manner intracytoplasmic sperm injection (ICSI) could be carried out simultaneously on both vitrified and fresh oocytes. In this series, embryo transfer was performed on day 3. All embryos not transferred on day 3 were cultured until day5 and then freezed. The Cryolock vitrification device was used for oocyte vitrification .Embryo quality was assessed on day 3 and at the blastocyst stage by using consensus scoring system.

Statistical Analysis

The chi 2 test was used for quantitative variables. Statistical significance was defined as $p<.05$.

RESULTS

In the present study , 30 donors and 30 recipients were evaluated. **Table 1** shows age, number of oocytes retrieved , number of oocytes received and BMI for donors and recipients. One hundred fifty eight vitrified oocytes were fertilized normally after ICSI (77.2%) whereas the fertilization rate for fresh oocytes was 83.1% ($p>0.05$). Values for abnormal fertilization and degenerated oocytes after ICSI were not statistically significantly different among vitrified and fresh oocytes as shown in **Table 2. Table 3** summarizes embryo development of fresh versus frozen oocytes. For day 2 development, the cleavage rate, and embryo quality were similar for both groups. In the vitrified group, 76.5 % of zygotes underwent cleavage on day 3,whereas for zygotes from fresh oocytes the cleavage rate was 83.5% ($p>.05$).Good quality embryos from vitrified oocytes was 79.8% and in those derived from fresh oocytes was 80.1% on day 3 ($p>.05$). Development rates for blastocyst were nearly identical (47.6% and 46.4%) for vitrified and fresh oocytes. **Table 4** shows the clinical results .In 15 cases, only embryos from vitrified oocytes were transferred. In remaining 15 cases, only embryos derived from fresh oocytes were transferred. Pregnancy rate per transfer observed in the vitrified group was 40.1% and 46.7% in the fresh group but the difference was not statistically significant ($p>.05$).

DISCUSSION

The present study compared the outcome of vitrified versus fresh oocytes. It is reassuring that the results are comparable to those achieved using standard IVF treatment. Vitrification procedures circumvent two of the major limiting factors for achieving optimal cryopreservation: chilling injury and ice formation . Chilling injury can be defined as the irreversible damage after exposure of cells to low temperatures, from -15_C to-5_C before the nucleation of ice. This detrimental event affects mainly the cytoskeleton and cell membranes.Chilling injury can be minimized during vitrification by use of high cooling rates **(7).** The velocity of the process is dependent on the volume of the vitrification solution (smaller volume of the sample, higher the cooling rate). On the other hand, direct contact with liquid nitrogen also increases the cooling rate. To avoid ice formation, the vitrification technique makes use of high cryoprotectant (CPA) concentrations **(7),** although such high concentrations are considered toxic to cells.Nonetheless , an appropriate, phased composition of CPA could mitigate the toxic and osmotic consequences of highly concentrated CPAs **(8).** In this way, a combination of two or three of these agents can decrease the individual specific toxicity. The most common mixture used for this purpose consists of ethylene glycol, DMSO, and sucrose **(8).** To optimize the results, in addition to an appropriate selection of CPAs, it is helpful to use these agents at lower concentration, while maintaining the necessary concentration to achieve vitrification. By dramatically increasing the cooling rate, the CPA concentration could be reduced. As a result, a high cooling rate avoids chilling injury and allows the reduction of the concentration of CPA, thereby preserving the cells at nontoxic concentrations of cryoprotectant. Several approaches fit these conditions. The "minimum drop vitrification" method proposed by Arav **(9)** has been tested successfully for bovine and porcine oocytes. It uses a very small volume of vitrification solution, and samples are placed on a special device that is cooled very quickly **(9).** Other methodologies using low volumes for loading the samples have been developed. In the present study , 205 out of 216 (95.2%) oocytes survived after warming.Yoon et al. **(10)** reported the birth of two infants derived from vitrified oocytes using electron microscope grids. Three years later, the same group reported that 325 of 474 (68.6%) oocytes survived after warming, and 142 (43%) of those were fertilized normally.Pregnancy and implantation rates were 21.4% and 6.4%, respectively. Kuleshova et al. **(11)** reported a live birth after the transfer of one chromosomally normal embryo as assessed by FISH analysis, generated from a vitrified human oocyte using the openpulled straw system. A very recent study also reports two ongoing pregnancies after vitrification of human oocytes applying this method **(12),** although this study was performed with a low number of oocytes. The oocyte survival rate was 75% (18 of 24 oocytes), the fertilization rate was 77.7%, the PR per transfer was 33.3%, and the implantation rate was 21.4% (3 of 14 replaced embryos). In the present study, we applied the Cryolock vitrification device to our oocyte donation program. In the present study, the first step to assess the integrity of the survived oocytes was their capability to fertilize normally after vitrification. It was noticed that the fertilization rates were not significantly different between groups, as this value for vitrified oocytes was 77.2%, whereas for fresh oocytes it was 83.1%. These observations were also similar to the obtained by other reseachers with different vitrification systems **(12)** .This suggests that oocytes conserve their capability of fertilization after being cryopreserved by all of these improved strategies.We also observed a similar rate of cleavage on day 2 (93.9% vs. 96.7%) and on day 3 (76.5% vs. 83.5%) after ICSI, in both vitrified and fresh oocytes, respectively.Moreover , blastocyst development rate was 47.6% for the vitrified group, similar to 46.4% observed for fresh oocytes, showing that vitrified oocytes conserve intact their potential to reach the blastocyst stage. This fact constitutes a manifestation of normal development and for instance, the absence of damages that could compromise the viability. To assess further the influence of the vitrification procedure on embryo development, we have also evaluated embryo quality in both early and blastocyst stages. Embryo quality of day 2 embryos, was 83.3% for vitrified oocytes and 70.4% for fresh ones. For day 3 and blastocyst stage, embryo quality was similar between groups (79.8% vs. 80.1% and 76.1% vs. 80.1% for vitrified and fresh oocytes). In addition, these data indicate that vitrification does not impair cleavage rate and embryo quality, nether for day 3 nor for blastocyst stage. Pregnancy rates per transfer with vitrified oocytes (40.1% and 46.7%, respectively) are similar to those observed with fresh oocytes **(13).** Applying the Cryotop method to human oocytes, Kuwayama et al. have reported a 91% survival rate, 81% cleavage rate, and a 50% blastocyst rate, with a 41% PR per embryo transfer, resulting in 11 live births **(14).** Katayama et al. **(15)** have reported excellent survival, fertilization, and cleavage rates of 94%, 91%, and 90%, respectively .

CONCLUSION

To conclude ,the present study showed that fertilization and pregnancy rates are similar to IVF/ICSI with fresh oocytes when vitrified oocytes are used as part of IVF/ICSI .Evidence indicates that oocyte vitrification and warming should no longer be considered experimental.

Table 1 – Donor and recipient data

Charecteristics	Donors	Recipients
Number	30	30
Age (years)	25.6 ± 3.2	34.8 ± 2.4
No. of oocytes	475	-
Mean oocytes received	-	16.79 ± 3.5
BMI(kg / m^2)	21.2 ± 2.4	22.1 ± 3.7

Table 2 – Oocyte distribution, survival and fertilization

	Vitrified	Fresh	p value
M II oocytes No. (%)	216 (51.4)	204 (48.6)	0.251
Survival No. (%)	205 (95.2)	-	-
No. of injected oocytes	205	204	-
Normal fertilization No. (%)	158 (77.2)	169 (83.1)	0.118
Abnormal fertilization No. (%)	8 (3.8)	10 (5.1)	0.358
Degenerated oocytes No. (%)	6 (2.6)	5 (2.4)	0.708

Table 3 – Embryo quality

	Vitrified	Fresh	p value
Cleavage rate Day 2 embryos	148/158 (93.9%)	163/169 (96.7%)	0.088
Good quality Day 2 embryos	123/148 (%)	115/163 (%)	0.068
Cleavage rate Day 3 embryos	113/148 (76.5%)	136/163 (83.5%)	0.089
Good quality Day 3 embryos	90/113 (79.8%)	107/136 (80.1%)	0.945
No. of embryos undergoing extended culture	81	106	-
Blastocyst rate No. (%)	39/81 (47.6%)	49/106 (46.4%)	0.758
Good quality blastocysts No. (%)	30/39 (76.1%)	39/49 (80.1%)	0.612

Table 4 – Clinical results

	Vitrified	Fresh	P value
No. of transfers	15	15	-
No. of embryos transferred (Mean ± S.D)	32 (2.1 ± 1.2)	30 (2 ± 0)	-
Pregnancy rate per transfer	6/15(40.1 %)	7/15(46.7%)	0.233

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