Oocyte cryopreservation for non-medical reasons

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Abbreviation

- SOF: social oocyte freezing
- AMH: antimüllerian hormone
- FSH: follicle-stimulating hormone
- LH: luteinizing hormone
- AFC: antral follicle count
- IVF: in vitro fertilization
- IVM: in-vitro maturation
- GnRH: gonadotropin releasing hormone
- hCG: human chorionic gonadotropin
- E2: estradiol
- PROH: propanediol
- DMSO: dimethyl sulphoxide
- EG: ethylene glycol
- HEFA: Human fertilisation and embryology authority
- ESHRE: European society of human reproduction and embryology
- ICSI: intracytoplasmic sperm injection
- SART: Society for Assisted Reproductive Technology
- ASRM: American Society for Reproductive Medicine
- PET: birth rate per embryo transferred
- PTC: birth rate per treatment cycle

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Summary

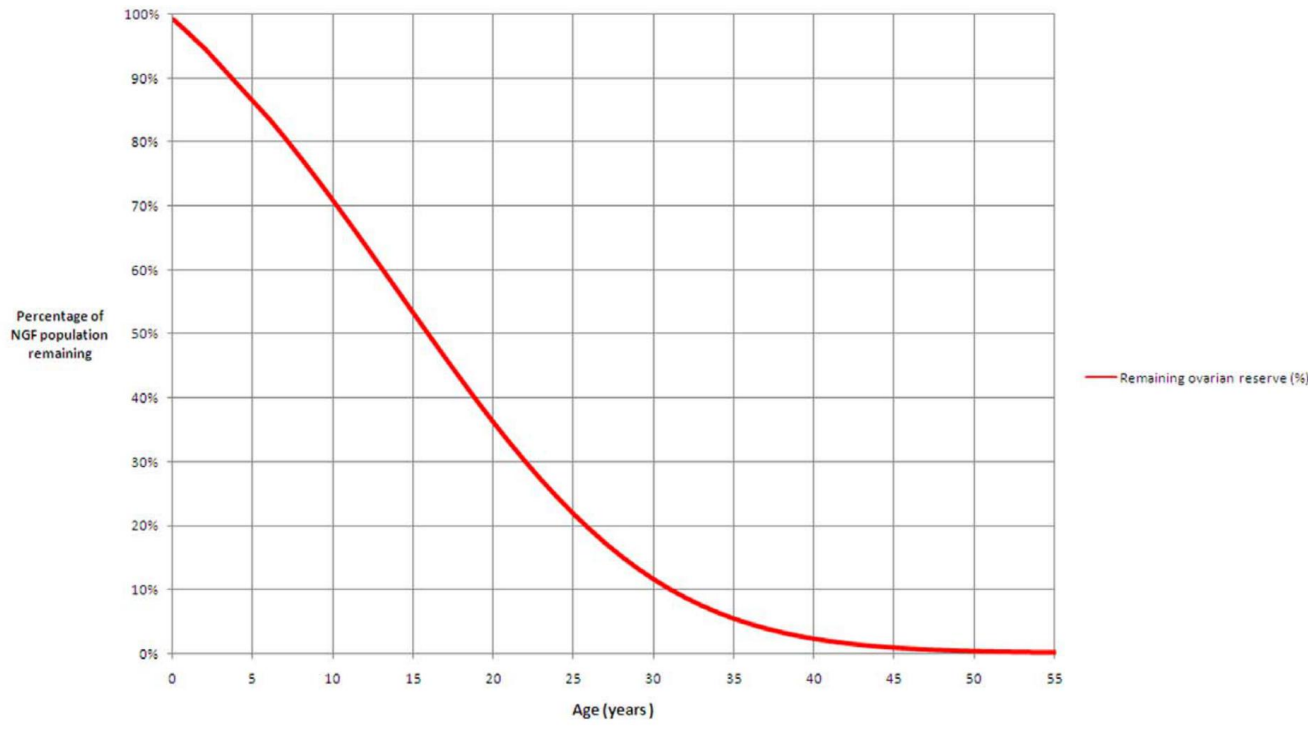
"Oocyte cryopreservation for non-medical reasons," also referred to as "oocyte banking for anticipated gamete exhaustion" by Stoop et al. is when oocyte cryopreservation is used for non-medical purposes. It is a reliable and effective technique for women who desire to preserve fertility. Studies have pointed out that there is a high “no use” rate which is when patients don’t come back for oocytes since they have found a partner in the meantime and got pregnant spontaneously. The two procedures - slow freezing of oocytes and vitrification are described in the article together with a short summary of the evolution of oocyte cryopreservation. Through the evaluation of numerous studies and reviews, it is safe to conclude that the awareness of social oocyte freezing is rising in the general population. The success rate of oocyte cryopreservation is dependent on many factors such as the age of the woman at the time of freezing/thawing, the number of oocytes frozen, the level of anti-müllerian hormone at the time of freezing, etc. Although the process of social oocyte freezing brings advantages to many women, it is important to inform them about risks such as ovarian hyperstimulation syndrome, the possibility of treatment success as well as risks that are brought by late maternity. It is still too early to make any definite conclusions on the outcome of social oocyte freezing, and even though there are significant advancements in the cryopreservation of mammalian oocytes, a lot regarding the molecular and biochemical mechanisms is still to be discovered.

Sažetak

Krioprezervacija jajnih stanica zbog nemedicinskih razloga omgućava očuvanje plodnosti za žene koje zbog socijalno-ekonomskih razloga žele odgoditi trudnoću. Istraživanja su pokazala da postoji visoki postotak oocita koji se nikad ne upotrijebe, što dovodi do niske isplativosti postupka. Pacijentice se ne vraćaju po oocite jer su u međuvremenu pronašle partnera i spontano zatrudnjele. Dva postupka, polagano zamrzavanje oocita i vitrifikacija, opisana su u ovoj radnji zajedno s kratkim sažetkom razvoja krioprezervacije jajnih stanica. Temeljem brojnih provedenih studija u općoj populaciji, može se sa sigurnošću zaključiti da svijest o društvenom zamrzavanju oocita raste. Uspješnost krioprezervacije jajnih stanica ovisi o mnogim čimbenicima kao što su dob žene u vrijeme zamrzavanja, broj zamrznutih oocita, razina Anti-Müllerovog hormona u vrijeme zamrzavanja, itd. Iako ovaj postupak nudi različite mogućnosti, pacijentice je važno informirati o rizicima poput sindroma hiperstimulacije jajnika, stopi uspješnosti liječenja, kao i rizicima koje donosi trudnoća u odmakloj dobi. Iako je recentni napredak u krioprezervaciji oocita sisavaca impresivan, još se mnogo toga tek treba otkriti o molekularnim i biokemijskim mehanizmima, a samim time je i prerano donositi bilo kakve definitivne zaključke o ishodu zamrzavanja jajnih stanica iz socio-ekonomskih razloga.

1. Introduction

An increasing number of women decide to postpone their first pregnancy due to the current social trends, until a later period in life. For example, the average age at first labour in England and Wales increased from 25.2 in 1980 to 30.2 in 2006(14). By the time they opt to have a child, the vast majority of women become infertile, this is largely owing to a decrease in the ovarian reserve through the aging process. Women have roughly 2,000,000 oocytes at birth, which are continuously lost throughout life; by the time of puberty, this number decreases to around 400,000; when the woman reaches her late 30s, her oocytes have decreased to about 27,000(48). Figure 1 illustrates the descend in ovarian reserve over years (63). Multiple indicators may be used to indirectly assess the ovarian reserve.



Firgure 1. Change in ovarian reserve according to age, NGF-non-growing follicle (Wallace et al. 2010)(63).

Currently, the antimüllerian hormone (AMH) and the antral follicle count (AFC) are of better predictive value than the day-3 follicle-stimulating hormone (FSH)(50). According to Sills et al., the ovarian reserve is a crucial component in determining the likelihood of spontaneous conception and the assisted fertilization success rate, the lower the ovarian reserve, the lesser the conception chance(49). Not only there is a decreased ovarian reserve in women with advanced age, but also it can lead to poor pregnancy outcome. As demonstrated in a Japanese study by Shigeki et al. the rate of adverse neonatal outcomes begin to increase at a maternal age of 30(13). It is therefore important to have technologies to assist in fertility preservation while the ovarian reserve is still optimal. Numerous techniques for fertility preservation have been developed throughout the years, namely sperm cryopreservation for males as well as embryo and oocyte cryopreservation for females. Ovarian tissue cryopreservation is still considered to be an experimental method even though there have been case series published of live births in these patients. currently experimental. Oocyte cryopreservation is a relatively novel technology in achieving fertility preservation. The first live birth from this innovation only came in the year 1989(2). “Social oocyte freezing” alludes to oocyte cryopreservation with practically no underlying medical reasons, thus bringing an opportunity for healthy women to postpone their motherhood. Various terminologies are used for “social oocyte freezing”, one frequently acknowledged is “elective egg freezing”, some likewise favor the use of “fertility preservation”, “non-medical egg freezing” as well as “oocyte banking for anticipated gamete exhaustion”(1). Two general techniques are engaged in SOF, controlled slow freezing and ultrarapid cooling by vitrification(2). There has been a large improvement in the survival and fertilization rate of oocytes after the introduction of vitrification(3). Despite advances in technology, several parameters such as the age of the woman at the time of cryopreservation, the amount of oocytes preserved, and the phase of the oocytes at the time of preservation all contribute to the success rate of SOF(4). In this thesis, we will

perform an extensive overview of the literature together with the analysis of the obtained results pertaining to the SOF approach.

In oocyte cryopreservation, three general groups of candidates have been recognized. First group of patients are those with certain medical diseases, due to the gonadotoxic effects of chemotherapy or radiation, it is a wise choice to preserve fertility prior to treatment for individuals with certain medical disorders, such as cancer patients. Because some surgical treatments, such as gender reassignment surgery and endometriosis surgery, might reduce the chances of pregnancy, oocyte cryopreservation is recommended. In addition, individuals with genetic diseases that might cause fertility loss such as Turner syndrome, X chromosome deletion, and fragile X prematuration are likewise recommended to have their oocytes preserved at an early stage of life. The second category of patients are those who are unable to have their embryos cryopreserved for religious or ethical concerns, with the major issue being whether freezing or thawing of human embryos increases the risk of aberrant or dysfunctional births. The last group of patients are those who choose to delay parenthood for various social reasons, which will be discussed in detail in the following sections(12).

2. Elective oocyte cryopreservation

The postponement of motherhood in elective oocyte cryopreservation can be impacted by a variety of lifestyle factors. It is more appealing to younger women who have an unfulfilled desire for children. In fact, as demonstrated by Lockwood (2011), there has been a phenomenon that researchers refer to as “flight from motherhood" rather than a "delay till later in life" in recent years(62). According to a research conducted in Melbourne by Pritchard et al., the most prevalent reason for selecting SOF is the lack of a suitable spouse at that particular moment or any commitment issue, and elective oocyte cryopreservation can be done in the hopes of buying time to find a suitable partner (Figure 2)(56).

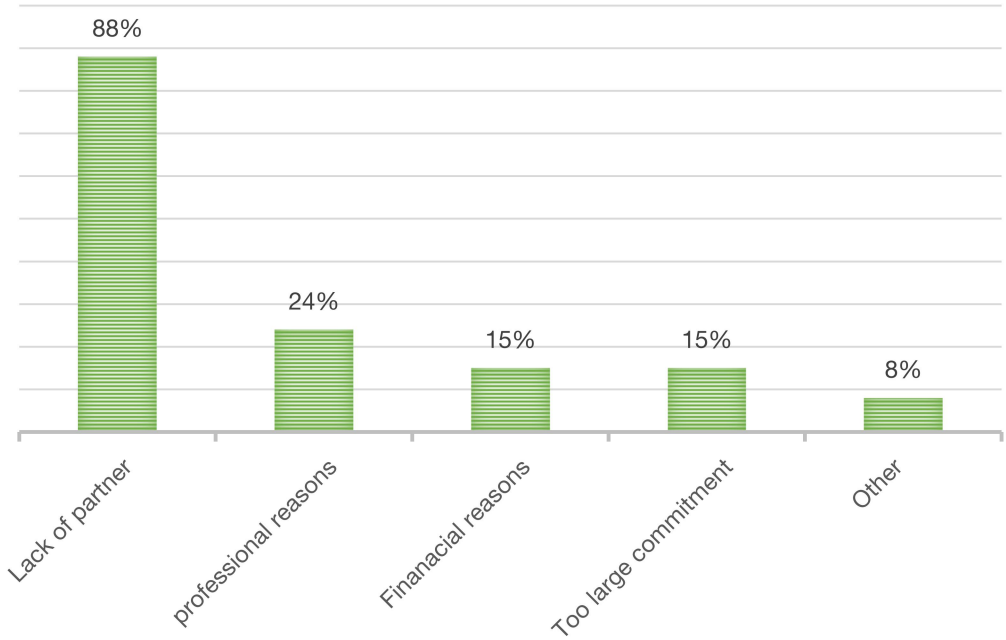


Figure 2: Reasons why women choose oocyte cryopreservation for non-medical reasons, Pritchard et al. 2016 (56)

Other reasons for delaying childbearing include a desire to wait until women believe they have acquired a suitable degree of maturity, financial security, or emotional support, as well as education or career advancement. Some studies also report that the feeling of empowerment of one's reproductive autonomy is one of the reasons women choose SOF(58). Furthermore, according to a UK study, motivations also include avoiding guilt if childlessness becomes an issue in a relationship(57). Therefore, nationality, ethnicity, and religious background all have an impact on population subjectivity(15)(18). These factors tend to postpone first pregnancies in more developed societies which in term leads to decrease in the overall number of children a woman will have. Candidates of SOF are generally highly educated, financially stable and are able to afford the treatment cycles(Nekkebroeck et al. 2010)(53). According to the study conducted by Gold et al., women were feeling pressured by their biological clock and considered SOF as an "insurance policy", additionally all participants obtained at least a bachelor's degree(25). Similarly, Kanters et al. published a recent research that found strikingly similar results(26). Since the American Society for Reproductive

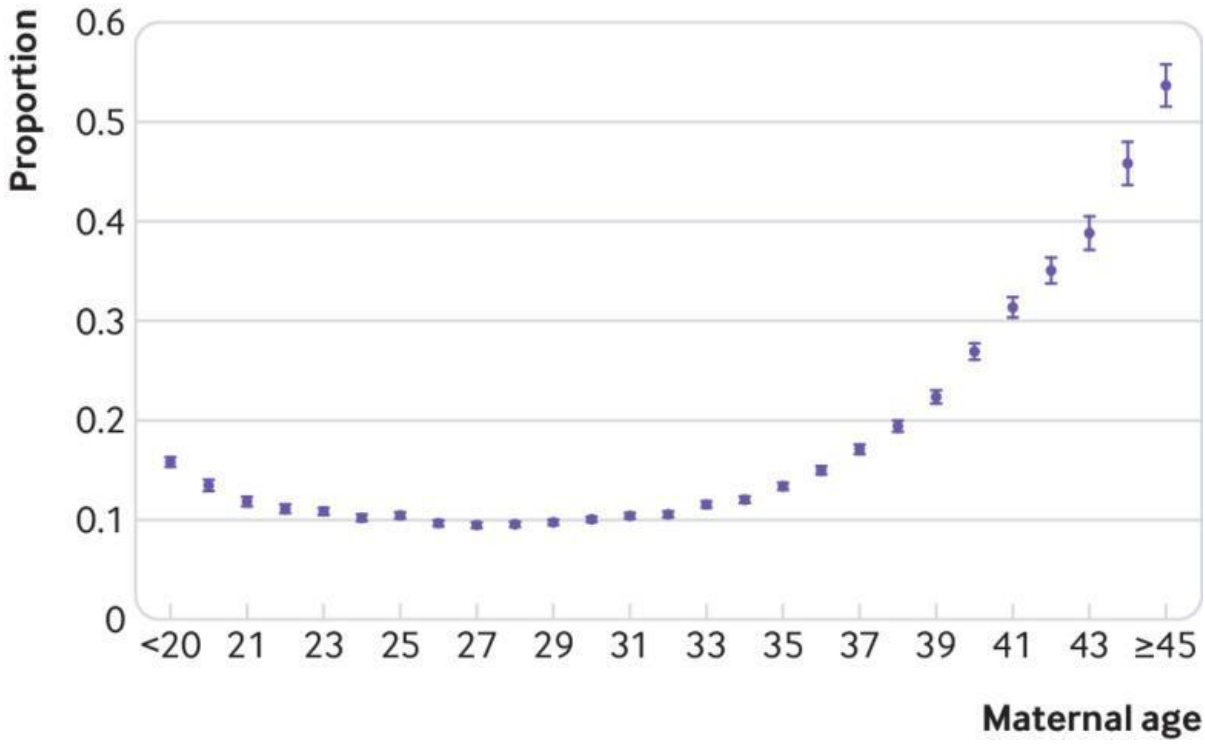


Figure 3: proportion of miscarriage based on age, magnus et al. 2019 (65)

Medicine (ASRM) designated SOF non-experimental in 2012, it has shown to be a fantastic tool for making age-related fertility decline less concerning. Fertility decline accelerates beyond the age of 35(2), with a decrease in ovarian reserve, oocyte quality, and chromosome integrity. Furthermore, the incidence of miscarriage is over 40% for women over the age of 40, and 75% for those over the age of 45(20). Figure 3 demonstrate the upward trend of miscarriage based on women's age(65). The higher risk of miscarriage is mostly owing to a rise in chromosomal abnormalities in women's oocytes; however, the specific process is unknown. Research has suggested that older oocytes are more likely to have abnormal spindles(64). Therefore elective oocyte freezing is an excellent technique for "freezing time" for female fertility. The success rates for genuine conception have improved owing to new vitrification procedures, which are more effective than the previous approach of slow freezing. An increasing number of countries are allowing oocyte freezing for non-medical reasons including many developing economies such as India and The Philippines. The price of oocyte cryopreservation may differ in different countries. According to the "human fertilization and embryology authority" the average expense in collecting and frozen of oocytes is 3,350 pounds with an addition of 500-1,500 pounds for medication and 125-350 pounds for storage per year in the UK without the subsequent ICSI treatment included(3). While in the USA the average price can be between 30,000-40,000 dollars. There is no doubt that the procedure is costly and the entire cycle of oocyte cryopreservation is funded by the individual herself. There may be some modifications in insurance policies related to elective oocyte freezing in the future, some studies provide insight into the policy renewing process. One study was conducted by Johnson et al., which evaluated 656 women's views on the funding of oocyte cryopreservation using an online cross-sectional survey. Results demonstrated that the majority supported elective oocyte freezing to be partially funded by the government(28). While In 2014, Facebook and Apple stated that they will pay the cost of SOF for their workers in

order to help them advance their careers(21). Each female employee is offered an reimbursement of up to 20,000 US dollars, which can assist young female employees advance in their careers. Companies can, however, be asked a number of questions about the frozen oocytes. For example, Can an employee still use her frozen oocytes if she no longer works for the company? Does the company's choice put pressure on employees, making them believe they should postpone their motherhood? Regardless of the difficulties that may arise as a result of the company's choice, it is nevertheless considerate of firms to reimburse for elective oocyte freezing(66).

2.1 Ethical considerations

Despite the fact that embryo cryopreservation is the most well-known method of fertility preservation, oocyte cryopreservation is currently the most suitable option for single reproductive-age women who want to postpone motherhood for personal reasons. In the case of embryo cryopreservation, the main ethical question is whether freezing and thawing human embryos results in a greater rate of aberrant or faulty births, causing harm to the offspring. Those who question specific ethically connected difficulties in SOF mostly focus on damages to women themselves. Ovarian hyperstimulation syndrome is one of the most serious medical hazards. Other concerns linked with oocyte retrieval include haemorrhage, pelvic infections, trauma, etc. Some have also suggested that ovarian stimulation may be linked to an increased risk of breast and uterine malignancies, although further studies are required in this area(40). According to “the European society of human reproduction and embryology (ESHRE) task force on ethics and law” the oocytes should only be stored to an age in which it is considered acceptable for the achievement of a pregnancy(19), because the success rate of oocyte freezing for women in their 40s is much lower in addition to increased risky pregnancy outcomes, such as pre-eclampsia, increased risk of miscarriages and premature births. Another ethical concern is the possibility of

damage to the welfare of children, as older or elderly parents may die early, leaving young children behind which can lead to possible negative psycho-social implications for the children(18). Additionally, gender equality may be one of the concerns associated with SOF; in comparison to males, women may have an earlier loss of fertility, which Godsten et al. referred to as "biological inequality"(59). Because oocyte cryopreservation has just recently been deemed "non-experimental," and sperm cryopreservation has been commercially accessible for a long time, a feminist argument might be made that using SOF could help women achieve reproductive autonomy and gender equality (60).

2.2 Public attitude

Oocyte cryopreservation for non-medical reasons is becoming more well known among women throughout the world. According to a study of 930 female students conducted at the University of Padova in Italy, 34.3 percent of students said they had heard about the possibility of SOF and knew what it meant, and 19.5 percent were in favor of the process. The majority of students who were married at the time of study answered "no" to the hypothesis of oocyte freezing, and those who were engaged also contributed to a high percentage of students who answered "don't know" to the assumption of oocyte cryopreservation, leaving only a small proportion in favor of the procedure. Furthermore, medical students were more open to the possibility of oocyte cryopreservation than healthcare students(15). An online anonymous cross-sectional survey of 129 female medical students in Singapore was designed to examine their knowledge and attitudes toward SOF. Of the 129 students, 36.4 % had heard of SOF, 70% out of these had considered oocyte freezing, but only 48.9% were still considering it after reading an information leaflet about SOF. The success rate of oocyte cryopreservation, the process of oocyte retrieval, and the cost of the treatment were all included in the information leaflet, which gave students a basic understanding of the procedure and prompted them to reevaluate their options(16). The concept of SOF is also

highly supported by the general population, however the indication for SOF is associated with considerable disparities in public support. According to a survey conducted in the United States, including 1064 participants of different genders, age and race. The majority (72%) of participants supported elective oocyte freezing due to career advancement, followed by (63%) delayed childbearing owing to a lack of partner, while those who voted for lack of funds contributed the least proportion(58%)(61). Furthermore, the attitudes towards oocyte cryopreservation for non-medical reasons are influenced by many factors such as gender, socio-cultural background, etc. A German cohort study with a total of 643 participants including all genders, showed that there are obvious links between patients' views regarding SOF and their socio-cultural background, age, gender, and reproductive issues(22). Equally important, Stoop et al. found that women with higher academic background were more likely to select SOF, and that the same group of women were also more likely to donate their oocytes(19). Additionally Lauren W. Milman et al. conducted one of the biggest cohort studies in the United States. It was suggested that characteristics such as Asian ethnicity, single status, and infertility had a significant effect in the decision to freeze oocytes(23). Certainly that the decision making in choosing SOF depends on factors like age of the woman, number of children the woman already has, personal beliefs based on socio-cultural background and many more. However, we may reasonably assume that SOF awareness is growing, and that its demand will continue to grow steadily in the future.

2.3 Success rate

Oocyte cryopreservation, particularly by vitrification, has shown to be an effective procedure, resulting in subsequent pregnancies comparable to IVF with fresh oocytes(12). oocytes that have been frozen with the method vitrification, when compared to the slow freezing method, has significantly higher oocyte survival rate (81% compared to 46%) and pregnancy rate (38% compared to 13%)(54).

There is no strict freezing protocol that guarantees a high probability of viability following thawing(24). However, the success rate of oocyte cryopreservation can be contributed to many factors such as maternal age, the number of oocytes frozen, time to retrieve the oocytes, temperature at freezing/thawing procedure, types of cryoprotectants used, and the amount of time that oocytes are exposed to them, these factors are discussed in the following text. Age at the time of oocyte cryopreservation is one of the most important factors in influencing the success rate. In accordance with a retrospective cohort study by Maslow et al., young age at the time of freezing, a high AMH level, low total gonadotropin usage, and a high peak estradiol level were the main contributors to the success rate in SOF. The best timing for women to consider SOF is 31-35 years because it is after the 20s when they still have time to find a suitable partner and before the time of actual age-related fertility decline(36). A recent prospective study investigating the effectiveness of oocyte cryopreservation using the vitrification method concluded that the fraction of vitrified oocytes resulting in a live birth was 8.2% in women under 36 years, and 3.3% in women 36–39 years. This was a more than 50% decrease in success rate with a three years increase of age, signifying the importance of maternal age at the time of oocyte cryopreservation(55). Gold et al. concluded in their study, the majority of the women that come for SOF are around 36-40 years which belongs to the age group that will result in a lower success rate(25). There is an obvious variation in live-birth rate based on women’s age at freezing, as shown in figure 4 published by Human fertilisation and embryology authority (HEFA), both the birth rate per embryo transferred and the birth rate per treatment cycle are the lowest at the older age of 40-42(31).

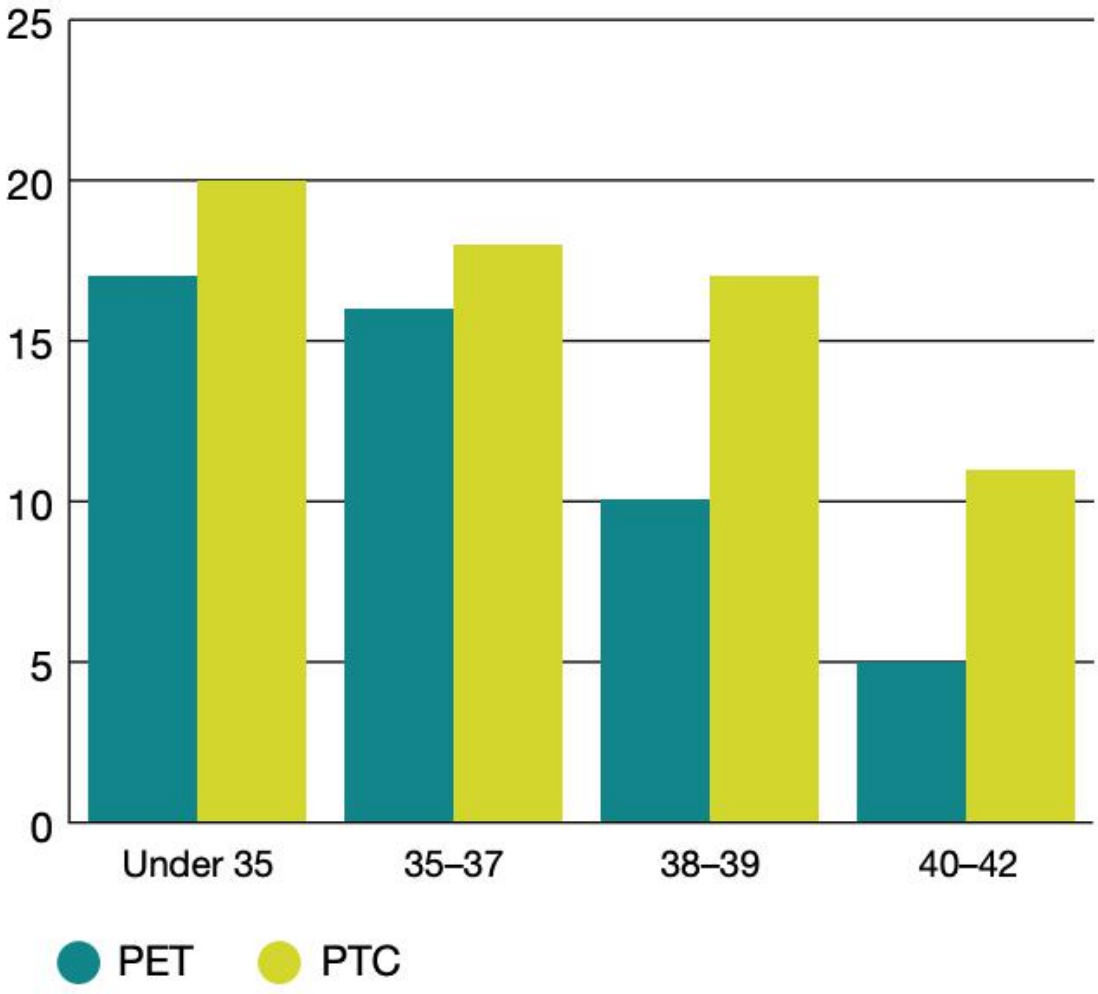


Figure 4: Birth rate by age at freeze cycle according to HEFA(31)

Despite the fact that oocytes frozen at this age group result in fewer pregnancies, they undoubtedly will still benefit from this procedure due to accelerated age-related fertility decline. A review by Walker et al. also suggests that there is an optimal age to perform elective oocyte. According to the study, the best live birth rate following elective oocyte freezing happens before the age of 37 or when anti-mullerian hormone levels are higher than 1.995 ng/dL(27). The number of oocytes frozen is also a crucial factor in elective oocyte cryopreservation success. Although there is no clear formula for determining the precise number of optimal oocytes for freezing, researches have shown that a particular quantity of oocyte frozen is preferable to achieve an ideal success rate. According to a meta-analysis by Oktay et al., there is a 4.5% pregnancy rate for each oocyte

thawed after vitrification, this yields to a requirement of 22 oocytes for each successful clinical pregnancy in a female population of 32.3 years old in average(29). The SART and ASRM practice committee stated that the informed consent form should include information regarding the approximately 4% success rate in each thawed oocyte following virtification(30). However, as previously said, the number of oocytes frozen is not the primary measure of oocyte freezing success; the value can vary greatly depending on a variety of parameters, one of the most important of which is age. Doyle et al. discovered that 14 oocytes at metaphae II are needed for patients at 30-34 years old, 15 would be needed for 35-37 years old and 26 are required for those at 38-40 years old in order to obtain a 70% likelihood of one live delivery following thawing from vitrification. Based on the findings, it is practically impossible to achieve a pregnancy rate of 70% if 26 oocytes are required for women approaching their 40s, this is due to a significantly lower ovarian reserve compared to women younger than 35. Furthermore, they have a reduced sensitivity to FSH stimulation, which means that even if they have adequate amount of follicles, these follicles do not respond to stimulations and remain small in the ovaries. Results of the study by Doyle et al. is shown in figure 5(39). According to data published by HEFA, the majority of women who undergo a thaw treatment cycle are over the age of 44, with only 17% being under the age of 35(31). As a result, the majority of patients who select SOF will not have the best outcome in the future. The maturity of oocyte at the time of retrieval may also contribute to the live-birth rate following thawing. Generally, oocytes at metaphase II are frozen, these oocytes are exceedingly fragile due to their water content, large size and chromosomal arrangement. Various investigations have revealed that intracellular ice generation during the

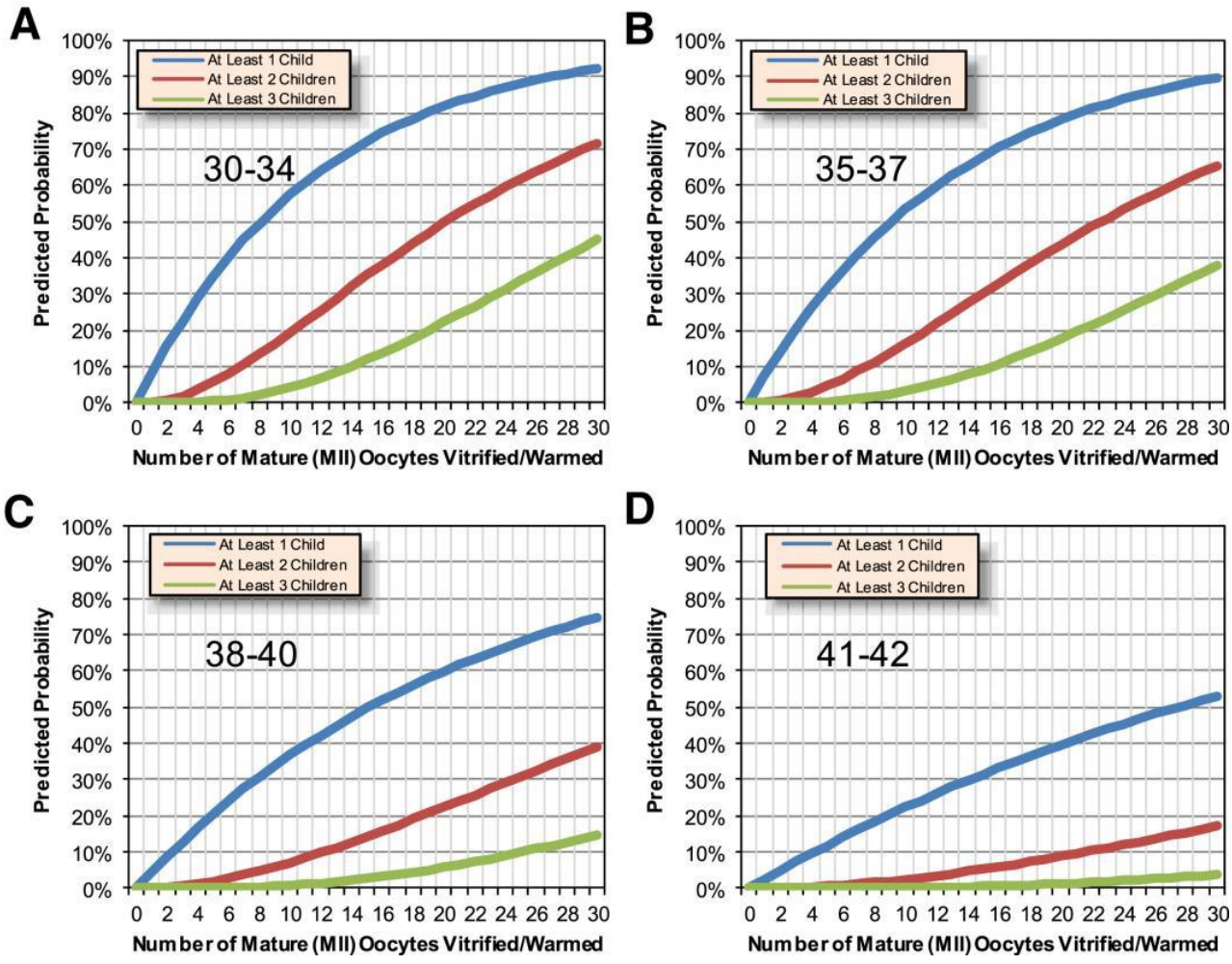


Figure 5: predicted success rate of SOF based on number of oocytes and maternal age published by Doyle et al. (39)

freezing or thawing process disrupts the meiotic spindle structure, which is related to the meiotic spindle lining up along the equatorial plate on metaphase chromosomes. Similar to that number of oocytes required is not a definite factor in influencing the success rate of oocyte freezing, the disruption of the meiotic spindles in metaphase II oocytes is likewise influenced by multiple factors such as the age of the women and cryopreservation techniques. Oocytes at the germinal vesicle stage can be used as an alternative to metaphase II oocytes. At this stage, they do not present a freezing-sensitive meiotic spindle and are shown to have higher survival rates and lower abnormalities compared to metaphase II

oocytes(32)(33). However, according to Lane et al., oocytes at this stage can be paradoxically more sensitive to freezing injury possibly because of a specific cytoskeletal formation and lower cell membrane stability. Despite the fact that germinal vesicle stage oocytes are less likely to have meiotic spindle disruption and have a higher thaw survival rate, the inefficiency of the maturation process in vitro results in a success rate similar to that of metaphase II oocytes(33). Further information in regard to the slow freezing method, the vitrification method, and cryoprotectants used was described in previous sections.

2.4 Current trends

Patients can decide whether to thaw their oocytes in the future and inject sperm into the oocytes that have survived. If the oocytes are not used for whatever reason, the patient who saved them have a variety of choices for putting their oocytes to good use. The oocytes can be donated for research purposes to aid in the study of infertility reasons and the development of novel treatments to treat it, or they can be donated for use by other patients. If a patient does not want to use her oocytes in any manner, she can simply discard them. The oocytes are put in a warmer temperature setting or in water to allow them to naturally thaw. Patients should be informed that the oocytes can only be stored for up to 10 years by HEFA regulation. There is a higher “no use” rate in younger patients because of their higher chance of natural conception. A retrospective study was conducted by Blakemore et al. on patients who received at least one cycle of treatment of SOF from January 2005 to December 2009. A total of 231 patients and 280 cycles were analyzed of which 38.1% returned to thaw their oocytes for further ICSI and embryo transfer(34). As stated in a review by Zachery et al., patients who had SOF treatment cycles had a high incidence of "no usage" (58.9%) and 62.5% of patients who returned to thaw their oocytes were with a spouse while the rest 37.5% chose to use the donor sperm(27). Patients that were more than 38 years old at the time of oocyte freezing were more likely to utilize their stored

oocytes in comparison to those less than 38 years old according to Leung et al(38). In another observational study conducted by Garcia-Velasco et al., 26 out of 560 patients that had planned oocyte cryopreservation returned for thawing of their stored oocytes, yielding an only 4.6% returning rate over the five years period(35). By analyzing the variability in returning rates in various studies, it is safe to assume that the returning rate is also influenced by many factors like ethnicity, socio-cultural background, and the presence of a suitable spouse similar to the decision rate in SOF. In the study conducted by Cubo et al. in 2018, an increase in the utilization rate from 4% to 22% was noted over the 11 years of study(37). Therefore it is also clear that there is a raise in awareness of oocyte cryopreservation for non-medical reasons in general populations.

2.5 Advantages and disadvantages

Planned oocyte cryopreservation followed by IVF and embryo transfer, without a doubt, widens women's right to establish a family and can help women avoid some of the most prevalent concerns, such as age-related fertility loss. It allows women to become genetic mothers at an advanced age, ensures them to prioritize their studies and careers. At the same time, it avoids the possible chromosomal abnormalities associated with

aneuploidy in embryos. Additionally, it is beneficial for women who have ethical concerns related to other forms of fertility preservation like embryo cryopreservation, and provides enough time for the women to find a suitable partner(40). Furthermore, many women postpone pregnancies after 37 years old in order to advance in their career in developed countries, resulting in a significantly drop in pregnancy rates. The procedure might help to offset the reduction in birth rates in those countries(3).

However, it is also important to inform the patients prior to the retrieval of oocytes.

Potentially there can be some serious risks such as ovarian hyperstimulation syndrome as well as bleeding and infection that can happen due to the process of oocyte retrieval. Patients can experience breast tenderness, nausea, fatigue, abdominal pain, headaches in mild to moderate ovarian hyperstimulation syndrome. Nevertheless, a small percentage of patients (0.1%-2%) may experience symptoms of severe ovarian hyperstimulation syndrome that can result in dyspnea, hypercoagulability, vomiting, and abdominal pain that may require hospitalization(40). Ovarian hyperstimulation may be prevented with the IVM procedure in which immature oocytes are harvested and together later on matured in-vitro, however, this procedure is still considered to be experimental(41). Patients should be aware that advanced maternal age at the time of delivery poses great risks for a variety of pregnancy complications such as gestational diabetes, cesarean section, preterm delivery of infants with low birth weight and preeclampsia(44). It has been stated in studies conducted by Jolly et al. in 2000 and Cleary-Goldman et al. in 2005 that there is an increased risk of perinatal mortality, infants with low birth weight, and preterm delivery among pregnant women aged 35 years or older(42)(43).

There is limited data on children born from thawed oocytes. According to Munne et al. there is no increased rate for chromosomal abnormalities or congenital malformations following vitrification compared to infants born from the IVF procedure(45). Issues related to child risk are mainly associated with advanced maternal age rather than being born from vitrified oocytes.

Another downside of oocyte cryopreservation, for both medical and non-medical reasons, is the high expense of each cycle, as well as the subsequent storage and ICSI fee. As stated in the earlier section, the expense of each cycle of oocyte cryopreservation varies depending on the country and the clinic, as well as the quantity of medications needed for ovarian stimulation(46). The health insurance

does not cover the expenses of social oocyte freezing. Therefore, the entire process is considered an “out of pocket” expense” that must to be paid by the women themselves, although there are some movements in changing that and trying to cover the process in the insurance plan(17). Various clinics usually offer financing plans to help their patients navigate the expenses for oocyte cryopreservation and the subsequent IVF process. Large companies like Facebook and Apple offer their female employees a $20,000 benefits for oocyte freezing which is symbolic in the era of social oocyte freezing since it may motivate more companies to do so(47). Therefore it is expected that in the future, at least for countries which already reimburse IVF expenses, SOF treatments will be covered at least partially.

3. Procedure of oocyte cryopreservation

Oocyte cryopreservation involves women undergoing procedures that allow their oocytes to be retrieved, frozen, and kept for possible usage in the future. When they opt to use the stored oocytes, the oocytes will be thawed, fertilized, and developed embryos are transferred into the uterus. Generally, 10-15 oocytes are required, if this number is not reached in one cycle, more cycles will be performed. Women over 37 years old frequently need to undergo more than 2 cycles to ensure such number of oocytes are cryopreserved. However, as mentioned in the previous section, the success rates of oocyte cryopreservation many vary depend on maternal age and the number of oocytes frozen. According to a retrospective cohort study conducted by Maslow et al.. In order to achieve a success rate of 50%, 6 oocytes are needed if the woman is less than 35 years old, and 50 if the woman is more than 42 years old(36). As a result, the number of cycles required in oocyte cryopreservation is also dependent on patient's age.

Firstly, women should be tested for infectious diseases such as HIV, Hepatitis B, and C just to ensure that their oocytes are stored separately if infected with these agents. Ovarian reserve testing is also routinely performed to assess the quality and quantity of oocytes. This is commonly done using the AFC assessed with ultrasound, the amount of AMH, day 3 FSH, and estradiol (E2)(51). Women then must go through the same oocyte retrieval procedure as in in vitro fertilization (IVF) before freezing their oocytes, generally oocytes at metaphase II are frozen and the procedure takes around 2-3 weeks(4). The oocyte retrieval procedure starts with the stimulation of ovarian follicle development using exogenous follicle-stimulating hormone (FSH), and the LH surge is prevented with a gonadotropin-releasing hormone (GnRH) agonist or antagonist, allowing physicians to schedule oocyte retrieval. The choice of drug is primarily based on the patient; however, because of its shorter duration and lower risk of ovarian hyperstimulation syndrome, the antagonist regimen is becoming more routinely

adopted(51). Once when three follicles have developed to a diameter of 18-20mm, human chorionic gonadotropin (hCG) is given to accomplish the ultimate maturity, then the oocytes are retrieved 36 hours later using ultrasound-guided transvaginal aspiration(5). Oocytes retrieved at metaphase-II should be dealt with extreme caution due to their fragility owing to high water content, large size, and specific chromosomal arrangement(52).

3.1 Cryopreservation methods

Once oocytes are retrieved, they are ready to be frozen. The slow-freezing approach and the ultra-rapid vitrification procedure are the two ways of oocyte cryopreservation currently available. The formation of ice crystals during the freezing process might compromise the integrity of cells due to the low surface area to volume ratio of human oocytes. To avoid this, cryoprotectants are added, which are compounds that aid in cell dehydration, allowing water to be replaced in the cells and preventing the development of ice crystals(6). In addition, cryoprotectants also help to reduce the freezing point(7). The speed of freezing should be closely monitored and manipulated to prevent ice crystal formation while simultaneously allowing for dehydration. It's also critical that the entire procedure be completed quickly enough to avoid cryoprotectant toxicity.

3.1.1. Slow freezing

Slow freezing, is a process that takes a relatively long time and results in a temperature drop of 0.3-2°C per minute(8). Slow cooling neutralizes the negative consequences of ice crystallization, osmotic pressure, and freezing damage. The rate of cooling is 1°C per minute between -5 and -9°C. When the seeding temperature is established, at which point ice crystallization is produced at -6°C, the oocytes are not further cooled for 10-30 minutes. They will be cooled at a rate of 0.3-0.5°C per minute afterward. In the end, oocytes are ready to be kept in liquid nitrogen when the temperature decreases to between -30 and -150°C, which is the temperature considered to have ceased all biological activity(7).

3.1.2. Vitrification

In the vitrification method, a greater amount of cryoprotectants is utilized, typically a mixture of propanediol (PROH), dimethyl sulphoxide (DSMO), and ethylene glycol (EG)(8). The essential concept is that vitrification causes the solution to freeze quickly enough to inhibit ice crystallization, resulting in so-called glass-like solidification(7). The time spent exposing oocytes to cryoprotectants must be reduced to avoid toxicity, and the rate of freezing must be reach -1500°C per minute until a temperature of -20,000°C is reached(8). Both technologies have the potential to impair the capacity of cells to grow normally after being frozen and to alter oocyte cellular activities. Huntington Medicina Reprodutiva in Sao Paulo and Rio de Janeiro, Brazil, did a prospective randomized research to see which of the two procedures is more effective. Patients who had their oocytes cryopreserved had nine oocytes frozen at random using either the slow freezing or vitrification method. The results showed that the vitrification/warming method had a significantly higher oocyte survival rate, fertilization rate, and cleavage rate following fertilization than the slow freezing/thawing method(10). Furthermore, a meta-analysis performed by Kalliopi et al. also demonstrated a higher survival rate of cleavage stage embryos in vitrification method than that of slow freezing(67).

3.2 The evolution of oocyte cryopreservation

The first pregnancy utilizing the slow freezing fast thawing procedure was in the late 1980s(2). However, the success rate was minimal, with only five live births in over a decade utilizing the slow freezing approach. The first live birth in oocyte cryopreservation following vitrification was reported in 1999(12). In 1937, Luyet was the first to publish a major paper on vitrification, at an epoch when fast cooling was common but there was little or no intracellular cryoprotectant. Despite the fact that cryoprotectants had been used before Luyet's time, the specific role they played in preventing freezing damage remained unknown. They solely used

it to dehydrate cells before cooling to reduce the amount of water that needed to be vitrified. The phase of fast cooling with little but careful cryoprotection begins next. Pierre Boutron was the first to propose utilizing increasing amounts of cryoprotectants in vitrification, although his efforts were mostly experimental. In 1981, Fahy explored vitrification with high cryoprotectant concentrations and slow cooling/warming rates, focusing on the necessity to prevent cryoprotectant toxicity(11). While the technique of oocyte cryopreservation is undoubtedly more advanced than before, given that it is still a relatively new technology, many more studies need to be conducted in order to attain a greater success rate and lower cost.

4. Conclusion

Oocyte cryopreservation for non-medical reasons is undoubtedly a safe and successful procedure that can help numerous women to postpone motherhood. Despite the fact that embryo freezing is the traditional process, social oocyte freezing appears to have a significant benefit over embryo freezing due to various ethical considerations and the lack of a male partner to provide sperm at the time of preservation. The procedure itself is becoming increasingly well-known among the general population and presumably, the utilization will increase together with the increase in awareness. Patients should always be educated about the success rate based on age, the number of cycles they should do, as well as other scientific factors that can influence the outcome. Certainly, the expense of the procedure, the limited amount of years that those oocytes are allowed to be stored in the bank, the potential risk of the procedure and the possible risk of late maternity should also be explained to patients.

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