3 Vitrification of Oocytes: General Considerations

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Cryopreservation • Fertility • Oocyte • Survival • Vitrification • Warming • Fertility preservation • Oocyte cryopreservation • Oocyte survival rate

Introduction

Cryopreservation of human cells is an integral part of assisted reproductive technology (ART). Sperm freezing is an established procedure and has been so for decades. As the rates of implantation with in vitro fertilization (IVF) improved, fewer embryos were being transferred owing to growing concerns of multiple pregnancies, and the cryopreservation of the supernumerary embryos was perceived as a necessity. The first successful report of human embryo freezing that generated a pregnancy was in 1983 [1]. Many rapid advances and improvements in the techniques of embryo freezing were made since, and a near consistent success rate was achieved with embryo freezing. Embryo freezing rapidly became established as a routine procedure in the ART lab.

Surprisingly though, enthusiasm for the cryopreservation of human oocytes remained conspicuous by its absence. Chen reported the first human birth using cryopreserved oocytes as early as in 1986 [2]. However, the failure to reproduce this early success became a major setback and the possibility of offering oocyte cryopreservation as a routine procedure remained remote. For a long time, it was considered asan experimental procedure at best. Significant resurgence of interest in the human egg freezing came about in recent times owing to its perceived potential to preserve fertility. Vitrification was applied to oocyte cryopreservation techniques, which produced more consistent and reproducible results. Kuleshova et al. reported the first birth from vitrified human oocytes [3]. Various studies followed, which showed superiority of vitrification over slow freezing in the post-thaw survival of vitrified oocytes [4, 5]. Vitrification was proposed as an alternative to conventional slow-freezing methods to avoid chilling injury

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and ice crystal formation. Recent studies have established that oocyte vitrification and subsequent warming are producing clinical results comparable to the results achieved by fresh cycles [6, 7].

Latest advances in cryobiology have led to a newer option or protocols available for the oocyte cryopreservation in terms of technique used, media composition, and various storage devices used for vitrification. This has resulted in an improved survival of cryopreserved oocytes. The American College of Obstetricians and Gynecologists (ACOG) endorses modern procedures to cryopreserve oocytes and no longer considers it experimental. The chapter aims at giving an overview of modern oocyte cryopreservation protocols and its indications and applications in current times.

Indications for Oocyte Freezing

- Loss of reproductive function in young women due to chemotherapy, radiation, and/or surgery for cancer
- Addressing the ethical and legal issues involved in embryo freezing
- Oocytes cryobanking for egg donation program
- In cases of premature menopause and then loss of ovarian function
- Women who want to delay childbearing
- Prevention of ovarian hyperstimulation syndrome
- Male factor infertility or inadequate seminal samples

Present trends show an increasing emphasis on health checkup among the general population, resulting in more aggressive cancer screening and earlier detection of cancers. This has meant earlier treatment and better survival rates. Advances made in the field of cancer treatments also have contributed to the increase in the population of cancer survivors. It is estimated that by 2015, one in every five cancer patients will survive and one in every 250 individuals will be a cancer survivor [8]. Chemotherapy and radiotherapy are known to decrease the fertility potential. The survival for these cancer patients comes at a cost of their fertility as chemotherapy has an adverse effect on ovarian function, and it is raising a new concern about their fertility [9]. Cryopreservation of ovarian tissue has been tried, but the potential risk of reintroducing cancer cells remains a matter to be seriously considered and counseled about. Embryos may be cryopreserved in patients with partners. The ethical and legal issues need to be discussed with the couple. Oocyte vitrification is the logical choice for the unpartnered and those with reservations with embryo freezing. Oocyte cryopreservation holds promise of fertility preservation for the cancer patients. Oocyte preservation should be recommended to those about to start gonadotoxic drugs or radiotherapy after adequate counseling. There has been no increase in the number of anomalies in children born from fertilizing the frozen oocytes demonstrated by a compilation of various studies. The pregnancy rates following oocyte cryopreservation although limited is however reassuring thereby making it a very attractive option for the cancer patients. A newer approach of cryopreserving immature oocytes from the luteal phase of the cycle has been recommended to prevent delay in the start of chemotherapy [10]. In vitro maturation (IVM) of the frozen oocytes prior to fertilization is then performed, while certain investigators have recommended IVM prior to the cryopreservation.

An application of oocyte vitrification likely to gain immense popularity is the establishment of donor oocyte banks for egg donation program. The oocyte donation practiced today involves the use of fresh oocytes, which requires the synchronization of periods required between the donor and the recipient. This either restricts the choice of donors for the recipient or causes delays in the cycle for clients who are very particular about the donor choice. These delays and restrictions increase the cost of treatment. Occasionally, unexpected events in a donor's life have led her to being unable to donate at a particular time, causing immense frustrations and loss of money for the recipient. Vitrifying oocytes and creating an oocyte bank can overcome these restrictions, much along the same lines as a sperm bank. The recipient is assured of a donor of her choice at

any time that she plans her cycle while the donor is also benefitted since she can donate eggs at her convenience. Donor oocyte banking potentially offers more choices for donor selection and more flexibility in the timing of treatment cycles and eventually cost reduction.

Assisted reproduction involves controlled ovarian stimulation and creating many embryos. As the rate of implantation increased, the number of embryos transferred started decreasing amid growing concerns of multiple pregnancies and associated complications. Cryopreservation of supernumerary embryos while a logical option raised ethical and legal concerns. There were clients with ethical and moral reservation with cryopreserving embryos, while certain countries like Germany and Italy continued to remain reluctant to endorse embryo freezing and even outright forbidding it. Legally too, there have been conflicts arising owing to a lack of definition or even a clear consensus with regard to the status of the embryos. There were some assigning the embryos the status of a human being with legal rights, while others consigning the embryos a status of an unborn child with no legal rights.

These dilemmas are of particular concerns in present times where transfer of fewer embryos is the trend and certain medical situations which demand the cryopreservation of all embryos as in prevention of OHSS a potentially life-threatening situation. Cryopreservation of oocyte addresses many of the ethical and legal concerns, and a well-established oocyte preservation program will provide viable option in many of these situations.

Every woman is born with a finite oocyte reservoir, and with age, there is an increased depletion in numbers also termed as "gamete exhaustion" along with the deterioration of oocyte quality. In women with a family history of early menopause, oocyte depletion is much faster than usual. Oocyte freezing can be an effective tool to preserve their eggs.

Certain genetic conditions like Turner mosaic are associated with premature menopause, and oocyte cryopreservation has been suggested in this group; however, the risk of transmission of the congenital anomalies and the safety and efficacy of the process need adequate consideration. For women who wish to delay childbearing, oocyte cryopreservation may be considered an option. However, the data for this group is very minimal with little data available of its efficacy. Promoting the use of oocyte cryopreservation in this group may give false hopes and may encourage delaying childbirth in women. The counseling in this group needs to address each of these issues and the potential risk of child bearing at a later age.

Challenges Associated with Oocyte Vitrification

Oocyte cryopreservation is technically more challenging than embryo cryopreservation. This is because the oocyte is a very sensitive cell. Shape, size and cell number are important factors affecting survival during cryopreservation and explain why it has taken so long to introduce oocyte cryopreservation even though the first embryo was cryopreserved 40 years ago.

Factors Affecting Oocyte Survival

(a) *Spherical shape*: Perfect sphere of the oocyte slows down permeation and equal distribution of cryoprotectant in the oocyte. This continuous concentration gradient results in longer exposure of oocyte to cryoprotectants, which may lead to toxic damage in one part of the oocyte offering less than optimal protection in the other. Therefore, the changes in the shape at the equilibration step due to osmotic effect may provide some type of benefits, but the cytoskeleton damage of the oocyte may also be triggered [11].

(b) *Size*: Size is a very important parameter in cryobiology. The larger the cell, the more difficult to cryopreserve it. Oocytes are the largest cell of the human body. Size of the oocyte widely affects crystal formation and the slow dilution or accumulation of toxic cryoprotectants, thus increasing the challenge of survival.

Fig. 3.1 Principle of vitrification

$$\text{Successful vitrification} = \frac{\text{Cooling and warming rate} \times \text{viscosity}}{\text{Volume}}$$

(c) *Low cell number*: Since oocyte is a single-cell structure, there is no backup to regenerate from serious injuries as compared to multicellular embryo, which can survive up to 50 % loss of its cells [11].

(d) *Osmotic shock:* The osmotic shock that can occur during dilution may result in extensive swelling, rupture of the membrane, and immediate death of the oocyte.

(e) *Cryoinjuries*: The oocyte is highly susceptible to cryoinjuries to the cytoplasmic content and the nuclear spindle [12]. Chilling injury occurs at high temperature and induces irreversible damage to the cytosolic content, membrane, and zona pellucida. Hardening of the zona due to premature cortical granule release may result in decreased fertilization rate. Therefore, a very careful approach has to be applied for oocyte freezing.

Due to these varied difficulties associated with oocyte vitrification, for many years, oocyte vitrification was considered experimental, and, therefore, embryo cryopreservation was the only established option for fertility preservation in female cancer patients [13].

Vitrification

Vitrification is a process of cryopreservation which involves exposure of oocyte to high concentration of cryoprotectants and ultrarapid cooling to solidify the cell into glass-like state without the formation of ice crystals. In the vitrification process, cooling rate in the range of 2,500–30,000 °C/min or greater is used, which results in the water of the cells to transform from the liquid phase to a glassy vitrified state. During the vitrification process, an oocyte is placed in a small volume of the vitrification medium and is then cooled at an extremely rapid rate. High osmolarity of the vitrification medium allows rapid dehydration of the cells, and immersion into liquid nitrogen solidifies the cells. This ultrarapid freezing eliminates the formation of damaging ice crystals in the cells. Though the high concentrations of cryoprotectants are toxic to oocytes, its toxicity can be minimized by increasing the cooling rate and also to prevent chilling damage [14]. For successful vitrification, a very high cooling and warming rates are required to reduce the concentration and toxicity of cryoprotectants (Fig. 3.1).

Time Schedule for Vitrification of Oocytes and Fertilization

Oocytes can be vitrified 2–4 h after the oocyte retrieval. Oocytes are stripped of cumulus cells by hyaluronic acid treatment. By stripping the cumulus cells from the oocytes, maturity of the oocyte can be assessed. A study by Chian et al. on bovine oocyte vitrification has reported that cells without cumulus had a higher survival rate and superior embryonic developmental capacity after warming as compared to oocytes vitrified with cumulus cells [15].

The meiotic spindle of the oocyte is very sensitive to cryoprotectants and low temperature [16, 17]. Oocytes analyzed immediately after thawing displayed severe disorganization or disappearance of spindles [18]. Although the spindle transiently disappears immediately after thawing, it reorganizes/reforms with good morphology after 1–3 h of in vitro culture at 37–38 °C in the majority of mature oocytes [19]. Inseminating oocytes soon after thawing, when there is serious spindle disorganization, adversely affects fertilization outcome and growth of embryos [18]. The optimum time for intracytoplasmic sperm injection (ICSI) for human oocytes is between 37 and 41 h post human chorionic gonadotropin (hCG). The time interval between oocyte thawing and ICSI is crucial for normal fertilization and subsequent development. Optimizing the time of ICSI, which involves giving adequate time for spindle recovery after warming,

Table 3.1 Types of cryodevices

Device	Vitrification system	Description	Merit
Electron microscope copper grid [20]	Open	Small copper disc with a fine mesh	It improves heat conduction to oocyte cytoplasm thereby prevents osmotic damage cause by increased concentration of vitrification solution
Open pulled straw [21]	Semi-closed	Pulled straw inner tip diameter approx. 0.8 mm	Renders high cooling and warming rate (>20,000 ° C/min)
			Easy loading process, "autoloading" by the capillary forces of OPS
Closed pulled straw [22, 23]	Closed	Pulled straw	It gives high survival rate and preserves spindle fiber
Cryoloop [24, 25]	Open	A thin nylon loop (0.5–0.7 mm in diameter)	Easy to perform and demonstrates stages of development of oocytes and day 3 embryos. Lower concentration of cryoprotectant is used
Cryotip [26]	Closed	Finely pulled plastic straw (250 μm inner diameter)	This system eliminates the potential for embryo contamination during cryopreservation and storage
Cryoleaf [26]	Open		Easy to handle and efficient storage of oocytes. Vitrified cells are protected from mechanical stress and contamination by a closed cover system
Cryotop [27]	Open	A fine polypropylene strip attached to a plastic handle and equipped with a cover straw	Cryotop technology is the minimal volume approach for vitrification. Easy to load and store oocytes or embryos
Cryotec [28]	Open and closed	A strip attached to a plastic handle and equipped with a cover straw	It is a relatively newer approach in minimal volume methods and is a very efficient technique in terms of survival embryo quality, implantation rates, and clinical pregnancy

while at the same time avoiding undue delay, which could result in oocyte aging related damage, is essential for the success of the program. Therefore, considering competing aspects of oocyte aging and spindle recovery is essential for a successful oocyte cryopreservation program. Hence, oocyte recovery can be performed at 34 h post hCG. Vitrification of oocytes can be performed at 2 h after oocyte recovery, and ICSI is being performed at 3 h post thaw, adding up to 39 h post hCG.

Vitrification Devices

Various open and closed system cryodevices are available today with their advantages and drawbacks as described in Table 3.1. Most vitrification protocols use an open system in which oocytes are directly exposed to liquid nitrogen to maximize cooling and minimize formation of ice crystals. Closed system device involves indirect contact with liquid nitrogen. With the use of open vitrification methods, theoretic infectious disease transmission is one of the concerns as the oocyte is directly exposed to the contaminated liquid nitrogen. Studies have suggested that infectious pathogens can survive in liquid nitrogen [29, 30]. For safety issues, various methods to sterilize the liquid nitrogen have been developed [31]. The hypothetical risk of contamination may also be possible in closed system during the warming procedure [31, 32]. Although the infectious disease transmission with the use of open vitrification system was of great concern, but there are studies which suggest that it was never observed in reproductive tissues [33].

Current advances in cryopreservation have resulted in devices that hold minimal amount of

cryoprotectant media containing the specimen, referred as minimum volume approaches. High cooling and warming rates along with the minimal volume methods help to avoid chilling and fracture injury. Therefore, scientists have made great efforts to find new methods using minimum volume techniques to increase the thermal change (Table 3.2) [20–28].

Vitrification: Cryotech Method (Cryotech, Japan)

Dr. Masashige Kuwayama has introduced various novel vitrification techniques. His minimal volume vitrification techniques are used worldwide producing excellent results. Cryotech vitrification "a minimal volume approach" is the latest and innovative method of oocyte and embryo cryopreservation introduced by Dr. Kuwayama. It is a safe and extremely efficient method of vitrification. Protocol of cryotech vitrification has been optimized to preserve oocytes and embryos of any developmental stage. With major improvements in the solutions and tools, cryotech vitrification has shown excellent results in terms of oocyte survival, fertilization rate, and pregnancy rate.

Table 3.2 Minimum volume methods

Vitrification method	Volume (ul)	Cooling rate (°C/min)	Warming rate (°C/min)
OPS [21]	1–2	16,700	13,900
Cryoloop [25]	<1	20,000	
Cryotop [26]	<0.1	23,000	42,100
Cryotip [26]	1	12,000	24,000

Cryotech Method of Vitrification

Cryotech vitrification includes equilibration step where oocytes are placed in equilibration solution and exposed to the lower strength of the cryoprotectants. Oocytes are incubated until they have recovered from osmotic shock. The equilibration results in dehydration of the cell and its permeation with cryoprotectant, which is visible by changes in the shape of an oocyte. The shape of an oocyte changes as it undergoes transient shrinkage with the loss of water and then regains its original shape as the cryoprotectant permeates into the cells (Figs. 3.2 and 3.3).

After the completion of the equilibration step, oocytes are then placed in vitrification medium which is a solution having higher strength of cryoprotectants. The exposure time of oocytes in the vitrification medium is very short, approx 60 s. This is followed by oocyte loading on cryotec (filmstrip), a carrier device, and direct plunging in liquid nitrogen. To ensure maximum cooling rate (23,000 °C/min), cryotec should be submerged in liquid nitrogen with continuous vertical movements. Cryotec is then covered with the cap still under liquid nitrogen. This helps in avoiding mechanical damage during storage (Figs. 3.4, 3.5, 3.6, 3.7, and 3.8).

Storage

After the vitrification process, the vitrified oocytes in cryotec are stored in liquid nitrogen until it is to be warmed and fertilized by a sperm. Vitrification thus involves a technique where the oocytes can be vitrified without damage and can be safely preserved for future use.

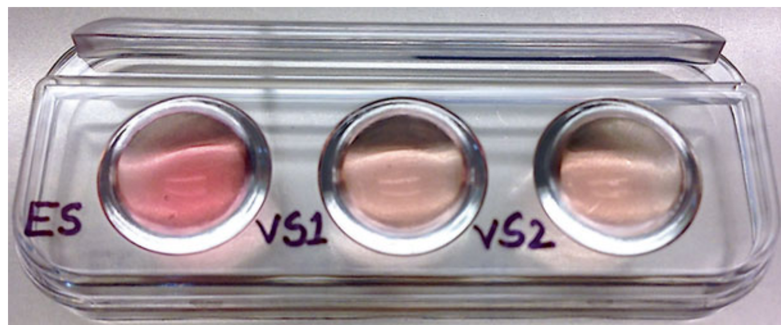

Fig. 3.2 Cryotec vitrification plate with solutions *ES* - Equilibration Solution, *VS1* - Vitrification Solution 1, *VS2* - Vitrification Solution 2

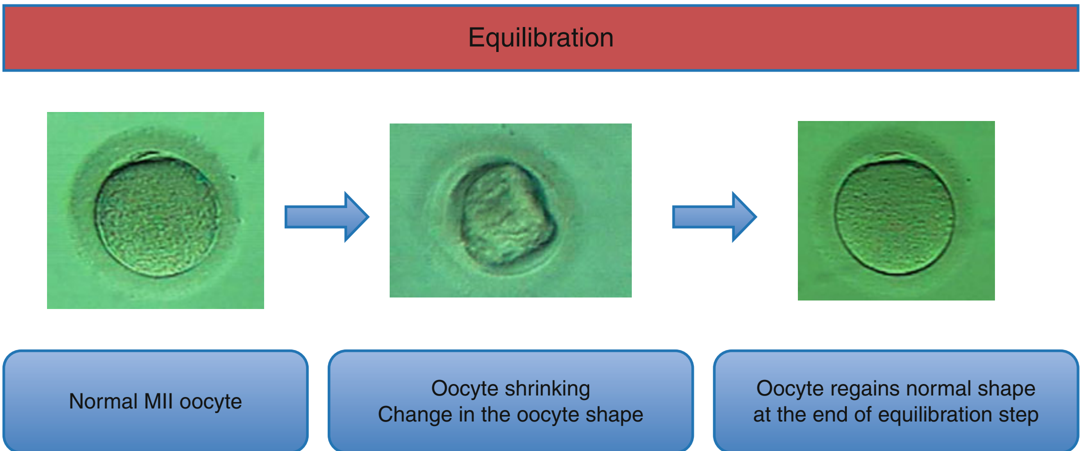

Fig. 3.3 Equilibration step

Fig. 3.4 Cryotec vitrification plate with cryotec placed in the groove

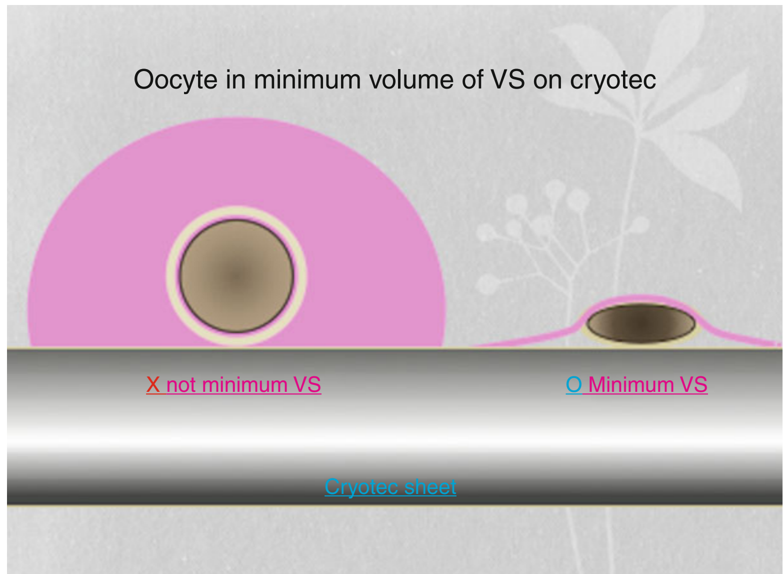

Fig. 3.5 Loading the oocyte with minimum volume
ES - Equilibration Solution,
VS - Vitrification Solution 1,
VS - Vitrification Solution 2

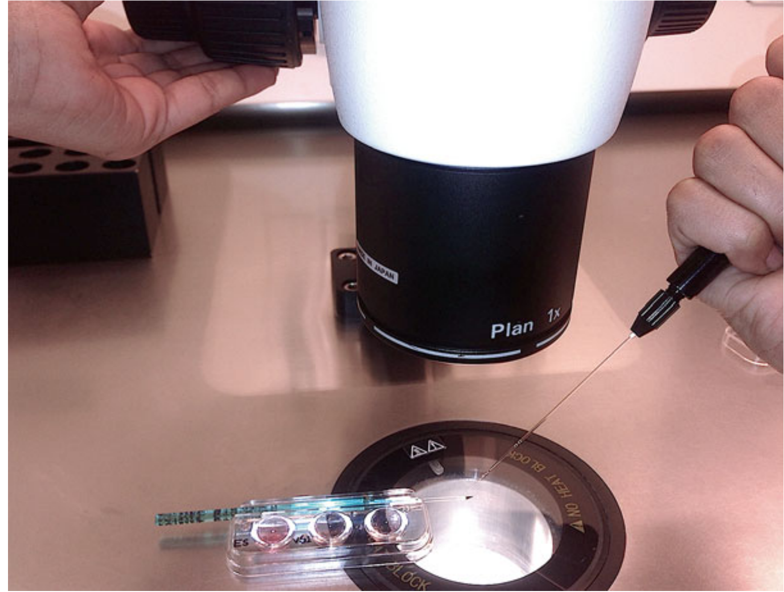

Fig. 3.6 Loading the oocytes onto the cryotec

Fig. 3.7 Dipping the loaded cryotec into liquid nitrogen

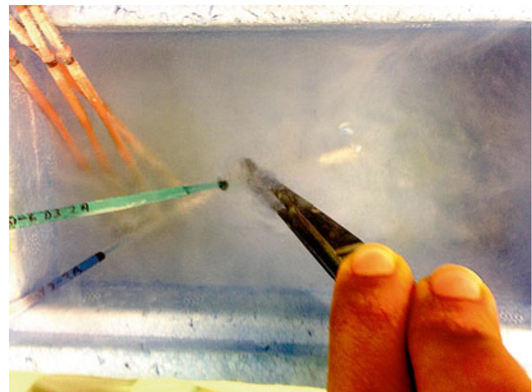

Fig. 3.8 Covering the loaded cryotec with the sleeve under liquid nitrogen

Warming

Warming is the reverse of the entire process of vitrification. Warming involves the immediate transfer of the vitrified oocytes to a pre-warmed (37 °C) environment. In warming, vitrified oocytes are exposed to hypotonic solutions of decreasing strengths of warming media in a stepwise manner to remove the cryoprotectant. Oocyte undergoes transient volume expansion during the removal of the cryoprotectant and gradually rehydrates.

Cryotech Method of Warming

For warming, the protective cover of the cryotec is removed and the film-strip holding the vitrified oocyte is quickly immersed into the warming solution which is maintained at 37 °C. This results in achieving extremely high warming rate (42,000 °C/min). After the incubation period of 1 min, oocyte is placed in the dilution solution for 3 min. The oocyte is then washed in washing solution for 5 min. Survival judgment is made at the end of this step depending on the recovery of

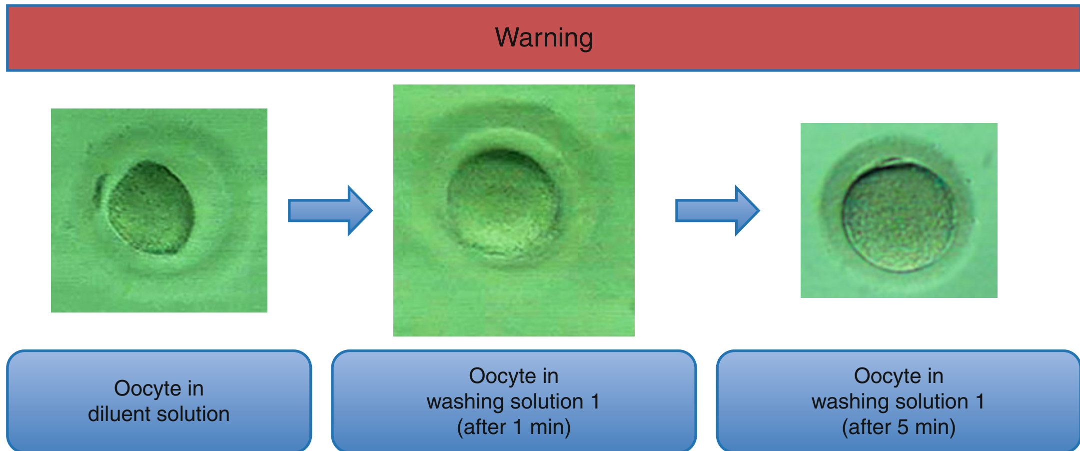

Fig. 3.9 Changes in oocyte shape during the warming process

the shrunken oocyte. The oocyte is again washed in washing medium and then placed in the culture medium (Fig. 3.9).

After warming, the oocytes can be fertilized using intracytoplasmic sperm injection (ICSI), as zona hardening is reported after warming [34].

Advantages of Cryotech Vitrification Technique

The Cryotech method has various advantages as compared to other techniques, in terms of solution, tools, and technique used.

1. *Solutions*
   - There is no added serum and synthetic serum supplements in vitrification solution. Hence, risk of serum-derived virus contamination is avoided.
   - Vitrification solution is completely chemically defined and is stable for 1 year at 4–8 °C and 3 months at room temperature.
   - It contains hydroxylpropyl cellulose (HPC), which has optimal high viscosity providing better resistance to damage during cooling, storage, and warming.
   - Sucrose is replaced with trehalose, thus reducing endotoxicity due to sucrose.
2. *Tools*
   - The plate used for vitrification has a special holder for the cryotec. Thus, the focus remains the same while washing the oocytes in vitrification solution and placing them on the cryotec.
   - The carrier device (cryotec) is a filmstrip attached to a plastic handle, equipped with a cap to cover the filmstrip for safe handling and storage.
   - There is no blind space in wells of vitrification and warming plates, so the chances of losing the oocyte is very rare.

Clinical Outcomes

In various randomized controlled trials where the clinical outcome of cryopreserved and fresh oocytes was compared by using the similar vitrification protocol, it was observed that there is no significant difference in fertilization and pregnancy rates in cycles with fresh oocytes and vitrified oocytes [1, 2]. It was further supported by a recent meta-analysis of five randomized controlled trials from 2011 where the efficacy of oocyte vitrification was assessed. Parameters like survival rate, fertilization rate, embryo development, and pregnancy rate were assessed after comparing the two methods of vitrification and slow freezing. There was no difference found in pregnancy rate, top-quality embryo, embryo cleavage, and fertilization rate between the vitrified and the fresh oocyte groups. However, the

oocyte survival rate, fertilization rate, and cleavage rate were found to be higher in vitrified oocytes when compared to slow frozen oocytes. Also the top-quality embryos formed were higher in vitrified oocytes as compared to the slow frozen oocytes [35]. These studies suggest that vitrification is a simple and effective method for the cryopreservation of oocytes [36].

In view of the results reported across the globe, the American Society for Reproductive Medicine (ASRM) in 2012 declared that oocyte vitrification is no longer considered experimental. The report entitled "Mature Oocyte Cryopreservation" replaced a report released in 2008 that had stated that the technique was experimental and should only be offered in that context [35]. The report examined nearly 1,000 published papers and found sufficient evidence to "demonstrate acceptable success rates in young highly selected populations." The report does urge caution, however. The Committee points out that the age of the woman at the time of egg freezing is a very important factor.

Our Experience with Oocyte Vitrification

A comparative study to evaluate the outcome of oocyte vitrification using the Cryotech method, observed in an egg donation program with fresh versus frozen oocyte, was done at Rotunda – The Center for Human Reproduction. The data was evaluated over a period from January 2011 to December 2011. A total of 1,210 oocytes were obtained from 112 oocyte donors. The analysis included a total of 1,029 mature oocytes, 485 oocytes were used in fresh cycle, and the remaining 544 oocytes were vitrified using the Cryotech method. The vitrified oocytes were subsequently warmed using the Cryotech warming method. The warmed oocytes were fertilized by ICSI, and the embryos thus created were transferred into the recipients. A total of 171 embryo transfer cycles were performed on day 3 of embryo development. The parameters, which were assessed and compared between the two groups, included survival rate, fertilization rate, cleavage rate, embryo development, and the clinical pregnancy rates (Tables 3.3 and 3.4).

Table 3.3 Comparative data showing survival, fertilization, and cleavage rates between fresh and frozen–thawed human oocytes

	Fresh oocytes	Frozen–thawed oocytes	*P*-value
No. of oocytes	485	544	
Survival rate (%)	NA	94.5	
Fertilization rate (%)	83.0	80.9	NS
Cleavage rate (%)	96.5	94.4	NS

Table 3.4 Comparative data showing clinical outcome between fresh and frozen–thawed human oocytes

	Fresh oocytes	Frozen–thawed oocytes	*P*-value
No. of cycles	80	91	
Pregnancy rate (%)	56.3	54.9	NS
Implantation rate (%)	28.0	31.1	NS
Biochemical pregnancy rate (%)	8.8	8.0	NS
Missed abortion rate (%)	11.1	12.0	NS
Live birth rate (%)	45.0	45.1	NS

With these encouraging results, it is proved that Cryotech vitrification is an effective method that preserves the potential of vitrified oocytes to fertilize and further develop, which is similar to fresh oocytes. Excellent clinical outcome indicates that this technology can be applied successfully for the oocyte vitrification in various indications and egg donation programs. Thus, Cryotech vitrification holds a great promise in infertility management by offering a successful oocyte vitrification program.

Important Tips to Be Followed for Cryotech Vitrification

1. It is recommended to use a pipette with an inner diameter of 140–150 µm for the oocyte.
2. Close adherence to the recommended protocol of the manufacturer is absolutely essential to achieve optimal results.
3. The vitrification and warming process is to be done at RT.
4. The vitrification solutions must be tempered to 25–27 °C at least 1 h before using.

5. The plate used for warming and warming solution vial should be incubated at 37 °C at least 3 h before use (overnight incubation recommended).
6. Dilution and washing must be tempered to 25–27 °C at least 1 h before using.
7. Oocytes can be vitrified ideally 1 h after denuding.

Safety of Vitrification

There have been many concerns regarding the safety of vitrification. With vitrification techniques being used in thousands of labs all over the world, it is very essential to establish the safety of the method. IVF has potential risk. Not just oocyte vitrification, any kind of assisted reproductive technique has some potential risk. There are many risks involved like proteomic, metabolic, epigenetic.

A study on the metabolomic profile of embryos developed from fresh and vitrified oocytes has suggested that embryonic metabolomic profile is not disturbed by oocyte vitrification [37]. The influence of vitrification on the oocyte proteome of in vitro-grown germinal vesicle (GV) as well as metaphase II (MII) oocytes was assessed in a study on mouse oocytes. Transient changes in mitochondrial activity by vitrification occur at the pre-antral stage, but proteome of in vitro-grown and matured oocyte is not affected [38]. Oocyte proteome and developmental potential may get affected [39] because of changes in cellular integrity and protein alterations with the use of high concentration of cryoprotectant in an animal model [40, 41].

In a study where messenger RNA (mRNA) contents in MII oocytes after slow-freezing/rapid-thawing and vitrification/warming protocols was compared with the fresh MII oocytes, it was found that mRNA abundance may decrease in both type of cryopreservation methods which may result in molecular injury. However, the mRNA content level in vitrified oocyte remains sufficient to sustain biological function [42]. In another study gene expression profile of human metaphase II (MII) oocytes in both slow freezing and vitrification suggests that both methods modify gene expression profile of MII oocyte [43]. Animal studies indicate that ART techniques are associated with epigenetic alternations, like DNA methylation, but it is not recommended to infer such findings to human embryology [44].

Various publications have shown that there is no disturbance of meiotic spindle and abnormal chromosomes from previously frozen oocytes [45, 46]. Recovery of the meiotic spindle occurs after cryopreservation in both conventional and vitrification technology [47–49]. Thus, it is suggested that there is no increase risks of disturbances in spindle formation or chromosome segregation with vitrification of oocytes [50]. Chromosomal abnormalities are generally associated with age. Various data has revealed that there is abnormal meiotic spindle in older women in terms of chromosomal alignment and the microtubule matrix that comprise the meiotic spindle. In younger population of females, a well-organized meiotic spindle with fully aligned chromosome is observed. Such study has shown that high prevalence of aneuploidy is observed in older females as their meiotic spindle is altered with age [51, 52].

In a study conducted to check the neonatal outcomes of babies born using vitrified oocytes, a total of 58 reports were reviewed from 1996 to 2008. Of the total 936 newborns, 1.3 % had birth anomalies. No difference was noted in the birth defect rates when compared with naturally conceived babies [53]. A similar study has revealed no difference in birth weight or congenital anomalies among those born from vitrified oocytes as compared to children conceived after fresh IVF [54, 55]. A successful live birth has been reported from vitrified oocytes after 5 years of cryopreservation [56]. These findings suggest that there are no reported increase in miscarriages, chromosome abnormalities, and birth defect in the infants born from vitrified oocytes. With many recent studies now reporting similar findings, vitrification procedures have certainly become mainstream and it increases our confidence in oocyte vitrification. Although these data are reassuring, long-term health of children born from vitrified oocytes should be assessed mainly the epigenetic

effects of the treatment. Intracytoplasmic sperm injection (ICSI) used to fertilize the vitrified warmed oocyte may be associated with epigenetic effects [57].

In cancer treatment, ovarian reserves are compromised as chemotherapy, radiation, and surgery affect the reproductive system; oocyte cryopreservation offers a chance to preserve the reproductive capability of a female. Fertility preservation approaches should be discussed before the start of the treatment. If a woman is diagnosed with cancer at a young age, it is advisable to freeze the oocyte for use in the future. For girls who have not reached puberty, ovarian tissue can also be frozen. With the help of IVF or in vitro fertilization of cryopreserved oocytes and subsequent embryo transfer, many cancer survivors can have children. Oocytes can also be preserved after the treatment of cancer even though these are considered pretreatment options as in some cases viable eggs may be present after cancer treatment. In a study, no unusual cancer risk has been identified in the offspring of cancer survivors except in families with genetic cancer syndromes like inherited retinoblastoma [58, 59]. Thus, with the use of oocyte cryopreservation, reproductive capacity of a female can be prolonged and protected.

Conclusion

Oocyte cryopreservation by vitrification is emerging as a very useful tool in ART clinics with many applications. Children born from cryopreserved oocytes have not shown any increase in chromosomal abnormalities, birth defects, or developmental deficits. The success of oocyte vitrification has improved with advances in the vitrification technology, and the data obtained from various studies are reassuring. Current data supports the idea of an efficient oocyte vitrification program in all ART laboratories.

Technology advances rapidly in the area of assisted reproduction, considering the demand for assurances of success, and we can expect that oocyte cryopreservation techniques will continue to become more sophisticated and easier for embryologists to use. The scope of its use and application is only likely to increase in the times to come. Many more interesting and fantastic dimension of research is also currently being done on animal gametes, where newer techniques are under study and will no doubt have some applicability for human endeavors as well, somewhere in the distant future.

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