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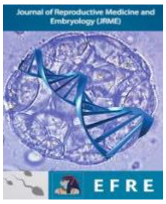

Improving Oocyte Freezing for Advanced Maternal Age

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Abstract

Recent advancements in reproductive medicine, specifically in cryobiology and oocyte freezing, have opened new possibilities for women seeking fertility preservation to overcome age-related infertility. This Mini Review aims to outline the expected benefits fertility preservation for non-medical reasons offers to women of advanced maternal age. The review will address current challenges, including determining the optimal number of vitrified oocytes for successful outcomes in planned oocyte cryopreservation, and the need for standardization in the vitrification process. Furthermore, this mini-review will explore concerns and ethical dilemmas arising from the increasing trend of women postponing motherhood, emphasizing the importance of aligning women's expectations with available scientific data to prevent misconceptions and false expectations. Additionally, it will touch upon the welfare of parents and children in light of the increasing age gap among them. Lastly, future developments aiming to enhance the efficiency and accessibility of fertility preservation via automation and the use of AI will be presented.

Keywords: Fertility preservation; Vitrification; Egg cryopreservation.

Introduction

In recent years, reproductive medicine has witnessed groundbreaking advancements, in the field of cryobiology and oocyte freezing. The introduction of vitrification in 2005 (1), was soon followed by reports highlighting its higher efficiency in cryopreserving both embryos and oocytes compared to the slow freezing method (2-3). The increasing body of positive published experience led in 2013, the ASRM Practice Committee to declare that vitrification should not be considered experimental (4). In the subsequent years, the field of assisted reproduction (ART) entered a new era where vitrification became a fundamental technique in

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the daily lab practices and the method of choice for cryopreserving both embryos and oocytes. Gradually, two new treatment options emerged, for fertile women whose fertility was threatened due to medical reasons (e.g. oncological patients) or age. Fertility preservation for medical and non-medical reasons entered ART and transformed consultation and lab practices.

In 2018 ASRM described planned oocyte cryopreservation as an ethically permissible medical treatment that may enhance women's reproductive autonomy and promote social equality' (5). Oocyte vitrification offered women, for the first time ever, the option to safely preserve their oocytes and fully maintain their biological competence during the cryostorage, for a viable pregnancy. Over the years vitrification became increasingly popular among healthy women who wish to maintain their reproductive potential (fertility preservation) as globally an increasing number of women choose to postpone motherhood for later in life when infertility due to age-related causes is common (6).

Vitrification: A Game-Changer

Vitrification is a rapid freezing method known for its ability to significantly reduce ice crystal formation. Ice crystal formation during vitrification, in the case of the single-cell oocyte is synonymous to cell death and oocyte's degeneration. Vitrification transforms oocytes into an amorphous glassy state inside and outside the vitrified cell. This crystal-free state is achieved by exposing the oocytes to vitrification media containing high concentrations of cryoprotectants; aiming to rapidly replace via osmosis the majority of water molecules from the oocytes' intracellular compartments and cytoplasm with cryoprotectants. Dehydration is followed by ultra-rapid cooling (>10.000C/min) leading to the glass-like solidification of the cell's contents, in which the oocyte's structural and functional integrity is fully preserved (7).

The vitrified oocytes remain in the cryostorage, ideally submerged in LN2, until the warming process. During warming the vitrified oocytes are removed from their storage location and following the standard operating procedures are submerged in a prewarmed medium containing high sucrose, followed by washing steps in media with a gradually lower concentration of sucrose. The warming process aims to the oocytes' rehydration via osmosis and gradual replacement of the cryoprotectants with water (8). For many, the warming process is more important than cooling as ice crystal formation may also take place during warming and cause cell death (9). The expected recovery rates after oocyte warming range from 80 to 90% (10).

Nowadays, the majority of fertility clinics globally, offer oocyte cryopreservation services to women who wish to preserve their oocytes for future use. A successful vitrification program heavily relies on the operators' experience and expertise, therefore, it is critical for the clinics and Lab managers to ensure that a proper and active total quality management system is in place, and the whole program from stimulation, oocyte vitrification, warming and performance of the embryos produced from the vitrified oocytes, is intensely monitored and optimized as a whole and as per operator basis. Every fertility clinic offering oocyte vitrification to their patients should be able to present their patients with their own KPIs in relation to the international standards and make sure that the prospective patients are fully aware of what to expect after warming before they proceed with the treatment for oocyte preservation (11-13).

Oocyte Vitrification for Advanced Maternal Age: The Challenges

Ovarian aging is a natural and physiological aging process characterized by loss of quantity and quality of oocyte or follicular pool. Follicular microenvironment plays a critical role in the acquisition of nuclear and cytoplasmic maturation which determines the oocyte's competence for supporting embryonic development and a viable pregnancy. As female age advances these fundamental processes are irreversibly disturbed and women over 35 years of age (14-15), are faced with a lower chance of pregnancy and live birth and a higher risk of miscarriages and birth defects due to this age-related decline in oocyte quality and overall competence for a viable pregnancy. Specifically, increased risk of chromosome segregation errors or recombination failure during meiotic divisions are the leading causes of oocyte aneuploidy associated with maternal aging (16). Moreover, the ovarian microenvironment and the stress that is induced by environmental pollutants and a poor diet, along with other lifestyle factors, impact oocyte quality and function and contribute to accelerated oocyte aging and infertility (17).

In recent decades women adapted to new roles and career goals in modern society and as a result, there is a global trend towards delayed motherhood. It was recently reported that the average age when women have their first child globally has significantly increased (18). This recently achieved 'record' comes to direct conflict with female reproductive physiology as postponing motherhood may increase involuntarily childless as well as pregnancy complications in case of a pregnancy at advanced maternal age leaving them with almost no chances of a live birth over the age of 45 with their own eggs.

Assisted reproduction for women of advanced maternal age, to overcome age-related infertility is linked to several challenges such as a low number of oocytes, higher cancellation rates, increased risk for additional rounds of stimulation, only a small fraction of the oocytes retrieved are euploid, low chances for a successful outcome and a viable pregnancy (19).

One of the biggest challenges when it comes to oocyte vitrification is the maternal age at the time of vitrification as the age is strongly and negatively associated with the expected prognosis after warming. For women undergoing fertility preservation before the age of 35 a higher oocyte yield, with fewer ovarian stimulation cycles, and higher live birth rates are expected which reinforces the importance of age on fertility preservation. Fertility preservation in the form of oocyte cryopreservation at a younger age seems to provide a good alternative for women who are planning to postpone motherhood for later in life. For these women oocyte vitrification at a younger age, will provide them with the option of having genetically related offspring in the event that their life circumstances prevent them from having a baby before the age after which a significant decline in fertility occurs. While it is clear that for fertility preservation cycles, the main prognostic factor for success rates is the age at the time of oocyte vitrification, most patients proceed with cryopreservation at the age of 36–39, with the mean age at the time of freezing being 37–38 and 80% of patients being above 35 (20-22).

Oocyte Vitrification for Advanced Maternal Age: How many Oocytes?

When it comes to planned oocyte cryopreservation a common question and concern women have is about the ideal number of oocytes they need to store to secure their dream of motherhood.

Several groups attempted to address this concern by analysing their own experience and data (23). In particular, Doyle et al. (2016) introduced 'vitrified oocyte to live-born child efficiency' to describe the ability of a frozen-thawed oocyte to result in live birth as a tool to guide decision-making regarding expected success rates (24). According to this tool it was estimated that for advanced maternal age women the highest probability of live birth from cryopreserved oocytes is obtained at the age of 36-38. In women less than 38 years of age 15–20 stored mature oocytes were required for a 75% chance of having one child. For women in the age group of 38–40, the number of stored mature oocytes increased to 25–30 oocytes for a 70% chance. The number increased further for women aged above 42 where 61 eggs are needed to achieve similar success rates. This later scenario surpasses the biological ability of these women in most of the cases even if they are willing to undergo several stimulation cycles. In the same context, Goldman et al. (2017) attempted to develop a user-friendly mathematical model to serve as a counseling tool for predicting, the optimal number of vitrified oocytes and the probability of a woman having at least one, two, or three live birth(s) (25). These evidence-based tools can be adjusted taking into consideration parameters both related to patient characteristics and the unit's expertise. More recently, Cascante et al,

(2022) reported a 70% final life birth rate (FLBR) per patient who stored more than 20 mature oocytes before the age of 38, FLBR fell to 33% for women who achieved to store >20 mature eggs at the age of >41 (26).

Oocyte Vitrification for Advanced Maternal Age: Shaping Realistic Expectations

The widespread tendency towards delaying motherhood and the advances in laboratory technologies and cryobiology are encouraging women to consider oocyte vitrification and, by doing so, increase their reproductive autonomy. However, elective oocyte vitrification in relation to the expected prognosis for a life birth is still not clear as the confounding parameters are many and only a limited number of cases after oocyte warming have been reported (27-28). Moreover, despite the well-documented decrease in fecundity that occurs as a woman ages, reproductive-aged women frequently overestimate the age at which a significant decline in fertility occurs and overestimate the success of assisted reproductive technologies (ART) to circumvent infertility.

The age at the time of freezing and the number of stored oocytes remain the two key factors that determine outcomes (24,29-32). It is estimated that on average, 20 oocytes are required to achieve a pregnancy with the minimum proposed number being eight to ten (26,29). However, for a given age, success rates will reach a plateau that cannot be surpassed regardless of oocyte number. In a multicenter retrospective study in Spain, it was demonstrated that in the most optimistic scenario, a 94,4% live birth rate was achieved for women who electively froze their eggs at 35 years of age or younger with 24 oocytes in storage (33). For the same age group, the cumulative live birth rate dropped to 42.8% with 10 oocytes in storage. The success rates recorded for women who cryopreserved oocytes at an older age (>35), were significantly lower. A 5.9% life birth rate was obtained for 5 oocytes and 17.3% for 8 oocytes. These data highlight two important facts women should be aware of: (i) as the age of planned oocyte cryopreservation advances the expected prognosis is fast dropping and (ii) planned oocyte cryopreservation should be considered as an option that increases the odds of a biological child later in life but it by no means can guarantee a life birth in any age group.

Future Perspectives

The widespread tendency towards the postponement of motherhood, the advances in laboratory technologies, and the ongoing awareness campaigns are encouraging women to consider oocyte vitrification to preserve their fertility. As a result oocyte cryopreservation for non-medical reasons is becoming one of the fastest-growing fertility treatment in the UK, increasing by 10% per year. It is estimated that there was a 240% increase from 2013 to 2018. In Spain, oocyte cryopreservation cycles for fertility preservation increased from 4% of total vitrification procedures to 22% in a 10-year period (33), in the USA, the number of cycles increased from 9607 in 2017 to 13,275 in 2018 (34). This new increasing trend demands a call to action on more than one level:

Awareness campaigns: Elective egg freezing for non-medical reasons provides a unique opportunity for women to mitigate the decline in their fertility with age, but highlights that women undertaking oocyte cryopreservation should only do so with a full understanding of the likelihood of success, as well as costs and risks (35). Several studies demonstrate a lack of awareness of how age impacts fertility for women (36,47). For women who wish to have children, fertility education is the key. Efforts to increase awareness among women about their reproductive options and the impact of age are of immense importance. Educational campaigns and personalized counseling play crucial roles in informing women about the possibilities and limitations of oocyte freezing before they decide if fertility preservation is a good option for them (38). Natural conception at a young age should always be the preferred way, and couples who have decided to have children should start trying early. For those, though, that conventional family planning is not an option, fertility preservation offers a good alternative to avoid age-related infertility (39-40).

Monitor-Standardize-Optimize: The delicate nature of the vitrification technique during cooling, storage, and warming makes continuous monitoring essential to ensure a highly-performing vitrification program. Optimizing oocyte survival rates post-warming is crucial especially in the group of women of advanced maternal age. Losing oocytes due to technical issues during the vitrification process, may have a profound effect on the overall prognosis and increase cancellation rates (41). As performance variations among operators and labs are still a common phenomenon (42) which indicates the lack of standardization it is upon each fertility clinic to take all the steps and ensure adequate and well-trained personnel (43), consistent results, and safe storage conditions (44).

A promising era towards standardization of vitrification is the use of automation with minimal reliance on manual techniques. Initial results of automated systems for oocyte vitrification have shown promising results (45); however, more studies are needed until these systems can be used in daily practice.

Artificial Intelligence: A promising era for improving prediction accuracy and better treatment decisions may be the development of an artificial intelligence (AI)-driven selection process for advanced oocyte morphology assessment. An AI-based tool for oocyte morphology assessment can be especially useful for personalized fertility assessment and planning for fertility preservation as it may augment the precision of the prediction algorithm (46).

Ethical Dilemmas: The systematic trend of pushing family planning timelines to later stages in life raises concerns about how realistic these newly adapted perspectives of family life may be. Especially in light of well-established risks associated with pregnancy and advanced maternal age. Addressing these concerns is essential not only for the well-being of parents and children but also for a better understanding of the expected changes these new trends may bring into society (47). As scientific developments advance and reshape women’s reproductive choices in life the development of public awareness campaigns and appropriate support systems and healthcare strategies are essential.

Conclusions

Elective oocyte cryopreservation, within the context of advanced maternal age, presents both promises and challenges. Fertility preservation empowers women and allows them to achieve greater social, psychological, and financial stability before embracing motherhood. It is evident that elective oocyte cryopreservation aligns with the principles of social-gender equality and enhances women's reproductive autonomy. However, it is vital to dispel misconceptions related to fertility preservation, as it by no means can guarantee a live birth in any age group. The current body of research, particularly concerning the post-warming experiences of oocytes in women of advanced age, remains limited. Additional studies are essential to accurately estimate the expected prognosis for different age groups based on oocyte retrieval numbers and maternal age at the time of pick-up. Moreover, the rapidly evolving nature of this field mandates ongoing research and development for improved outcomes and accessibility, since despite existing controversies, planned oocyte cryopreservation remains a strategic response and the method of choice for age-related infertility.

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