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Editorial

Oocyte Cryopreservation: Who, how and what to Expect

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Summary

Oocyte cryopreservation is gaining widespread clinical acceptance. The main indications include: a) Fertility preservation in cancer patients or other fertility-impairing conditions; b) Patients at risk of premature ovarian failure; c) Oocytes banking for donation programs; d) Cases with no sperm available at the time of oocyte harvesting; and e) Cases where for religious reasons patients prefer to cryopreserve gametes instead of embryos. Oocytes cryopreservation has recently been proposed also for women that wish to postpone their reproductive plans at later age for career or social reasons. The utilization of oocyte preservation in this setting is appealing also from an ethical perspective, allowing maintenaince of reproductive autonomy and rights at later age, thus avoiding the stigma of childlessness or resorting to oocytes donation to fullfil the desire of motherhood. However, despite increasing reports about the safety (hundreds of documented births and reassuring data on obstetrical and neonatal safety), the 2009 practice committee opinion of the American Society for Reproductive Medicine (ASRM) still considers oocyte cryopreservation an experimental procedure requiring an investigational IRB-approved protocol. It is anticipated that soon the ASRM by reviewing the accumulating worldwide evidence of high survival and pregnancy rates, comparable to those obtained with fresh oocytes, will remove the label of experimental.

Introduction

Since the first birth through assisted reproduction in 1978 the number of couples seeking infertility treatment has increased exponentially. This is due in part to patient awareness and acceptability of fertility treatments, but also to increased success rate, wider availability, costs-coverage, and a general acceptance of ART as a safe method for conception. Oocytes are probably the most complex of the human cells; they are genetically determined in number, reaching a maximum or peak at 24 weeks of intrauterine life and then progressively decreasing to about 400,000 at birth. Throughout women's reproductive life, the number of the oocytes is progressively depleted and at the same time their quality deteriorate. Oocyte cryopreservation could be seen as one of the most innovative methods to safeguard against the age-related degenerative changes and to extend fertility opportunities [1]. That female aging is associated with reduced probabilities of pregnancy has been known since ancient times. In fact, Abraham and Sarah are probably the first known example of age-related infertility. When unable to conceive, Sarah asked her handmaiden Hagar to carry a child for them. Abraham had intercourse with Hagar and she subsequently gave birth to a boy, Ishmael, who she then gave to Sarah to raise.

Oocyte cryopreservation can stop the biological clock. The majority of studies aimed at perfecting this technology have been carried in Italy [2]. In response to legal rules banning embryo freezing, many Italian IVF centers had to learn, improve and offer oocyte cryopreservation [3]. Although most of the studies utilized the methodology of slow freezing, vitrification has been recently showed to have better survival and fertilization rates, with embryo quality and implantation rates comparable to those seen with fresh oocytes [4]. Oocyte cryopreservation has been applied in experimental trials in Italy before a very restrictive law regulating ART treatment took effect in March 2004 [3]. This law banned embryo cryopreservation and imposed the use of only 3 oocytes for insemination and the transfer of all the resulting embryos. This law forced the routine use of oocyte freezing for most of the Italian ART programs so to safeguard their patient's cumulative pregnancy rates. In the five years period the Law was applied, until May 2009, most of these restrictions were removed by the Italian Constitutional Court, a very large experience and improvement of oocyte freezing results were observed in several programs. In 2008 a single large ART unit reported [5], 1270 thawing cycles in 833 couples, using different slow freezing - rapid thawing protocols, showing the exponential growth in number of cycles with oocyte cryopreservation and thawing with an overall pregnancy rate of 12.3% per transfer (144 pregnancies).

The aim of this article is three fold: 1) To review who are the ideal candidates for oocyte cryopreservation; 2) To report on how to cryopreserve; and 3) To provide a reappraisal on what are the clinical results of this procedure.

Indications to oocytes cryopreservation - The "Who" of freezing

a) Patients at risk of premature ovarian failure which may result from: ovarian diseases such as cysts, benign tumors and recurrent or large endometriomas requiring ovary removal; and chemotherapy or radiotherapy to treat cancer or other systemic diseases. With the today improvements in cancer treatment protocols, more patients are experiencing a long-term survival. Chemotherapy and total body irradiation in preparation for bone marrow transplantation, are associated with significant gonadal toxicity. The great majority of cancer striking women of reproductive age are lymphomas (both Hodgkin and non Hodgkin), leukemia and breast cancer. Since it is not possible to assess the level of the gonadotoxic risk of chemotherapy with certainty, it is always recommended to consider fertility preservation options (oocyte or embryo freezing). Breast cancer is the most frequently diagnosed malignancy among women, with 25% of cases occurring

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prior to menopause and 7% diagnosed in women younger than 40. Over 90% of all breast cancers are diagnosed at local/regional stages, with a 98% 5-year survival rate for those with local disease and 84% with regional disease [6]. Breast tumor are hormone sensitive in about 60% of patients, therefore conventional gonadotrophin-stimulated IVF treatment have been modified to mitigate the rise of oestradiol levels (by using protocols with aromatase inhibitors). Short-term follow up on the use of these modified regimens in a small cohort of patients (including both oestrogen-receptor positive and negative tumours) found comparable disease-free and survival rates as compared with those not undergoing fertility preservation procedures [7]. Fertility preservation strategies have become paramount in the lives of reproductive-age women battling malignancy and represent an integral component in cancer management to improve their post-cancer quality- of-life. Embryo cryopreservation has been for a long time the only option offered but was underutilized due to many disadvantages. A sperm source is required; male gametes are often not available to young single women except through an anonymous donor. A number of ethical, religious and social issues have been associated with the creation and storage of embryos, especially in the face of a malignancy. For women that are not married or in a stable relationship, oocyte cryopreservation is the best alternative to preserve future fertility. Both embryo or oocyte cryopreservation require ovarian stimulation and retrieval, taking an average of 12 days [4,5].

b) In several other conditions such as autoimmune diseases, severe endometriosis, as well as many genetic or familiar conditions, oocyte preservation should be considered in a multidisciplinary approch to conditions predisposing to ovarian failure.

c) Patients requesting infertility treatment with oocyte donation can also benefit from oocyte cryopreservation. In fact, the current use of fresh oocyte donations is challenged with difficulties of synchronization, long waiting periods and lack of quarantine measures, as for sperm donations. Oocyte donation through egg cryobanking could provide the possibility of distributing oocytes among two or more recipients, without facing difficulties of endometrial synchronization among multiple recipients and by so doing the whole treatment can become more economical and affordable.

d) When sperm is not available at the time of oocyte retrieval every ART structure should be able to perform oocyte preservation to afford this infrequent, but not rare condition.

e) In addition, oocyte cryopreservation has been seen as a successful alternative for storing the excess of oocytes during the ART therapies, thus avoiding ethical, moral and religious dilemmas and reducing the number of embryos stored for future use. Approaching ART treatment for many couples is a conflict between their religious or personal beliefs and the desire of creating a family. Storing oocytes is an option that every ART [8] center has to offer.

f) Egg freezing, especially if proven safe and successful, is clearly the next step in the intersection of female reproduction, aging, and the labor market. Even if major regulatory bodies in Europe and the United States believe oocyte cryopreservation to be a still an experimental technology, others like Israel views it as a standard medical procedure [9]. Taken that fertility is age dependent, one could consider freezing oocytes as an act of preventive medicine, allowing women to avoid egg donation and the burden of ineffective fertility treatments at older ages. In addition, oocyte freezing provides additional benefits to women, as it allows them to maintain their reproductive autonomy while increasing their chances of genetic motherhood. Several studies from around the world [10,11] have shown that young people (men and women alike) lack knowledge about the natural limits of human fertility, and display an optimistic bias. In addition, a recent survey on the attitudes towards nonmedical egg freezing in Belgium showed that a third of the respondents (women aged 21- 40 years) considered themselves potential users of this new technology for nonmedical uses [12]. It is also important to mention that while there is a great belief in new reproductive technologies, as professionals we must also inform patients that "fertility insurance" gained by egg freezing is not a warranty of future successful reproduction. It is therefore suggested that policy makers and worldwide strive for better education in this area [9].

a) fertility preservation in cancer patients or other fertility-impairing conditions;
b) patients at risk of premature ovarian failure (genetic reasons as Turner mosaic, Fragile X, balanced translocations, mosaicisms, etc,) or family history;
c) oocytes banking for donation programs;
d) cases with no sperm available at the time of oocyte harvesting;
e) cases where for religious reasons patients prefer to cryopreserve gametes instead of embryos; oocyte banking in countries where there is forbiddance for embryo cryopreservation;
f) oocyte banking for women that desire to postpone motherhood (social freezing);

Table 1: Indications to oocytes cryopreservation

Indication to oocyte cryopreservation are summarized in table 1.

The "How" of freezing (The difficulties, the slow freezing and vitrification)

Only recently the technique of oocyte cryopreservation has been showing remarkable effectiveness. This complex, large, human cell, mostly comprised of water and thus highly susceptible to low temperature damages, has required many years of experimenting to finally prove that once frozen and thawed can produce consistently live births [13]. The first report of a pregnancy from a frozen oocyte was described by Chen in 1986 [14]. A few other births were achieved shortly afterwards, but for many years the reports remained sporadic. Many technical problems had to be solved. Gook [15] suggested for the first time that Intracytoplasmic Sperm Injection (ICSI) could improve fertilization rates in frozen oocytes caused by premature cortical granule release and zona pellucida hardening. However, in spite of several live births [16] with ICSI, there were other issues that affected the efficiency of the procedure. These were centered around the various protocols of slow freezing methodology. For many years, centers have experimented different concentrations of cryoprotectants at the time of freezing and thawing in order to improve the oocyte survival rates. For oocyte cryopreservation with slow-freezing method, cryoprotectant solution usually consisted of 1.5 M membrane-permeating cryoprotectant (i.e. propanediol) and 0.1 M - 0.3 M sucrose. It has been reported [17] that an increase in sucrose concentrations could benefit the survival of frozen-thawed human oocytes and some reports had shown improvements in success when compared to those from the past two decades [18]. However the success rates remained sub-optimal, with highly variable fertilization rates. An overview of the literature shows that most studies use a similar freezing and thawing procedure (slow-freezing rapid-thawing), similar seeding points and cryoprotectants (1,2 - propanediol and sucrose). The history of cryopreservation has been recently and elegantly reviewed by Gosden [19], who praised the alternative methodology of vitrification (or rapid freezing) based on the premise of completely avoiding ice formation, as well as its simplicity, rapidity, and economy. Impressive clinical success rates at the Kato Ladies Clinic and other centers in the past decade have encouraged clinics around the globe to switch to vitrification for both oocytes and embryos [20]. In essence, vitrification involves equilibration of the specimen in a

cocktail of CPAs followed by plunging into liquid nitrogen. It normally requires molar concentrations threefold or fourfold higher than for slow freezing, and a very rapid rate of cooling below the glass Transition Temperature (Tg). Rewarming must also be ultrarapid to avoid ice nucleation. The major drawbacks of the technology are toxicity from the high solute concentrations - biochemical and osmotic - for which there are various strategies for mitigation. The CPA loading and unloading are performed stepwise and at 0-4°C, and by combining two or more CPAs to achieve the desired molar strength of 5.0 - 6.0 mol.L, their individual toxicities are proportionately reduced. However, not all CPAs are equally good glass-formers, and unfortunately the best tend to be more toxic. Vitrification cocktails have proliferated, some based on painstaking research and others on more homespun formulas. There is, however, no disagreement about the importance of cooling at the fastest possible rate to guarantee vitrification and, hopefully, avoid problems from chilling and in the cytoskeleton. A physicochemical trade-off exists between cooling rates and solute concentrations needed for vitrification, which can be fine tuned to reduce toxicity. Because cooling rates vary inversely with the mass of the specimen, a number of devices have been invented for vitrifying embryos and oocytes, some of which support cells in a mere film of moisture (< 0.1 mL). Most devices expose cells directly to liquid nitrogen (so-called open systems), but regulatory authorities like the U.S. Food and Drug Administration (FDA) demand greater safety using seals and jackets to maintain sterility, although greater insulation slows the rate of cooling.

Many approaches have been tried to join the benefits of the vitrification process with the lower CPA concentration used in slow freezing with interesting experimental results [21]. A recent meta-analysis [22], comparing slow freezing, vitrification and fresh oocytes, suggests that oocytes coming from vitrification/ warming cycles could result in better survival and fertilization rates than those coming from SF/thawing cycles. However, several important limitations should be considered with respect to this meta-analysis: a) only five studies have been included in this review, all of them presented an evident clinical heterogeneity regarding the inclusion criteria and basal characteristics of the samples b) The external validity of the study might have been limited to good responders because all the included patients had at least six MII oocytes after controlled ovarian stimulation, or they were oocyte donors. c) Statistical heterogeneity between studies was observed for some of the measures studied, especially for the oocyte survival rate. This could be related by the two different methods of vitrification used in the trials that reported this outcome. Open devices improve oocyte survival when compared with the closed ones. In four of the five studies randomization was not used to allocate embryos derived from cryopreserved oocytes to the recipients. This could introduce a selection bias in the studies that considered clinical variables, such as pregnancy and implantation rates. Prospectively randomized trials comparing the two methods are sparse and properly designed experimental trials are needed to definitively show an advantage of a specific method. While vitrification is the 'new' technique, this does not mean that slow freezing should be abandoned, at least for now. According to the considerations published in a recent review by Boldt in 2011, imagine a clinic with two embryologists that have four egg retrieval cycles on a given day. In two of the cycles, oocyte cryopreservation is to be performed and between the two patients there are 50 oocytes to be preserved. Given such a scenario, and the workload associated with vitrification of that many oocytes together with the other cases and associated workload (clearing of eggs for intracytoplasmic sperm injection, micromanipulation, etc.), slow freezing might be a more appropriate alternative, in that the bulk of the time spent for the freezing process is spent within the confines of a slow-freezing unit, rather than at the bench performing vitrification have advantages with respect to subsequent embryo [23].

The "What" of freezing (A reappraisal of the clinical results)

Clinical results of oocyte vitrification from a recent randomized study for recipients of oocyte doantion, showed that Ongoing Pregnancy Rates (OPR) were comparable to those with fresh oocytes [24]. These results were confirmed by another recent publication [25] comparing the clinical outcomes between two centers (Spain and USA) . In one center all the vitrified oocytes from one donor were warmed and allocated to a single recipient. In the second center (USA), oocytes from one donor were divided among several recipients (typically two-three recipients obtained oocytes from one donor, limiting the number of warmed oocytes to an average of six per recipient). Despite the lower number of oocytes per recipient in this programme, pregnancy rates were not different than those obtained with fresh oocyte donation.

In a multicenter trial involving 940 thawing, 8 Italian centers compared fresh and thawing results, using the same slow-freezing protocol. Data from the 8 partecipating centers is reported, showing how different mean age of the patients, number of oocytes retrieved, fertilization and pregnancy rate in the fresh cycle reflects the post thawing results [26]. Rienzi reported in 2010 a prospective randomized sibling oocyte study in infertile patients, analyzing the embryo development after injection of only 3 fresh versus warmed oocytes [27]. No differences were found between the two groups in terms of fertilization rate and embryo development. 124 patients were enrolled, 54 (43.2%) obtained a clinical pregnancy, an implantation rate 21.7% (69/318) was reported with an 11.1% early abortion rate and an ongoing implantation rate of 19.2%. Finally, it is extremely reassuring that the initial follow-up of over 1000 babies born form oocyte freezing has failed to detect an increase in the rate of congenital malformations or genetic conditions [28-30].

Discussion

The development of efficient methods of oocyte cryopreservation has been the latest major breakthrough in human IVF. Egg storage has the potential not only to circumvent several ethical, legal and storage problems associated to embryo freezing, as well as preserve female fertility in patients at risk of premature ovarian failure, or in women who are forced to postpone their motherhood for social or economical reasons. Despite the fact that with some protocols survival rates are reduced, fertilization rates, cleavage rates are not necessarily compromised, approaching the value normally achieved with embryos obtained from fresh oocytes. Although the largest experiences have been achieved with the application of slow freezing protocols, vitrification is emerging as a technique that could overcome slow freezing not only for oocytes freezing, but even for embryo and ovarian tissue [31]. Safety is also inferred by the evidence that comparable aneuploidy frequencies were observed in embryos obtained from fresh or frozen oocytes (28% and 26%, respectively), by performing a FISH analysis and employing specific probes for chromosomes 13,18,21, X and Y [32]. The establishment of oocyte banks could improve the safety of fertility treatments for women using oocyte donors by allowing improved screening of donors for potential transmittable diseases. Finally, patients who find gamete cryopreservation more acceptable than embryo cryopreservation could cryopreserve their oocytes, thus reducing the number of supernumerary embryos.

Today oocyte cryopreservation is still considered an experimental technique by the major regulatory bodies in Europe and the United

States [33-35]. A new Israeli policy views it as a standard medical procedure, highlighting its reported success [36]. Further, taken that fertility is age dependent, freezing eggs in advance could be an act of preventive medicine, allowing women to avoid egg donation and the burden of ineffective fertility treatments at older ages. Consequently, egg freezing is perceived beneficial to women, as it allows them to practice their reproductive autonomy while increasing their chances of genetic motherhood. Therefore, although other countries clearly differentiate between medical and nonmedical egg freezing, supporting the first, yet hesitant toward the second, Israel recent Governamental guidelines does not support such a clear distinction [10]. Various studies from around the world have shown that young people (men and women alike) lack knowledge about the natural limits of human fertility, and display an optimistic bias. In addition, a recent survey on the attitudes towards nonmedical egg freezing in Belgium shows that a third of the respondents (women aged 21- 40 years) consider themselves potential users of this new technology for nonmedical uses [37]. It is therefore suggested that policy makers worldwide strive for better education in this area, which would in part counter this problematic tendency, while considering oocyte freezing as a potential primary prevention for reproductive ageing [38].

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