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Annals of Reproductive Medicine and Treatment

Review Article

Oocyte Cryopreservation

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Submitted: 14 April 2017

Accepted: 29 May 2017

Published: 30 May 2017

Keywords
- Oocyte freezing
- Vitrification
- Egg banking

Abstract

Oocyte cryopreservation has achieved an important role in infertility treatment and is increasingly being used for various medical, legal and social reasons such as fertility preservation in women at risk of compromising fertility due to oncological treatment or chronic diseases, oocyte donation, and delaying childbirth, and eliminates several religious, ethical, and legal concerns of embryo freezing. Introduction of new 'vitrification' technique has made the success rates for actual conception more reliable than the earlier method of slow freezing and opened a new era for this technology. Due to the improvements in the techniques and clinical outcomes related with oocyte cryopreservation, American Society of Reproductive Medicine (ASRM) has also declared that oocyte freezing should no longer be considered experimental. Since then oocyte freezing and egg banking have been proposed for various new horizons of indications.

ABBREVIATIONS

ASRM: American Society of Reproductive Medicine

INTRODUCTION

Cryopreservation has emerged as an important developmental milestone in field of assisted reproduction and it has made this technique more effective as well as more flexible. Although cryopreservation of sperm and embryos have been performed successfully as a part of routine IVF procedures for a long time **[**1**],** it took more than 20 years for oocyte cryopreservation to evolve into a successful technique with acceptable clinical outcomes. This delay in evolution of technique can be attributed to challenges related to the structure of oocytes and freezing methods.

Oocyte cryopreservation has achieved an important role in infertility treatment and is increasingly being used for various medical, legal and social reasons. Availability of oocyte freezing and banking has evoked hope in those patients who have been diagnosed with cancers that might affect their fertility later. In 2013 American Society of Reproductive Medicine (ASRM) [2] has also declared that oocyte freezing should no longer be considered experimental. Since then oocyte freezing and egg banking have been proposed for various new horizons of indications. Oocyte freezing has evolved from being done for donor eggs & fertility preservation to now being used for nonmedical reasons like social egg freezing. Introduction of new 'vitrification' technique has made the success rates for actual conception more reliable than the earlier method of slow freezing and opened a new era for this technology. In 2007, our group at Lilavati Hospital & Research Centre, Mumbai (India) was one of the earliest groups in country to introduce the oocyte freezing using 'vitrification' technique. Since then we have performed freezing of more than 1000 of oocytes with subsequent successful pregnancy outcomes using this technique for women with various indications.

This review focuses on highlighting various indications, developmental milestones, clinical outcomes as well as various concerns related with oocyte cryopreservation.

Indications of oocyte freezing

There are several areas where an efficient oocyte cryopreservation program would be beneficial, including:

Fertility preservation in women with malignant or premalignant conditions, who have been provided a fairly good chance of survival with a normal post recovery life style due to progress in oncostatic treatments. However, these women are at risk of having menstrual disorders, infertility due to decreased ovarian reserve, and early menopause. Additionally chemotherapy can accelerate follicular depletion and radiotherapy can induce ovarian damage and significantly reduce the content of follicles and oocytes inside the ovary. Various established as well as experiential techniques are proposed for fertility preservation prior to chemotherapy and radiation therapy. Of these mature oocyte freezing is clinically accepted to yield the best results. Recently for cases such as breast cancer wherein it is not advisable to wait for the next menstrual period to start a stimulation protocol owing to urgency of cancer treatment, random-start ovarian stimulation protocol has been proposed. This protocol provides a significant advantage by decreasing total time for the IVF cycle, and in emergent settings, ovarian stimulation can be started at a random cycle date for the purpose of fertility preservation without compromising oocyte yield and maturity [3]**.** Oocyte freezing is very good option for

these women as they can immediately go for the stimulation cycle and egg retrieval. Embryos can be created at later stage when they find a suitable life partner & are in remission period and become ready for pregnancy.

Apart from malignant conditions, women with certain genetic **co**nditions such as BRCA 1 and 2 mutations may also be candidates for fertility preservation. These medical disorders are associated with high risk of ovarian cancer and prophylactic salpingo opherectomy may be recommended. In addition several genetic conditions have been associated with premature ovarian failure such as Turner's syndrome, fragile X permutation and deletion of X chromosome. Oocyte freezing provides an option for fertility preservation for women with these risk factors before ovarian failure ensues **[4].** Additionally, successful oocyte freezing/thawing technique would potentially be helpful in donor oocyte programs. Post menopausal women and women with poor ovarian reserve can achieve pregnancy with use of donor eggs. Egg banking negates the need of coordination and synchronization of the donor and the recipient cycles. Thus frozen Egg banking is an asset in providing greater range of choice in donor profile without wasting any time needed in the treatment process. It also allows quarantine of oocytes giving time for genetic and infection screening and acts as a back-up for fresh oocyte donation program. In a study Cobo et al demonstrated high oocyte cryosurvival, similar fertilization, embryo development, implantation, and pregnancy rates to those reported after fresh egg donation **[**5**]**. Another group of women who may be benefited from oocyte cryopreservation are women, who wish to delay the child birth i.e. for 'Social oocyte freezing'. Female fertility peaks between 20s and 30 and starts falling after the age of 30 years; oocyte freezing is the option to freeze the eggs before the biological clocks starts ticking. It allows women to freeze their eggs at the peak of fertility and create embryos at later stage when they find a suitable partner or when they are ready to pursue the family. Nonmedical egg freezing is to preserve women's most important ability to reproduce, as it provides a technical solution to a number of problems women face due to the extended education and waiting to establish their household and career [6]. Oocyte cryopreservation also provides a reasonable option in situations when husband is unable to give semen sample due to some unexpected problem or failure of yielding sperms in testicular biopsy on the day of oocyte retrieval. In such situations, many couples are not willing for the use of donor sperms. Oocyte freezing is relief giving option to such couples where wife's eggs are stored and thawed at a later date when sperms are obtained on subsequent testicular biopsies or sperms are obtained following multiple ejaculated samples **[**7**]**. Similarly in non obstructive azoospermia patients wherein the wife is an expected poor responder, multiple oocyte retrieval cycles can be performed with oocyte freezing and pooling. Once sufficient number (at least 10-12) of mature oocytes are pooled and frozen, testicular retrieval of sperms using surgical techniques such as TESE or micro dissection TESE is performed and the pooled thawed eggs are injected. A novel indication for oocyte cryopreservation is accumulation of oocytes for women with poor ovarian response**.** Poor responders or women with decreased ovarian reserve yield limited number of oocytes during IVF stimulation cycle. Oocyte pooling and egg banking is a practical option in these women, wherein multiple stimulation cycles are done and all the collected oocytes are thawed together and ICSI is performed to create the embryos thus contributing to increase in the inseminated cohort and creating a similar situation as in normal responders, thus achieving higher live birth rates [8]. Apart from several medical or non medical indications there are some legal/ethical reasons as well for oocyte freezing. In countries like Italy, where the law does not permits embryo freezing, oocyte freezing is a good option to save the extra oocytes during an IVF cycle and freeze them for later use.

Techniques of Oocyte Freezing

The two known techniques for oocyte freezing are slow freezing and vitrification.

In Slow freezing method extracellular ice formation drives cellular dehydration through an equilibrium process. The slow-freeze method relies on low initial cryoprotectant concentrations. In contrast Vitrification is a non equilibrium cooling method, which utilizes very high concentrations of cryoprotectants that solidify without forming ice crystals.

Due to the substantial toxicity related with high concentration of permeating cryoprotectant, the oocyte cannot be exposed to this temperature for long. So, a very short time is allowed for equilibration, which follows the plunging of oocytes directly into liquid nitrogen. Another factor which protect against ice-crystal formation, is an extremely rapid rate of cooling. This can be achieved by using novel cryovessels that allow direct contact between liquid nitrogen and the oocyte-containing solution. Vitrification has shown its popularity in freezing the oocytes due to its superiority to the slow-freezing method, in form of better oocyte survival rate, fertilization, and embryonic development in vitro. These results may be related to the fact that vitrified human oocytes incur less damage to spindle integrity and chromosome alignment.

Slow freezing Vs Vitrification

The first birth with frozen oocytes was reported in 1986 with slow freezing **[**9**],** but due to very low success rates, there were only five live births reported with this technique for over a decade. Oocyte cryopreservation using the technique of vitrification resulted in first live birth in 1999, reported by Kuleshova et al. **[**10**],**. However, following this only a few case reports and clinical studies were reported until 2005. A meta-analysis conducted by Oktay K et al in 2006 **[**11**]**, reviewed all the reports on live birth following oocyte cryopreservation and concluded that oocyte cryopreservation using slow freezing has given lower success than that of IVF with fresh oocytes. However, they could not provide a valid comparison of vitrification with either slow freezing or fresh oocyte cycles due to the limited number of reports with vitrification at the time of publication. After that several studies have demonstrated results in favor of vitrification. Nagy et al (2009) compared the outcomes of IVF with vitrified donor eggs with results of previous fresh donor IVF cycles by the same donors. Despite the lower numbers of eggs available to each recipient in frozen compared to the fresh cycles, fertilization rates, implantation rates, pregnancy rates per fresh transfer, and cumulative pregnancy rates were all as high in the vitrified egg cycles as in the prior fresh cycles **[**12].

In a particularly well-controlled study Cobo et al (2010) compared fertilization rates and embryonic development between sibling eggs by prospective randomization of donor egg cohorts into fresh IVF or post-vitrification IVF treatment groups. They found no significant differences in fertilization rates, day 2 cleavage, day 3 cleavage, day 3 embryo quality, blastocyst formation, or blastocyst quality [13]. In a similarly well-designed study, Rienzi et al (2010) prospectively randomized sibling eggs within cohorts retrieved from infertility patients to either fresh IVF or post-vitrification IVF. They found comparable fertilization rates, embryonic development, and embryo quality between the fresh and post-vitrification treatment groups [14]. In a recent Cochrane review of two RCTs with 106 participants it was found that Vitrification was associated with an increased clinical pregnancy rate compared to slow freezing. No data was available on live births or adverse effects [15]. Over the past 7 years, the improvement in oocyte cryopreservation technology especially with vitrification has widened its clinical applications. Considering its success rate on the basis of available evidence, American Society of Reproductive Medicine has recently stated that oocyte cryopreservation should no longer be considered experimental for women with medical indications, outlying elective oocyte cryopreservation [2].

Outcomes with Oocyte cryopreservation

Several studies have reported successful clinical outcomes with oocyte cryopreservation. In a prospective randomized study Cobo et al.[16], evaluated the outcome of oocyte vitrification using the Cryotop method, in an egg donation program by simultaneously evaluating embryos derived from vitrified and fresh oocytes coming from the same stimulated cycle. They observed similar fertilisation rates, day 2 cleavage rates, blastocyst formation for vitrified and fresh oocytes. Thus excellent clinical outcome indicates the possible use of this technology for egg donation programs, as well as a high potential for establishing oocyte banking.

In a meta-analysis on oocyte vitrification and post warming fertility outcomes, on comparing vitrified with fresh oocytes, no statistically significant difference was observed in fertilization, cleavage and clinical pregnancy rates, but ongoing pregnancy rate was reduced in the vitrified group (odds ratio 0.74), with heterogeneity between studies. The review highlights the scientific excellence that has been achieved over the decades. It suggests that cryopreservation is effective and safe, but individual units need to determine their safety and efficacy using vitrification techniques [17]. Despite the many scientific reports indicating successful reproductive outcomes, this technique has raised several concerns as well. One of the most important concerns is its relation with chromosomal abnormalities in children born through this technique, due to alteration of meiotic spindle integrity. However, studies have provided reassurance related to this concern. Noyes et al [18], reviewed 58 reports [1986 to 2008], which included 609 live born babies (308 from slow freezing, 289 from vitrification and 12 from both methods) and formed a database to verify live born infants born after oocyte freezing. No difference was observed in congenital anomalies compared with naturally conceived infants. They concluded that with more live born data accumulating, this procedure may become main stream as a fertility preservation option, particularly for women diagnosed with malignancy requiring cytotoxic therapy. In a recent RCT Forman EJ et al. [19], found that aneuploidy rates in embryos derived from vitrified oocytes were similar to those derived from fresh oocytes in young infertile women undergoing IVF with their own eggs. This report concluded that oocyte cryopreservation does not have an adverse effect on chromosome segregation during meiotic division.

Factors affecting success rate of oocyte cryopreservation

Several factors have been attributed to affect the success with oocyte cryopreservation. These can be related to patient (such as age, cause of infertility, donor/nondonor oocyte), stimulation protocols, number of oocytes, cryopreservation methods (slow-freezing and vitrification), and devices (cryotop, cryoleaf, cryotip), as well as indications for oocyte cryopreservation (medical, nonmedical, or IVF-related reasons) [20].

Although each and every factor described above can affect the success of oocyte cryopreservation, age remains one of the most important determination factors, which is due to age related decline in oocyte quality. A recent individual patient data meta-analysis also reported that live-birth success rates with cryopreserved oocytes show an age-related decline regardless of the freezing technique used, and an aged-based probability of live birth may be calculated for cryopreserved oocytes [21]. Another factor affecting the success is the available number of oocytes for freezing. An observational longitudinal cohort multicentre study performed by Rienzi et al in 450 couples, with 2721 warmed oocytes of which about 2304 of them survived cryopreservation (84.7%). A total of 128 deliveries were obtained (26.3% per cycle and 29.4% per transfer) and 147 babies were live born from 929 embryos transferred (15.8%). The forward logistic regression analysis on a per patient basis showed that female age, number of vitrified oocytes, and the day of transfer influenced delivery rates. Study concluded that more than eight vitrified oocytes are required to improve the outcome and delivery rates [22]. A latest study also supports the concept that at least 8–10 metaphase II oocytes are necessary to achieve reasonable success. Numbers should be individualized in women>36 years old. They suggest encouraging women who are motivated exclusively by a desire to postpone childbearing because of age, to come at younger ages to increase success possibilities. (Cobo et al.) [23].

Benefits and risks related with oocyte cryopreservation

Egg freezing refutes the need of donor and recipient synchronization and allows the eggs to be quarantine for infection screening. It avoids loss of surplus oocytes in countries where embryo freezing is illegal. Considering its advantages for cancer patients, it is presently a mainstream technique & is better than the still experimental ovarian tissue cryopreservation as it is not associated with the risk of reimplantation of malignant cells. However Even though this has become very popular, this can lead to false hope to those who are planning to delay pregnancy, as ovarian reserve decreases with increasing age. Other downside of egg freezing is that patient has to undergo surgical egg retrieval, and these frozen eggs need to undergo intracytoplasmic injection

for achieving fertilisation. There also are theoretic concerns related with infectious disease due to the use of open vitrification methods. However, infectious transmission has never been observed in reproductive tissues from this technique **[**24**]**.

There is also a need for further long term studies on congenital anomalies and health risk associated with egg freezing. As stated in the ASRM–SART guideline, "there are not yet sufficient data to recommend oocyte cryopreservation for the sole purpose of circumventing reproductive aging in healthy women because there are no data to support the safety, efficacy, ethics, emotional risks, and cost-effectiveness of oocyte cryopreservation for this indication" **[**2**].**

CONCLUSION

In the last few years' major advances in the field of oocyte cryopreservation especially with vitrification, have opened a new era in the field of ART. In the past it was indicated for women, suffering with various premalignant or malignant conditions, to prevent them sterilizing cancer therapies. However it has expanded its role in several non medical indications as well including the women at risk of reduced reproductive capacity due to age-related fertility decline and as a part of oocyte donation programs. With proven results of vitrification technique in terms of post thaw survival and pregnancy rates, oocyte freezing has gained popularity in egg donation services. It is also a viable alternative to embryo cryopreservation because does not carry the same ethical and legal issues. With expanded role and indications, oocyte cryopreservation will soon play integral role in infertility treatments.

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Cite this article

Pai HD, Palshetkar N, Talreja D, Pai RD, Palshetkar R, et al. (2017) Oocyte Cryopreservation. Ann Reprod Med Treat 2(3): 1016.