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Oocyte Cryopreservation for the Elective Preservation of Reproductive Potential

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1. Introduction

Cryopreservation has been a technique used in reproductive endocrinology and infertility medicine since the early 1980s. Embryo cryopreservation, specifically, has been widely used in many in vitro fertilization (IVF) programs worldwide. This method has been well studied and is a common strategy employed for storing supernumerary embryos after IVF cycles, among other applications. Oocyte cryopreservation, which involves cryopreservation of unfertilized human ova, is a newer procedure that is gaining popularity due to its many benefits, including delay of childbearing, fertility preservation for cancer patients, and avoidance of ethical, religious or legal dilemmas surrounding embryo cryopreservation. While this technique is still considered “experimental,” oocyte cryopreservation is rapidly gaining acceptance in the field of fertility preservation. The current chapter discusses the multifaceted reasons for delayed childbearing, the applications of oocyte cryopreservation, and technical aspects of this procedure. Additionally, arguments are presented to counter the “experimental” label of oocyte cryopreservation and obstetric and perinatal outcome data are analyzed.

2. The history of human oocyte cryopreservation

The first human pregnancy after cryopreservation and thaw of an 8-cell embryo occurred in 1983 (Trounson & Mohr, 1983). The first reports of mature human oocyte cryopreservation also occurred in the 1980s, with the first live birth after oocyte cryopreservation using a slow-freeze method being reported in 1986 (Chen, 1986). In this early case report, mature oocytes were cryopreserved using a slow-freeze, rapid-thaw method and DMSO was used as a cryopreservant (Figure 1). Chen achieved an egg survival rate of 80%, with an 83% fertilization rate in the thawed surviving oocyte population. This cohort of 40 oocytes ultimately resulted in one viable twin gestation. Despite this promising early work, significant advances in the field of oocyte cryopreservation did not occur until decades later.

Since the first reports of successful egg freezing, there have been many changes and advances in the protocols and techniques utilized to maximize post-thaw success rates. Alterations in cryopreservants and media have been tested and improved in the past three decades. Replacement of sodium with choline in the cryopreservation media has been shown to improve cryopreservation outcomes (Quintans et al., 2002; Stachecki et al., 1998). Alternative strategies, including trehalose injection have also been introduced in attempts to improve survival of cryopreserved oocytes (Eroglu et al., 2000; Jain & Paulson, 2006).



Courtesy of Reproductive Medicine Associates of New York, LLP.

Fig. 1. Mature human oocyte.

The introduction of vitrification led to the first live birth after this technique in 1999 (Kuleshova et al., 1999). Vitrification of embryos has been shown to be reliable (Kolibianakis et al., 2009) and vitrification is now routinely applied to oocytes. The efficiency of both oocyte cryopreservation methods has been studied and shows similar trends in improvement. While more data exists for slow-freeze cryopreservation, due solely to the number of years that this method has been available, vitrification data is equally promising. In a 2006 meta-analysis, Oktay et al. demonstrated live birth rates increasing after slow-freezing from 21.6% per transfer (from 1996 to 2004) to 32.4% (from 2002 to 2004). The vitrification data showed a similar trend, with a live birth rate of 29.4% before 2005 and 39% after 2005 (Oktay et al., 2006). Through the use of intracytoplasmic sperm injection (ICSI), many of the concerns about zona pellucida hardening from cryopreservation were bypassed. This ability to augment fertilization of thawed oocytes altered the outlook on oocyte cryopreservation and made this a more viable option for fertility preservation.

More recently, experimentation with cryopreservation of ovarian tissue through orthotopic or heterotopic transplantation has been attempted. The first child born after ovarian tissue cryopreservation was documented in 2004, to a woman who had a history of chemotherapy and radiation treatment for lymphoma (Donnez et al., 2004). Due to fewer studies on optimal cryopreservation protocols and methods of tissue selection, this technique has been considered more experimental than oocyte cryopreservation. In addition, optimal strategies for enhancing graft revascularization are limited in the literature and ideal tissue size has not been established. In a case series of 13 patients who underwent ovarian tissue cryopreservation due to various diseases requiring chemotherapy, large strips (8-10mm x 5mm) and small cubes (2mm x 2mm) of ovarian tissue were both effective in restoring ovarian function (Donnez et al., 2011). However, due to small numbers of human patients having undergone this procedure and a lack of standardized protocols, these outcomes are difficult to interpret. Furthermore, appropriate candidate selection for ovarian tissue cryopreservation has not been defined. Due to waning primordial follicle counts as women age, it has been suggested that ovarian tissue cryopreservation should be limited, at the very least, to women <40 years of age (Oktay, 2002). Some concern also exists about the risk of ovarian metastasis and the reintroduction of malignant cells upon transplantation of thawed

ovarian tissue. Minimal residual disease (MRD) in cryopreserved ovarian tissue of patients with leukemia has been demonstrated in humans, with the prevalence of MRD in chronic myeloid leukemia and acute lymphoblastic leukemia as high as 33% and 70%, respectively (Dolmans et al., 2010). Given these risks, strategies to effectively test cryopreserved ovarian tissue for evidence of MRD are required before this technique can be widely utilized in clinical practice. In light of these uncertainties, ovarian tissue cryopreservation is still in its infancy with regard to fertility preservation. Additionally, immature oocyte cryopreservation is being studied but is also in early experimental stages, according to the American Society for Reproductive Medicine (ASRM) (ASRM Practice Committee, 2008). This potentially new frontier is still being studied in primate models and preliminary human studies are ongoing.

These changes in cryopreservants, rates of freezing, fertilization, and protocols for cryopreservation have improved outcomes. As research funding, referring provider knowledge, and patient interest in oocyte cryopreservation increase, we can anticipate continued advancements in the field of fertility preservation.

3. The significance of human aging

Human aging has been well-studied and is a known contributor to the decline in fertility experienced by women. Female fecundity, or the ability to produce offspring, declines with advancing age. This is partially due to decreased numbers of oogonia, which have a steady rate of atresia from birth, with a more rapid decline around the age of 37.5 years. Numbers of oogonia, or primordial fetal oocytes, are maximal at 20 weeks' gestation, totaling between six and seven million. At birth, this number has already declined to one to two million; a mere 400,000 oocytes remain at the beginning of puberty. While this number still seems rather high, only around 500 of these oocytes are destined for maturation and ovulation. The remainder will be lost through a highly controlled system of follicular atresia and apoptosis (Williams Gynecology, 2008 Ed.), until around 1000 oocytes remain at the time of menopause (Figure 2). Since women now live longer, a larger portion of their lives are spent in reproductive senescence, and the need for reproductive assistance due to challenges associated with diminished ovarian reserve has increased (Faddy et al., 1992).

Recent research has suggested that there may be a population of oogonial stem cells, similar to that seen in males for lifelong spermatocyte production. Several studies have pointed toward the presence of mitotically-active germline stem cells in the mammalian ovary (Johnson et al., 2004; Pacchiarotti et al., 2010; Parte et al., 2011; Zou et al., 2009). Many groups have conducted experiments which have isolated stem cells capable of sustaining oocyte and follicle production *in vitro*. While these results are controversial and disputed by some (Byskov et al., 2005), the potential for regeneration of oocytes and follicular development throughout the female life span is an exciting and promising future area in assisted reproduction.

Mathematical models have been developed in order to generate prediction rules for numbers of remaining oocytes and reproductive capacity. Oocyte atresia appears to follow a bi-exponential pattern, with a more rapid decline in oocyte number occurring after a critical number of 25,000 follicles remain around the age of 37.5 years (Faddy et al., 1992). According to this model, around 1000 follicles remain at the age of 51, which corresponds to the median age of menopause in the general population. Other authors have studied histological samples to identify the rate of recruitment of non-growing follicles (NGF) in human ovaries from

prenatal samples through menopause (Wallace & Kelsey, 2010). This model suggests that up to 81% of the variance in non-growing follicles is due to age alone. Interestingly, the authors' mathematical model demonstrates an increased rate of non-growing follicle recruitment until the age of 14 years old, after which NGF recruitment decreases until the menopause. Using this best-fitting asymmetric peak mathematical model, it may be possible to predict ovarian reserve in women based on age and guide discussions of fertility preservation in women seeking information about oocyte cryopreservation.

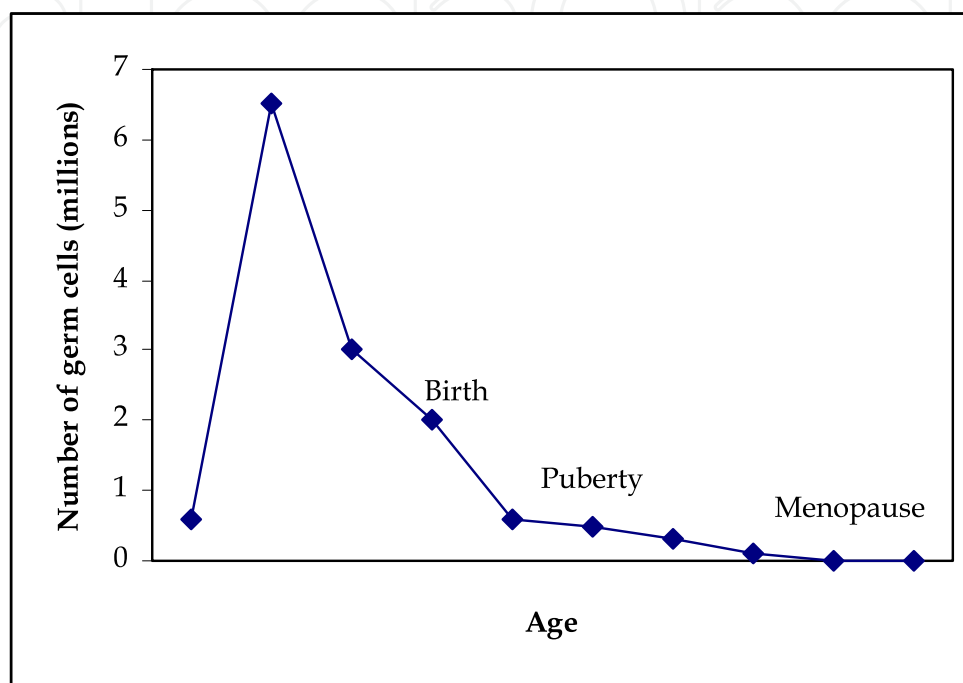


Fig. 2. Number of germ cells across the human female lifespan. Germ cells peak around 6 months post-conceptional age at a level of 6-7 million. At birth, this number has declined to around 2 million germ cells remaining in the infant ovary. Further decline occurs during the rest of the lifespan, with ~500,000 remaining at puberty and only ~1000 oocytes left at menopause.

Though we have some clinical tools to help predict a woman's reproductive capacity, including hormonal tests and the basal antral follicle count, the ramifications of human aging on reproduction are still variable and difficult to predict. Traditionally, elevated levels of basal follicle stimulating hormone (FSH) and abnormal estradiol (E2) levels have been used to guide physicians who are assessing ovarian reserve. FSH is measured in the early follicular phase of the menstrual cycle, when luteal inhibin levels decrease. Classically, it is measured on day 3 after the onset of menses. Studies have shown that a day 3 FSH level above 15 mIU/mL predicts significantly lower rates of pregnancy (Scott, 1995). Concomitant measure of E2 levels may decrease the rate of false negatives when FSH values are used alone. Estradiol should be thus be measured concurrently with day 3 FSH testing. The basal antral follicle count (BAFC) has also been used widely in the field of reproductive endocrinology and infertility to help predict ovarian reserve. BAFC <4 has a specificity of 98.7% when predicting non-pregnancy following IVF (Gibreel et al., 2009). BAFC may therefore be an appropriate measure of ovarian reserve in women undergoing infertility evaluation. Meta-analysis has also shown that BAFC of less than 4 has a sensitivity and specificity to predict cycle cancellation of 66.7% and 94.7%,

respectively. Additionally, women with a BAFC of less than 4 are 37 times more likely to have their cycle cancelled (Gibreel et al., 2009).

A newer marker for predicting ovarian reserve is anti-Müllerian hormone, or AMH, which has been in the literature since the early 2000s (Gruijters et al., 2003). Serum AMH levels are constant throughout the menstrual cycle, unlike FSH or E2, and are not affected by other hormone levels. Because of these relatively constant levels, AMH may be useful for predicting ovarian response to stimulation cycles for IVF; its predictive power seems to be similar to that of the BAFC (La Marca et al., 2009). Additionally, AMH is secreted in primary, preantral, and small antral follicles, which are thought to comprise the pool of ovarian reserve (Figure 3). This endocrine marker is secreted by granulosa cells and reflects the transition of resting primordial follicles to growing follicles (Sowers et al., 2008). Additionally, AMH levels diminish as an FSH-dependent dominant follicle begins to develop (Broekmans et al., 2008), reinforcing its role as a marker of preantral and small antral follicles in the pool of ovarian reserve. AMH is not, on the other hand, expressed in atretic follicles. Therefore, its levels are directly correlated to the number of viable, growing follicles that remain in the ovary. Levels of AMH decline in a predictable fashion as women near the menopausal transition, which has been studied in concordance with declining levels of inhibin-B and increasing levels of FSH (thus reinforcing the soundness of this marker as a predictor of declining ovarian reserve) (Sowers et al., 2008). There is a statistical association between AMH and FSH levels in assessing ovarian reserve. Singer et al. compared the correlation between these two hormones and found that serum AMH level is highly predictive of baseline FSH level. Using these two serum marker levels in combination may prove to be a useful predictor of ovarian reserve (Singer et al., 2009).

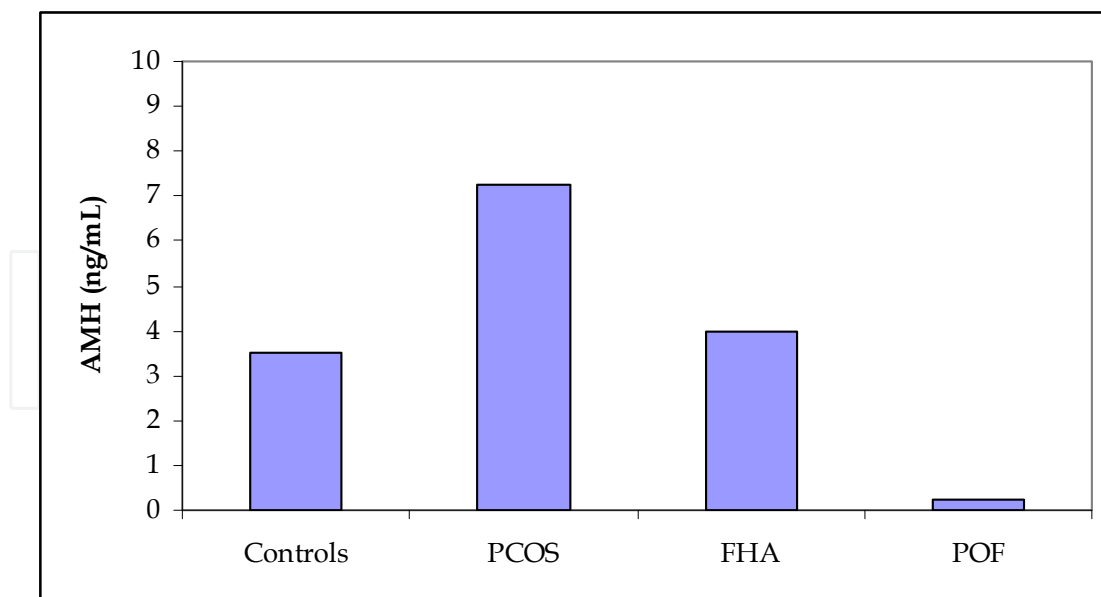


Fig. 3. Mean AMH plasma levels in patients and controls. Women with PCOS have significantly higher levels of plasma AMH and women with POF have significantly lower levels, when compared to controls and women with FHA ($p < 0.05$). PCOS: Polycystic Ovarian Syndrome; FHA: Familial Hypothalamic Amenorrhea; POF: Premature Ovarian Failure. Adapted from Broekmans et al., 2008.

In our NYC-based infertility clinic, women presenting for new oocyte cryopreservation consultations were retrospectively evaluated. Of the 519 women presenting for new patient consultation, approximately 1/3 initiated oocyte cryopreservation cycles. The best predictors of successful oocyte cryopreservation cycles were (in order) BAFC, day 3 FSH, and age (all $p < 0.05$) (Barritt et al., 2010). Importantly, providers must remember that all of these tests and models attempt to predict the *quantity* of oocytes available for future reproduction. Unfortunately, tests to predict oocyte *quality* are still lacking. Models incorporating multiple variables may end up being the best predictor of ovarian reserve and ART cycle success, though many still consider age the best predictor of ovarian reserve and reproductive potential.

The risk of aneuploidy is increased in older oocytes, which leads to higher rates of chromosomally abnormal fetuses and spontaneous abortion. Approximately 15-20% of pregnancies end in spontaneous abortion, or miscarriage (Barron, 1968). Maternal age has long been recognized as a risk factor for pregnancy loss. Risk of chromosomal abnormalities, decreased fecundity, and prevalence of comorbid medical illnesses rise with increasing age – all of which may lead to spontaneous abortion (Barron, 1968). Aneuploidy is thought to affect around 20% of human oocytes (Jones, 2008). Some hypothesize that rates of aneuploidy increase with age through a “two-hit” pathway: nondisjunction followed by an inability of the oocyte to detect the chromosomal abnormality. Nondisjunction, or inappropriate chromosomal separation during meiosis I, is a leading cause of aneuploidy and increases with maternal age. Oocytes from older women may have decreased cohesive bonds between chromosomes, further predisposing them to meiotic errors (Jones, 2008). Additionally, as oocytes age, they may be unable to detect errors in recombination and sister chromatid separation.

It has been well-documented that infertility rates increase with age and that reproductive aging is primarily related to oocyte age. One prospective study demonstrated infertility rates increasing from 8% in women aged 19-26 years to 13-14% in women aged 27-34 years, and ultimately to 18% for women aged 35-39 years (Dunson, 2004). Similarly, there is a decline in success rates of fresh-cycle, non-donor oocyte IVF as a woman ages. Live birth rates per embryo transfer have been documented around 47.5% for women <35 years old, with a progressive decline to 17.0% in women 41-42 years of age, according to 2009 data from the Society of Assisted Reproductive Technologies (SART) (SART, 2009). In light of this data, strategies to preserve fertility for young women are paramount.

4. Changing demographics of reproductive-aged women

In the United States, there has been a notable shift in the demographics of reproductive-aged women. Many women are delaying childbearing in the setting of career pursuits and shifting societal expectations of gender roles. An analysis of Danish fertility rates from 1980-2001 showed an increase in the mean age of childbearing of 3 years over the 21 year period (Hvidtfeldt et al., 2010). There appears to be a global shift in reproduction leading to delayed childbearing and increased maternal age.

Reasons for delaying childbearing are multifaceted and complex. Perceived career threats are a very real and prevalent issue in young women of reproductive age (Willett et al., 2010). For women in professional programs, fear about extension of training, loss of future career

opportunities and concern for pregnancy complications are all significantly higher than in men. These concerns lead to choosing between career training and childbearing, thus risking subfertility by delaying reproduction for the sake of a woman's profession. Studies at our center have evaluated motivations for and trends in elective preservation of fertility in women seeking care at a New York City infertility clinic. Women seeking elective egg freezing were likely to have a high level of education, with all women having at least a bachelor's degree and 75% holding a master's or professional degree. These women were all single, nulliparous, and the majority expressed a desire to be sure they had taken advantage of all reproductive opportunities (Gold et al., 2006). Half of women interviewed described being pressured by their "biological clock" and many wanted to freeze eggs as an "insurance policy," though did not anticipate needing to use them. Interestingly, the mean patient age was 39 years old and 65% of women had reported only recently learning about egg freezing technology. In a multicenter analysis, more than 3000 women called to inquire about fertility preservation. Of these women, those who actually completed a cycle had a significantly higher average age of 37.1 years; patients who were older than 35 had fewer cycles that resulted in the recommended number of metaphase II oocytes for cryopreservation (Frank Sage et al., 2008). This may suggest an inadequate awareness of the age-related decline in fertility that occurs as part of normal human aging. Most studies on reproductive outcomes after oocyte cryopreservation (including oocyte survival rates, fertilization rates, and number of pregnancies) have analyzed women under the age of 35 (Jain & Paulson, 2006). Because of this limitation in the body of literature on oocyte cryopreservation, providers should ideally cryopreserve oocytes in women <35 years of age. As oocyte cryopreservation becomes more publicized and accurate information about declining female fertility is disseminated, the mean age of cryopreservation may decrease.

Trends in the local and national economy have been studied in relation to elective medical procedures, including oocyte cryopreservation. Costs of oocyte and embryo cryopreservation have been evaluated through the LIVESTRONG database of 154 participating reproductive centers. For the average patient, the cost of oocyte cryopreservation is around \$7,800, compared to an average of \$9,300 for embryo cryopreservation (Beck et al., 2010). The costs of fertility preservation are variable based on geography and center. In a New York City private IVF program, annual per capita income showed significant positive correlation with new consults for oocyte cryopreservation. Additionally, as annual unemployment rates increased, the number of new consults significantly decreased (Flisser et al., 2009).

Oocyte cryopreservation has many social and ethical advantages over embryo cryopreservation. Embryo cryopreservation remains the standard recommendation for fertility preservation according to ASRM guidelines, mainly due to the amount of literature studying this technique. Single women, however, may encounter social issues with freezing embryos. The option to extend fertility without the need for a male partner or sperm donor is frequently appealing to women who are not in a long-term relationship. The discomfort of anonymity associated with sperm donors is eliminated with egg freezing. Other potential issues include decisions regarding paternity and legal obligations for patients who undergo directed sperm donation, strategies for disposing of embryos if a woman gets married later in life, and how to handle the disposition of embryos if the egg donor dies and does not have explicit advanced directives in place (Jain & Paulson, 2006). These dilemmas are all circumvented with oocyte cryopreservation. Additionally, infertility centers avoid the often difficult task of synchronizing cycles between oocyte donors and recipients, in the case of

third party reproduction (Oktay et al., 2010). Improvement in coordination of care, costs, and the ability to quarantine oocytes for infectious disease testing are benefits of oocyte cryopreservation for egg donors.

Fertility preservation for cancer patients undergoing potentially sterilizing chemotherapy and radiation has been a widely accepted application of oocyte cryopreservation. Management of all of the gynecologic cancers has the potential to affect ovarian reserve. Cervical cancer often requires pelvic radiation and endometrial cancer is frequently treated with hysterectomy and bilateral salpingoophorectomy. Therapy for breast cancer, the most common cancer in women in the United States, commonly utilizes cyclophosphamide, which has well-known ovary-toxic effects and leads to premature ovarian failure (Oktay & Sönmezer, 2007). Ovarian stimulation is necessary for both oocyte and embryo cryopreservation for these patients; stimulation protocols have been developed to avoid excessive estrogen exposure in women with estrogen-responsive cancers. For patients who do not need to immediately initiate chemotherapy (or other therapies that may affect the ovary), cryopreservation is a viable option for fertility preservation. In a retrospective data analysis of a NYC infertility clinic from 2005-2007, women presenting for pre-cancer treatment oocyte cryopreservation cycles were evaluated. The average time between initial consultation and completion of the cryopreservation cycle was 37.2 ± 22.5 days, and a mean number of 17.8 oocytes were retrieved across the 4 patients studied (Barritt et al., 2008). Early referral to a fertility center is vital, as patients will require 2 weeks of stimulation after menses in order to retrieve oocytes for cryopreservation. Many oncologists are supportive of their patients' desire to preserve fertility, even in light of the potential delay of chemotherapy and need for gonadotropin stimulation. Women who require immediate initiation of chemotherapy or pediatric cancer patients may benefit from ovarian tissue cryopreservation, though studies of this technique are still quite small and this strategy has not yet been widely used (Oktay & Sönmezer, 2007).

Reproductive endocrinologists approaching the patient interested in elective fertility preservation need to recognize the demographic shifts and societal attitudes toward oocyte cryopreservation. The wide variety of applications of oocyte cryopreservation, including delayed childbearing, ethical opposition to embryo cryopreservation, improvement in third party oocyte donation and fertility preservation for cancer patients, all highlight the advantages of this emerging reproductive technology.

5. Technical aspects of oocyte cryopreservation

Oocyte cryopreservation is a delicate and complex process. Mammalian cells are generally stored at a temperature of -196°C , at which no biological activity takes place. Cryopreservation must transform human oocytes from a biologically active system at 37°C to an inert structure at -196°C ; oocytes are most vulnerable during this temperature transition. Membrane permeability and kinetics vary throughout the developmental cycle of the oocyte; metaphase II oocytes have demonstrated higher post-cryopreservation survival in a mouse model (Gook & Edgar, 2007). There are three main goals of oocyte cryopreservation: avoidance of ice crystal formation, avoidance of solution effect, and avoidance of osmotic shock (Jain & Paulson, 2006). As water freezes and expands to form ice, *crystal formation* causes shearing forces on organelles and increases intracellular pressure. Additionally, as water transitions from its liquid to solid form, any solutes dissolved in liquid water are excluded from the ice. This can lead to very high, if not toxic,

levels of non-liquid solutes and electrolytes, known as *solution effect*. Further damage to intracellular proteins can occur in the presence of these toxic levels of intracellular substances during cryopreservation. Finally, *osmotic shock* can occur in the setting of rapid rewarming, during which rapid free water shifts lead to cell shrinking and swelling to accommodate alterations in extracellular osmotic pressure. These three goals are achieved through the use of different cryoprotectant chemicals. Cryoprotectants facilitate oocyte cryopreservation by generating an osmotic gradient by which water can exit the oocyte. Permeating cryoprotectants are able to enter the oocyte, thereby preventing cell shrinkage during osmosis of water to the extracellular space.

Two protocols for oocyte cryopreservation exist, slow-freeze methods and vitrification. While slow-freezing is the most widely used and has been studied more in the literature, recent studies in embryos suggest that vitrification may have improved post-thaw survival rates, though it is still not clear whether there are significant differences in clinical pregnancy rates. These methods are discussed here and are analyzed in light of recent evidence of comparative efficacy. In addition, methods for ovarian tissue cryopreservation are briefly discussed.

5.1 Slow freeze

Slow freezing has traditionally been the more widely-used technique for mature oocyte cryopreservation. This technique was first described in 1972 by Whittingham et al. after successful slow freeze and post-thaw survival of mouse embryos (Whittingham et al., 1972). The technology was first applied to human embryos in 1983, and resulted in successful post-thaw survival and pregnancy after cryopreservation (Trounson & Mohr, 1983), followed by live birth after mature oocyte cryopreservation in 1986 (Chen, 1986).

Slow freeze cryopreservation is achieved using initial low cryoprotectant concentrations to reduce toxicity while the oocyte is still metabolically active (Jain & Paulson, 2006). The temperature is lowered gradually, at rates between 0.3-2°C/minute. This slow rate of cooling allows retardation of the metabolic rate in the oocyte without accumulating toxic levels of cryoprotectant. Propanediol (PROH) and dimethylsulfoxide (DMSO) are permeating cryoprotectants which form hydrogen bonds with intracellular water molecules and prevent ice crystal formation, thus achieving the first goal of successful cryopreservation. Additionally, the presence of PROH dilutes electrolyte concentrations by remaining in solution (due to its low freezing point); this prevents solution effect, which is the second goal of cryopreservation. PROH is preferred to DMSO as a cryoprotectant, as it is thought to be less toxic to the oocyte (Renard & Babinet, 1984). Additionally, using 0.2-0.3M sucrose as a nonpermeating cryoprotectant during oocyte dehydration seems to improve post-thaw survival (Fabbri et al., 2001). "Seeding" the extracellular solution with an ice crystal occurs around -6°C, during which an ice front grows and excludes solutes, thereby increasing their concentration around the oocyte. This ice front can potentially cause intracellular damage to the oocyte if it comes in contact with the cell or can lead to gas bubble formation (Ashwood et al., 1988). The oocyte is maintained at -6°C for 10 to 30 minutes before being further cooled to -32°C. At this point, metabolic activity in the oocyte is extremely low and the cell is plunged into a Dewar vessel of liquid nitrogen to vitrify any remaining cryoprotectant solution (Figure 4). The Dewar vessel is capable of maintaining a near constant temperature for the frozen oocytes during storage.



Fig. 4. Liquid nitrogen Dewar vessel, used for freezing and storing cryopreserved oocytes. *Courtesy of Reproductive Medicine Associates of New York, LLP.*

Thawing of embryos occurs at a rate of 4-25°C/minute. A relatively rapid temperature transition is needed to prevent recrystallization of water in the cell. Nonpermeating cryoprotectants, such as sucrose or other disaccharides, are utilized to help prevent osmotic shock during thawing, as high levels of permeating cryoprotectants are present intracellularly (Jain & Paulson, 2006). This helps achieve the third goal of cryopreservation (Figure 5).

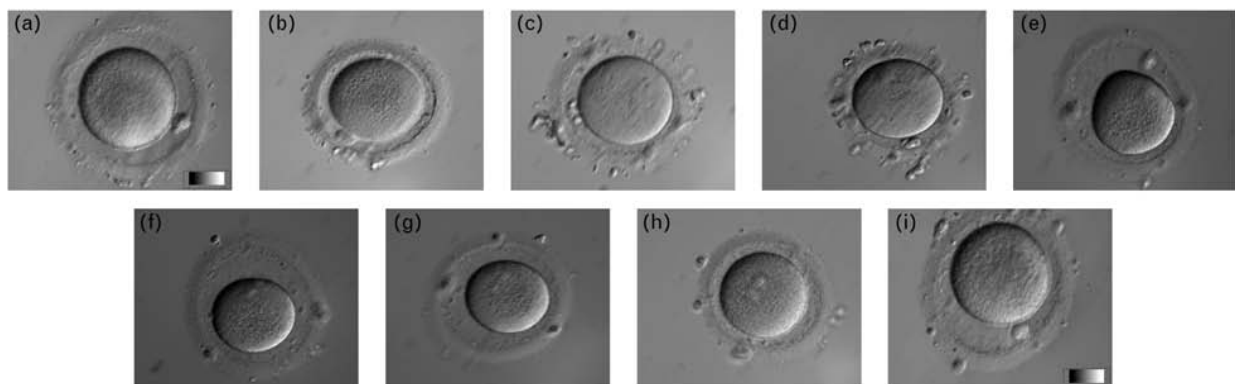


Fig. 5. Oocyte volume changes during freezing and thawing with slow-freeze protocol. (a) De-cumulated oocyte. (b)-(d) Oocyte undergoing freezing stages. (e)-(h) Oocyte undergoing thawing stages of slow-freeze protocol. (i) Oocyte after cryopreservation. *Images courtesy of Herrero et al., 2011.*

Slow freezing has limitations. First, this method is expensive and requires programmable freezing equipment that must be purchased by the IVF laboratory. This poses a substantial cost to many centers. Additionally, this method is extremely time-consuming, taking embryologists at least 90 minutes to successfully cryopreserve oocytes. Despite these drawbacks, this technique is still the most widely used and has the most literature available about tested protocols and outcomes.

5.2 Vitrification

Vitrification, which literally means “the act or process of converting into glass,” is an alternative method to slow freeze cryopreservation. This technique uses high concentrations of cryoprotectants and a rapid cooling rate to convert liquid intracellular water directly into a glassy, vitrified state.

This method of oocyte cryopreservation was first described in humans in 1986 (Fahy et al., 1986), with the first live birth after vitrification occurring in 1999 (Kuleshova et al., 1999). Oocytes are directly exposed to liquid nitrogen which practically eliminates ice crystal formation, due to the rapid cooling rate, around 20,000°C/minute. This minimizes the risk of physical damage to the oocyte from shearing of organelles or increased intracellular pressure. The oocyte is converted rapidly into an amorphous state (Figure 6). A plastic straw containing cryoprotectants and the oocyte is directly plunged into the liquid nitrogen. While initial studies of these “cryoprotectant cocktails” found them to be incredibly toxic, extensive evaluation has indicated that the combination with minimal toxicity is a combination of a high concentration of ethylene glycol (5.5M) and sucrose (1.0M) (Ali & Shelton, 1993). Further modification of the cryoprotectant protocols has decreased the concentration of ethylene glycol to 5.0M (Kuwayama et al., 2005a). Other groups have had high success with vitrification using 2.5M ethylene glycol, 0.5M sucrose and 2.1M DMSO (Gook & Edgar, 2007). These changes in methodology have led to continued improvement in vitrification outcomes, including improved oocyte post-thaw survival, fertilization rates, and pregnancy outcomes.

Some studies have reported the potential for disease transmission, especially viral illnesses, through direct contact with contaminated liquid nitrogen using open-carrier systems for vitrification (Bielanski et al., 2000, 2003), in which there is direct contact between the cryoprotectant media and liquid nitrogen. Closed-carrier system vitrification, in which oocytes are not in direct contact with liquid nitrogen, have been shown to have similar blastocyst survival, pregnancy rates, and live birth rates as open-carrier systems (Kuwayama et al., 2005b), without the theoretical risk of horizontal viral transmission (Bielanski et al., 2000). Closed-carrier systems cool at a slower rate (around 200°C/minute) but have similar rates of post-thaw embryo development, and may demonstrate similar efficacy (Jain & Paulson, 2006).

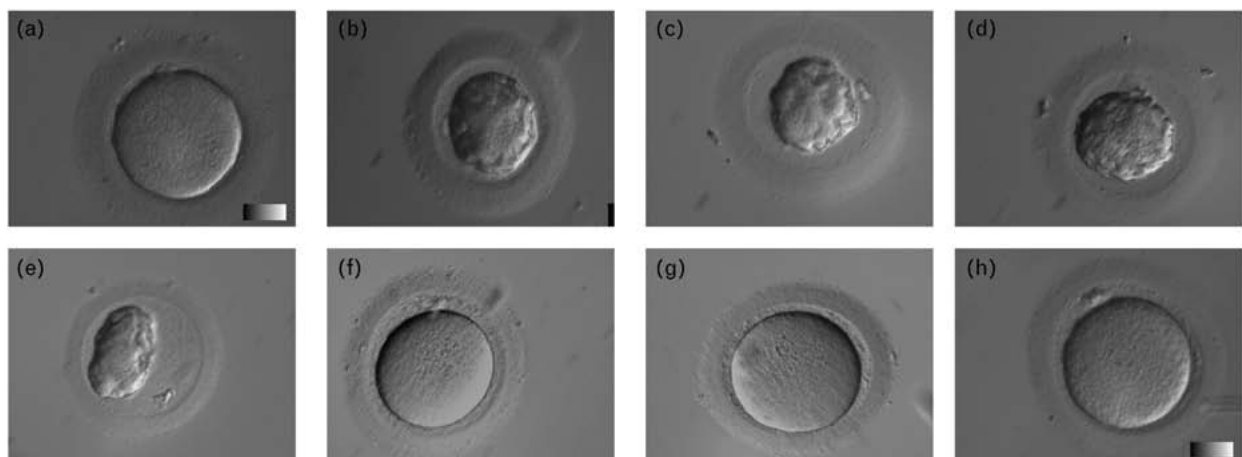


Fig. 6. Oocyte volume changes during vitrification and thaw. (a) De-cumulated oocyte before cryopreservation. (b)-(e) Oocyte undergoing vitrification. (f)-(g) Oocyte during warming phase of vitrification protocol. (h) Oocyte after cryopreservation. *Images courtesy of Herrero et al., 2011.*

Vitrification has its own drawbacks. This method, too, is very expensive for IVF centers to implement in terms of costs of freezing and thawing media. Additionally, this technique has a high learning curve, which must be considered. On the other hand, vitrification does not take as much time as slow freezing due to the rapid cooling procedure and does not require expensive embryology lab equipment. As vitrification continues to be used and data accrued about the success of this method, it is likely to alter the choice of cryopreservation protocols worldwide.

5.3 Slow freeze versus vitrification for oocyte cryopreservation

Slow freezing reports first began 13 years before literature on vitrification emerged. Both of these methods have demonstrated increasing efficiency over time, with continually improving live birth and ongoing pregnancy rates per transfer (Oktay et al., 2006). Although there is a lag in the data for vitrification outcomes, the number of babies born after vitrification is approaching that of slow freeze methods for oocyte cryopreservation (Noyes et al., 2009).

In a recent meta-analysis of randomized controlled trials (RCTs) comparing these two methods, vitrification was found to have better post-thawing survival rates for cleavage stage embryos (odds ratio [OR] 6.35, 95% confidence interval [CI] 1.14, 35.26) and for blastocysts (OR 4.09, 95% CI 2.45, 6.84) (Kolibianakis et al., 2009). A significantly higher number of embryos cryopreserved in the cleavage stage developed into blastocysts following vitrification. Clinical pregnancy rates, however, demonstrated no significant difference between slow freeze and vitrification protocols. This meta-analysis was undertaken to evaluate and summarize the available evidence for cryopreservation of human embryos, not oocytes. Additionally, the data amassed for this meta-analysis came from only 6 RCTs, only one of which commented on live birth rates. The authors, in light of this limited data, call for well-designed randomized controlled trials to further study differences between and advantages or disadvantages of these cryopreservation techniques.

Recently, a prospective randomized comparison of slow freeze versus vitrification for mature human oocyte cryopreservation was performed in Brazil (Smith et al., 2010). In this study, women with supernumerary oocytes retrieved (more than nine) were consented and randomized to either slow freeze or vitrification of these supernumerary oocytes. Demographic characteristics between the two groups of women were similar, including patient age, baseline laboratory values, and number of oocytes collected. Semen parameters were also similar between the groups and all oocytes were inseminated by intracytoplasmic sperm injection (ICSI). Oocyte survival after thawing was significantly higher in those having undergone vitrification. Additionally, a higher percentage of vitrified oocytes were fertilized (77% vs. 67% of slow freeze oocytes; $p < 0.03$) and more of these zygotes underwent cleavage from day 1 to day 2 (84% vs. 71%, respectively; $p < 0.01$). Perhaps the most important outcome for any assisted reproductive technology, however, is the rate of pregnancy. Biochemical and clinical pregnancy rates per thaw cycle were significantly higher in the vitrification group compared to the slow freeze group (46% vs. 17% and 38% vs. 13%, respectively; $p < 0.01$ and $p < 0.02$) (Table 1). Additionally, the two groups had similar rates of spontaneous abortion following embryo transfer. Perinatal outcomes were not evaluated by these authors. From case reports evaluating live births following oocyte cryopreservation, the average gestational age at delivery for slow freeze was 36.9 weeks, compared to 39 weeks' gestational age at delivery after vitrification (Noyes et al., 2009). This

data suggests improved efficiency and a clinical advantage of oocyte vitrification for elective fertility preservation. Reproductive endocrinologists should be aware of this recent data when considering the implementation of oocyte cryopreservation into their clinical practice and when counseling patients seeking fertility preservation.

| | Slow-Freeze | Vitrification | p-value |
|---|---------------|---------------|---------|
| Number of cycles | 30 | 48 | NA |
| Oocytes thawed | 238 | 349 | NA |
| Oocytes thawed per treatment (mean ± SE) | 7.9 ± 0.5 | 7.3 ± 0.3 | NS |
| Immediate post-thaw survival (%) | 159/238 (67%) | 281/349 (81%) | <0.001 |
| 4-hour post-thaw survival (%) | 155/238 (65%) | 260/349 (75%) | <0.01 |
| Fertilization (%) | 104/155 (67%) | 200/260 (77%) | <0.03 |
| Cleavage from Day 1 to Day 2 | 74/104 (71%) | 168/200 (84%) | <0.01 |
| Biochemical pregnancies per cycle (%) | 5/30 (17%) | 22/48 (46%) | <0.01 |
| Clinical pregnancies per cycle (%) | 4/30 (13%) | 18/48 (38%) | <0.02 |
| Clinical pregnancy per oocytes thawed (%) | 4/238 (1.7%) | 18/349 (5.2%) | <0.03 |

NA = not applicable; NS = not significant. SE = standard error. *Adapted and reproduced with permission from Smith et al., 2010.*

Table 1. Oocyte survival and function following slow-freeze or vitrification for cryopreservation

5.4 Ovarian tissue cryopreservation

Ovarian tissue cryopreservation (oophoropexy) and transplantation can also be considered for female children who will survive childhood cancers but have potentially sterilizing chemotherapy and/or radiation. Ovarian tissue cryopreservation was first described using a sheep model (Gosden et al., 1994). After oophorectomy, strips of ovarian cortex were cryopreserved using a slow-freeze protocol with DMSO. Ovarian tissue was cooled to -140°C before being plunged into liquid nitrogen and stored for 3 weeks. Tissue was thawed and grafted back into the same animal after removal of the remaining ovary, after which animals were returned to the pasture and normal husbandry conditions. This protocol has been followed in human studies of ovarian tissue cryopreservation (Donnez et al., 2011). After thawing, decortication of the patient's atrophic ovaries occurs before transplantation of cryopreserved tissue (Donnez & Dolmans, 2009). Return of ovarian function appears to occur between 3.5-6.5 months after transplantation, as evidenced by an increase in E2 and decreased basal FSH levels. In a small case study, the duration of ovarian activity after transplantation appears to be about 2-5 years (Donnez et al., 2011). Heterotopic transplantation of fresh ovarian tissue to the forearm has been successful in 2 cancer patients with return of ovarian function (Oktay et al., 2001). Forearm heterotopic transplantation of cryopreserved ovarian tissue has been successful in primates (Schnorr et al., 2002), and preliminary studies of this technique in humans are ongoing.

6. Is oocyte cryopreservation “experimental?”

The American Society for Reproductive Medicine (ASRM) published a committee opinion in 2008 which stated that “the experimental nature of oocyte cryopreservation suggests

potential for clinical application [...] it might therefore be acceptable [...] with appropriate informed consent under the auspices of an IRB" (ASRM Practice Committee, 2008). This "experimental" label was first published by ASRM in 2006. Some studies have looked at provider compliance with this ASRM practice guideline and likelihood of referral for oocyte cryopreservation. In a retrospective study of 530 IVF centers in the United States, 69% of these centers (365/530) were found to offer oocyte cryopreservation. Of these centers, only 62% do so under IRB approval, while 15% reported having an IRB pending and 18% did not use an IRB at all for oocyte cryopreservation (Beck et al., 2009). Compliance with ASRM guidelines was highest in the northeast (71%) and the size of the program was inversely related to the likelihood that oocyte cryopreservation occurred in conjunction with IRB approval. Still, these numbers indicate relatively high compliance with ASRM guidelines. In a different survey of healthcare providers at 5 United States IVF centers, physician preferences and recommendations were analyzed for practice patterns regarding oocyte cryopreservation. More than half of providers considered the ideal age for oocyte cryopreservation to be less than 35 years and 50% found it acceptable for a woman to preserve fertility in this way with a day 3 FSH value of <13 IU/L. A large proportion of providers were less likely to recommend egg freezing to patients with a low BAFC. Additionally, 89% of physicians were more likely to offer oocyte cryopreservation to their patients if there was a medical indication for the procedure, instead of elective reasons for fertility preservation (Luna et al., 2008). Providers recognized the emerging role oocyte cryopreservation will have in the field of fertility preservation. Thus, despite current reservations regarding which patients to refer for oocyte cryopreservation and ASRM guidelines, physicians view oocyte cryopreservation as a technique that will continue to be used with increasing frequency.

Discussion about the safety and efficacy of oocyte cryopreservation has focused on potential concern about meiotic spindle interruption from freezing, hardening of the zona pellucida (which may decrease rates of fertilization), and the potential risk of anomalies and abnormalities that may arise in the setting of a new technique without much outcome data. The meiotic spindle is a dynamic structure that forms during mitosis and meiosis to facilitate chromosomal segregation. Disruption of the meiotic spindle increases the risk of aneuploidy. These concerns were studied by Rienzi et al. by slow freezing oocytes and looking at the meiotic spindle using computer-assisted polarization microscopy (Rienzi et al., 2004). This technique allowed visualization of the spindle in real time by evaluating living oocytes. Previous studies had used electron microscopy or immunocytochemistry, which requires cell fixation and does not permit evaluation of dynamic spindle activity (reviewed in Eichenlaub-Ritter et al., 2002). Though spindles disappeared in oocytes during the thawing process, all surviving post-thaw oocytes were noted to have intact, functional meiotic spindles. Thus, it appears that cryopreserved oocytes are capable of reforming the meiotic spindle apparatus after thawing (Noyes et al., 2010). Hardening of the zona pellucida (ZP, the transparent glycoprotein envelope that surrounds a mature mammalian oocyte) is thought to occur due to premature cortical granule release during cryopreservation (Jain & Paulson, 2006). This release leads to early hardening of the ZP, which impedes penetration and fertilization by sperm. The advent of ICSI in 1992 introduced a solution to ZP hardening, in which the zona is bypassed by direct injection of the sperm into the oocyte. Additionally, vitrification of oocytes in calcium-free media

appears to reduce zona pellucida hardening and leads to increased fertilization. Embryos obtained from cryopreserved oocytes have a similar incidence of chromosomal abnormalities when compared to control embryos using fluorescence in situ hybridization (FISH) (Cobo et al., 2001). Multiple recent studies have evaluated pregnancy, live birth, and early childhood outcomes in children born after mature oocyte cryopreservation (Borini et al., 2007; Chian et al., 2008a, 2008b; Noyes et al., 2009; Oktay et al., 2006; Wennerholm et al., 2009); these have not documented an increased rate of congenital anomalies among children born after oocyte cryopreservation. These studies are discussed in more detail in the next section.

In light of these concerns about cryopreservation, the ASRM maintains that oocyte cryopreservation should be considered an experimental procedure. The ASRM specifically states that assisted reproductive technology (ART) procedures should be considered “experimental” until “the published medical evidence regarding their [...] overall safety and efficacy is sufficient to regard them as standard medical practice. [This] medical evidence can derive only from appropriately designed, peer-reviewed, published studies performed by multiple independent investigators” (ASRM Practice Committee, 2009). Other authors have supported this statement, by noting that “because the largest demand for oocyte cryopreservation most probably is going to come from women who wish to delay childbearing electively, it is quite likely that several years will be required before sufficient births have occurred to determine the true safety of cryopreserved oocytes” (Jain & Paulson, 2006). Some authors, however, argue against this labeling of oocyte cryopreservation, stating that a variety of commonly used assisted reproductive technologies have never been studied “under the auspices of an IRB” before implementation into standard practice (Noyes et al., 2010).

The safety of oocyte cryopreservation has been evaluated through studies of pregnancy, perinatal, and childhood outcomes, in which over 900 infants have been evaluated (Chian et al., 2000b; Noyes et al., 2009). There does not seem to be an increased risk for adverse pregnancy outcomes or congenital anomalies in pregnancies conceived after oocyte cryopreservation, thaw, fertilization and embryo transfer. Additionally, cryopreservation may introduce an extra safety measure with regard to quarantine for infectious disease, similar to protocols in place for cryobanking of donor sperm. By freezing donated oocytes, additional infectious disease testing can be done months after oocyte retrieval to ensure optimal embryo transfer and pregnancy outcome. In an early 2007 paper by Barritt et al., 4 oocyte donors underwent synchronous ovarian stimulation with 4 recipient patients with impaired ovarian reserve, elevated basal FSH, and prior unsuccessful IVF treatments (Barritt et al., 2007). The donors were given a complete medical examination in accordance with ASRM guidelines for oocyte donors, which included a full history, physical exam, BAFC, and cervical cultures. In addition, these women had serological testing for infectious diseases, including HIV, hepatitis B and C, syphilis, gonorrhea, cytomegalovirus, and a urine drug screen. While this initial workup for oocyte donors seems exhaustive, additional checkpoints for infectious disease testing after an extended period of cryopreservation will further prevent the spread of communicable disease and improve pregnancy outcomes by preventing congenital infections. Data continue to emerge supporting the safety and efficacy of oocyte cryopreservation and it is likely that the “experimental” label will soon be removed from this technique.

Multiple studies from different investigators and institutions have compared the efficacy of oocyte cryopreservation to fresh oocyte cycles. An early IRB-approved prospective study of four donor-recipient oocyte cycles by Barritt et al. demonstrated high pregnancy and implantation rates following slow-freezing and overnight storage before thawing. After ICSI, the authors demonstrated an 89.7% fertilization rate and 91.8% of these fertilized oocytes cleaved normally. Of 23 transferred embryos, 26.1% implanted and 75% of implanted embryos led to clinical pregnancy (Barritt et al., 2007). Cobo et al. performed a study in which fresh oocytes from the same donor were either inseminated directly or vitrified for at least 1 hour before thaw and insemination (Cobo et al., 2008). In comparing embryo quality and clinical outcomes, they found that vitrified/thawed oocytes produced embryos capable of a 47.8% ongoing pregnancy rate, which was similar to fresh oocytes. In addition, Grifo and Noyes performed an age-matched control study of 23 oocyte cryopreservation cycles and fresh oocyte control cycles. Fertilization rates, blastocyst formation, and pregnancy rates were not significantly different between these two matched groups (Grifo & Noyes, 2010). This indicates that frozen/thawed oocytes perform as well as fresh oocytes in ART procedures. Finally, Nagy et al. demonstrated high efficiency of egg cryobanking, with a 55% implantation rate and delivery of 26 live infants. Furthermore, their study showed that twice-frozen gametes (i.e. oocyte cryopreservation followed by fertilization and supernumerary embryo vitrification) can lead to pregnancy after embryo thawing (Nagy et al., 2009). The efficacy of oocyte cryopreservation has thus been established by multiple independent groups in the literature, strengthening the argument to remove its experimental status.

For female cancer patients, treatment regimens of intensive chemotherapy, ionizing radiation, and bone marrow transplantation can lead to premature ovarian failure, with direct impact on the number and viability of remaining oocytes. Gonadotropin-releasing hormone (GnRH) analogues have been studied as a method for fertility preservation before cytotoxic treatments. By suppressing ovarian function and, essentially, rendering the ovary quiescent, it is thought that chemotherapeutics and radiation would not be able to affect post-treatment ovarian function. Unfortunately, this strategy does not have well-documented efficacy in the literature (Maltaris et al., 2009). Additionally, studies are lacking that have documented resultant oocyte and embryo quality following a course of chemotherapy (ASRM Practice Committee, 2008). Consequently, oocyte or ovarian tissue cryopreservation may be more reliable methods of fertility preservation for female cancer patients.

Finally, it is important to consider the ethical dilemmas of embryo cryopreservation that are bypassed by using oocyte cryopreservation. These issues, while not directly related to safety and efficacy of oocyte cryopreservation, provide additional support for arguments about the importance of this method to avoid the moral impasses generated by embryo cryopreservation and storage. Embryo cryopreservation has legal implications worldwide. Ovarian stimulation cycles and IVF procedures frequently lead to supernumerary cryopreserved embryos. Over 400,000 embryos are currently stored in the United States alone (Hoffman et al., 2003), leading to high rates of embryo abandonment in IVF clinics. The issue of embryo disposal versus continued cryopreservation is one which IVF clinics deal with daily.

7. Long-term pregnancy and health outcomes after oocyte cryopreservation

Given the evolving nature of the technology and the heterogeneity of patient-population and cryopreservation techniques, the actual “success rate” of egg freezing is unknown. Review of the literature, however, suggests that the efficiency of cryopreservation appears to be improving. One analysis of slow-freezing demonstrated improvement in live birth rates from 21.6% per transfer from 1996 to 2004 to 32.4% from 2002 to 2004 (Oktay et al., 2006). Vitrification data shows a similar trend of improvement: 29.4% live birth rate before 2005 versus 39% after 2005. In a study out of McGill University Health Center, 38 women underwent ovarian stimulation and vitrification of retrieved oocytes. After cryopreservation for one full menstrual cycle, there was an 81% thaw survival rate, 75.6% of oocytes were successfully fertilized, and a 50% pregnancy rate per cycle started was achieved (Chian et al., 2008a). Ultimately, 39.5% of women who initiated ovarian stimulation and cryopreservation cycles gave birth to live infants. Nine of these births were singleton, while the remaining six deliveries were multiples (five twins and one triplet). While initial high rates of spontaneous abortion were documented after oocyte cryopreservation (Borini et al., 2004), these rates have declined with a corresponding increase in live birth rates.

Since the early 2000s, studies have begun reporting pregnancy and neonatal outcomes following oocyte cryopreservation. Borini et al. reported 13 children born after slow freeze cryopreservation in 2004. All babies born were found to have a normal karyotype and no malformations were seen in their study group. They did note, however, a 20% spontaneous abortion rate in their cohort of patients who had undergone oocyte cryopreservation cycles (Borini et al., 2004). In a later study out of Italy, 149 pregnancies occurred after using a slow freeze protocol for oocyte cryopreservation. This group, again, had a relatively high spontaneous abortion rate of 23.5% (Borini et al., 2007). Reports of live births following oocyte cryopreservation have also emerged from groups in China. Chian et al. found that neonates born after ovarian stimulation and oocyte vitrification were all appropriate birthweights, none weighed <2500g. Additionally, all singletons in their cohort were born at term, with a mean gestational age at delivery of 39 1/7 weeks (Chian et al., 2008a). The same group analyzed 165 pregnancies resulting in 200 babies born after vitrification of oocytes at three centers. In their study, multiple gestations were more likely to deliver in the late preterm period (between 34-37 weeks' gestation) - 57% vs. 22% of singleton pregnancies. This is consistent with current expectations for multiple gestations in the general population. Additionally, 74% of multiples in their study were low birth weight (LBW, <2500g), with 5% of the cohort being very low birth weight (VLBW, <1500g). This was in comparison to singleton neonates born after vitrification, only 17% of which were LBW and 0.7% were VLBW. These birth weights are not significantly different when compared to women who spontaneously conceived or had fresh IVF (Chian et al., 2008b). Birthweight was also analyzed in a systematic review of pregnancy outcome data after oocyte cryopreservation and found to be consistently within normal limits (Wennerholm et al., 2009).

Some concerns have been raised about the rate of malformation or congenital anomalies seen in babies born after any assisted reproductive technology. Epigenetic syndromes (such as Beckwith-Weidemann Syndrome and Angelman Syndrome) have been reported as more common, specifically after ICSI (Noyes et al., 2009). With regard to egg freezing, of 105 babies studied by Borini et al. in 2007, only 2 malformations were seen; one infant was born

with choanal atresia and the other with Rubenstein-Taybi syndrome (Borini et al., 2007). Chian et al. analyzed rates of malformations in their 2008 cohort of 200 babies born after vitrification. Overall, only 5 birth defects were noted, for a malformation rate of 2.5% (Chian et al., 2008b). This rate is consistent with that seen in spontaneously conceived pregnancies and those following fresh IVF (Tan et al., 1992). In the Chian study, 2 ventricular septal defects (VSD), 1 case of biliary atresia, 1 club foot and 1 skin hemangioma were described in neonates. In their systematic review of the literature, Wennerholm et al. found that children who underwent karyotype analysis after oocyte cryopreservation were all within normal limits (Wennerholm et al., 2009).

The largest study to date of congenital anomalies following oocyte cryopreservation was published in 2009 by Noyes, Porcu and Borini. In this literature review, the authors identified 936 infants born after oocyte cryopreservation. In this worldwide population of infants, only 12 of 936 had either a major or minor congenital anomaly, for a malformation rate of 1.3% (Noyes et al., 2009). Defects seen included 3 VSD, 3 clubfoot, 1 choanal atresia, 1 biliary atresia, 1 Rubenstein-Taybi syndrome, 1 Arnold-Chiari syndrome, 1 cleft palate, and 1 skin hemangioma; some of these defects have already been discussed from earlier studies (Borini et al., 2007; Chian et al., 2008b). No difference in rates of major or minor congenital anomalies was found when compared to the United States birth outcome data from the Centers for Disease Control and Prevention (CDC). The CDC reports major structural or genetic birth defects occurring in 3% of live births (CDC, 2011); the number of malformations seen after oocyte cryopreservation is, in fact, lower than this national average. Importantly, the birth defects amassed in this group mirror those seen most commonly in the general population. Additionally, the authors stratified the infants between those born after slow freeze versus vitrification protocols. There was no major difference in the rate of anomalies found after these methods of oocyte cryopreservation (1.1% versus 1.5%, respectively). No epigenetic syndromes were found in this international group of infants born after oocyte cryopreservation, though these have been reported for other types of ART.

Ovarian tissue cryopreservation, which has been less studied and is not as widely used as oocyte cryopreservation, has also resulted in successful pregnancies. The first birth after ovarian tissue cryopreservation and autotransplantation was documented in 2004 (Donnez et al., 2004). To date, there have been 13 infants born to 10 women after ovarian tissue cryopreservation (Donnez et al., 2011). Two of these women conceived and delivered two healthy infants in subsequent pregnancies from thawed, transplanted ovarian tissue. These 10 case-reports suggest that ovarian function may be restored anywhere from 2 to 5 years post-transplant of cryopreserved tissue. Women who received chemotherapy before taking measures to preserve ovarian tissue all had significantly decreased length of graft function, compared to those who cryopreserved ovarian tissue before initiating a chemotherapy regimen. All singleton gestations delivered at term, after 37 weeks' gestational age. Additionally, all of the infants born after this method of fertility preservation are alive and healthy, without any known congenital anomalies or perinatal morbidity (Donnez et al., 2011).

Studies of pregnancy outcome and neonatal well-being are extremely important with any new reproductive technology. Perhaps more crucial, however, is the ability to track and register pregnancies that arise out of oocyte cryopreservation cycles. The Human Oocyte

Preservation Experience (HOPE) is a phase IV, multicenter, observational registry in the United States that has been created to prospectively collect data on oocyte cryopreservation and subsequent outcomes (Ezcurra et al., 2009). The goals of this project are twofold: first, to evaluate the safety and efficacy of different oocyte cryopreservation techniques, and second, to assess the safety of these methods in relation to the babies resulting from cryopreserved oocytes. This initiative will follow 400 women over three years who are undergoing oocyte cryopreservation, thawing, and subsequent embryo transfer. Standardized data will be collected for all subjects, including demographic information, laboratory studies, and pregnancy outcomes. Additionally, all babies will be followed for the first year of life to evaluate perinatal and infant outcomes after oocyte cryopreservation. Studies of this nature are crucial for the validation of oocyte cryopreservation as a valuable method for fertility preservation in the United States and removal of its “experimental” categorization by ASRM.

8. Conclusion

A variety of ART strategies have been introduced over the past few decades without being deemed “experimental” or requiring IRB approval. Moreover, new procedures in ART have not historically been required to demonstrate improved efficacy over established protocols before being introduced into clinical practice (Noyes et al., 2010). One example is the introduction of ICSI in the 1990s (Palermo et al., 1995). Though ICSI is more invasive than conventional IVF, it was quickly embraced in the field and used widely for couples with severe male factor infertility after extensive informed consent. Other ART techniques, such as frozen embryo storage, prenatal genetic diagnosis (PGD), laser assisted hatching, and even human chorionic gonadotropin (hCG) agonist triggering of ovulation have not required implementation under the “auspices of an IRB.” Instead, informed consent documents highlight risks and benefits of these procedures and infertility centers are expected to honestly present data regarding success rates and outcomes. In light of these inconsistencies, it seems incongruous to require such stringent, IRB-approved regulations for oocyte cryopreservation, which has been shown to produce high survival rates. Clinics should be transparent about their experience, their site-specific pregnancy rates, and the associated perinatal outcomes.

Though oocyte cryopreservation was first introduced more than three decades ago, the past several years have yielded significant enhancement of techniques and documentation of efficacy. Current and future advancements have the potential to preserve reproductive potential for young women with cancer prior to gonadotoxic treatments as well as for those seeking elective preservation of their fertility. As stimulation techniques are simplified, costs are contained, safety and efficacy are documented, and more wide-spread awareness of the reproductive aging process is achieved, it is likely that the number of women who are able to benefit from this new technology will continue to increase.

9. References

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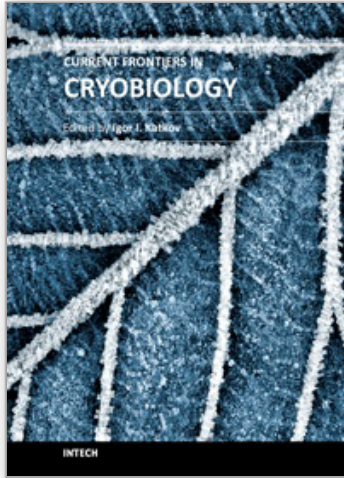
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Almost a decade has passed since the last textbook on the science of cryobiology, *Life in the Frozen State*, was published. Recently, there have been some serious tectonic shifts in cryobiology which were perhaps not seen on the surface but will have a profound effect on both the future of cryobiology and the development of new cryopreservation methods. We feel that it is time to revise the previous paradigms and dogmas, discuss the conceptually new cryobiological ideas, and introduce the recently emerged practical protocols for cryopreservation. The present books, "Current Frontiers in Cryobiology" and "Current Frontiers in Cryopreservation" will serve the purpose. This is a global effort by scientists from 27 countries from all continents and we hope it will be interesting to a wide audience.

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