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FERTILITY PRESERVATION

Oocyte cryopreservation: is it time to remove its *experimental* label?

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Abstract As more reproductive-age women survive cancer at the expense of gonadotoxic therapy, the need for viable fertility preservation options has become paramount. Embryo cryopreservation, often using donor sperm, has been the standard offered these women over the past 20 years. Preservation of unfertilized oocytes now represents an acceptable and often equally viable alternative, particularly for single women, due to technologic advances made in the past decade. Given such, oocyte cryopreservation's *experimental* designation and need for IRB approval should thus be revisited.

Keywords Oocyte cryopreservation · Fertility preservation · Cancer · Survival · Experimental

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Capsule As more reproductive-age women survive cancer, the need for fertility preservation is paramount. Oocyte cryopreservation success is increasing; thus, its *experimental* designation should be revisited.

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Introduction

Historically, human oocyte cryopreservation (OC) began in the 1980s with isolated and sporadic reports of successful pregnancies [1, 2]. Early concerns regarding damage to the meiotic spindle [3], loss of cortical granules leading to lowered fertilization rates [4, 5] and the low success rates of oocyte freezing/thawing as compared to the relative success of embryo cryopreservation caused a wavering of interest until the 1990s. Then, a series of studies indicating that reasonable oocyte thaw survival [6], fertilization [7, 8], embryos with normal karyotype [8–10], and viable blastocyst development [11] could be achieved led to renewed interest in OC technology. Reports demonstrating live births following application of OC appeared soon after [12, 13].

In the intervening years, variations to OC methods including changes in sucrose [14–16] and sodium [17, 18] concentrations in slow freezing media, along with the first report of successful oocyte vitrification [19] and the development of novel cryotools [20–23] have combined to provide consistently improved survival and pregnancy rates with OC. In addition, although depolymerization of the oocyte's meiotic spindle is known to occur, a number of studies have now shown that the spindle reforms in the majority of oocytes after thawing [24–29]. The advent of ICSI for insemination has overcome any concern regarding potential premature cortical granule reaction [11]. Work from several Italian groups (where oocyte cryopreservation has often been used because of legal restrictions on embryo cryopreservation) has been instrumental in establishing OC as a clinically valid procedure [30, 31]. Importantly, the most recent and largest survey of OC outcomes indicates that there have now been almost 60 case and series reports in the literature demonstrating that OC is a viable reproductive technology [32]. As more successful outcome

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data is reported, the technology's *experimental* label and the need for it to be performed under the auspices of an IRB should be reconsidered. We argue that OC could potentially now be deemed a standard ART procedure offered to appropriate patients after comprehensive informed consent once individual IVF clinics have established their own efficacy in the procedure.

Oocyte cryopreservation is not *experimental*

In a recent ASRM publication (June 2008), the Society defined an "experimental" procedure, indicating that one should be designated as such until "there is adequate scientific evidence of safety and efficacy from appropriately designed peer-reviewed published studies by different investigator groups". Three comparative studies regarding the efficacy of OC have now been published and several more are ongoing or in submission.

Grifo and Noyes [33] studied 23 OC cycles using either slow freezing or vitrification, and compared results after thawing/warming to age-matched controls. In the frozen/thawed group, survival, fertilization, blastocyst formation and pregnancy rates were no different than those obtained in the fresh group, indicating that frozen oocytes performed as well as fresh oocytes. These data have recently been updated to include 32 OC thaw cycles (mean age 31±1 y) resulting in 18 (56%) ongoing or delivered gestations [29].

Cobo et al. [34] performed a study of oocyte donors in which oocytes from a given donor were either inseminated fresh, or were frozen by vitrification for a minimum of 1 h and then thawed, inseminated, and cultured along with the fresh oocytes from that same donor cycle. They found no differences in fertilization or cleavage rates, or in the assigned embryo quality scores of the fresh and vitrified oocyte groups. In 23 cycles, embryos from frozen/thawed oocytes were transferred resulting in a 65.2% pregnancy, 40.8% implantation and a 47.8% ongoing pregnancy rate, similar to what was obtained with fresh oocytes.

Nagy et al. [35] performed a study in which oocytes from ten donors were cryopreserved by vitrification and subsequently thawed for transfer to a total of 20 recipients. They achieved an 87.5% survival, 87% fertilization and 68% blastocyst formation rate. Fifteen of the 20 recipients became pregnant after embryo transfer, with 26/47 (55%) transferred embryos implanting. These pregnancies resulted in delivery of 26 live born infants. Another two pregnancies were established from supernumerary embryos that had been created during the frozen egg cycle, then subsequently vitrified and thawed. Importantly, pregnancies from twice-frozen gametes (oocytes, then the resultant embryos) were established. When vitrified oocyte success rates were compared to those of fresh oocyte cycles using the same oocyte donors, all outcome parameters were similar, with some being more favorable in the frozen oocyte cycles.

Without central outcome registries, the concern regarding the relative safety of OC is being addressed by studies examining the obstetrical outcome of pregnancies from frozen oocytes. Chian et al. [36] reported on 165 vitrified oocyte pregnancies totaling 200 infants born, and showed no difference in mean birth weight or incidence of congenital anomalies in children born from frozen oocytes vs. those from either spontaneous conception in fertile women, or in women conceiving through fresh IVF. Noyes et al. [32] tabulated data from multiple centers around the world using either OC cryopreservation method and found that in 936 babies born from frozen oocytes, there was no apparent increase in the rate of congenital anomalies as compared to United States national statistics for natural conceptions as reported by the CDC. Continued worldwide reporting of healthy live born children from OC will reinforce this trend.

Regarding the introduction of new procedures in ART, historically there has never been a requirement for a newer technology to be as effective as an older one to be initiated. One could argue that a less effective procedure (as defined by a lower pregnancy rate) might be appropriate if the newer technology is either safer or represents the only acceptable alternative for a unique medical situation. An example where the use of frozen/thawed oocytes could potentially add safety is in donor oocyte treatment where oocytes could now be quarantined for the purpose of infectious disease risk (as is currently the standard when using anonymous donor sperm). A unique clinical situation where the use of frozen oocytes would be more appropriate than frozen embryos is that of a newly diagnosed female cancer patient who is not in a male-female relationship; in this instance, fertility preservation through OC may be her only suitable alternative. A condition that might necessitate using a less proven or even riskier technology is that of ICSI for male-factor infertility. Although standard IVF with inseminated sperm (considered less invasive than ICSI) could be argued as being safer than ICSI, if only testicular sperm is available, ICSI may be the more appropriate fertilization option.

The introduction of ICSI as a novel reproductive technology in the early 1990s has similarities to that of OC today. In the case of ICSI, with appropriate informed consent, this micromanipulation technique was embraced almost immediately and soon thereafter, was heralded as one of the greatest advances in our field, affording men with severe oligoazospermia (who would otherwise have required the use of donor sperm) the ability to produce genetically-linked offspring. Likewise, today, 19 years later, single women desiring biologically-connected offspring who are not in the position to do so at present, are in the

same situation; desiring the avoidance of donor gamete to achieve their reproductive goals. OC can potentially fulfill this desire and the acceptance of such is increasing.

Oocyte vs. embryo cryopreservation

Currently, embryo cryopreservation is the sole *approved* technology for female patients wanting to preserve genetic material for future use. This is due to the fact that embryo freezing is considered a standard procedure, whereas OC is not. When one reviews the data on these two techniques, however, it is uncertain whether oocyte freezing offers any less chance for pregnancy than embryo freezing in clinics with established OC success. Relative to fertility preservation measures, it would be difficult to design a study that directly compares OC to embryo cryopreservation, because in many centers embryo freezing is either banned by law, or patients request OC precisely because they are uncomfortable ethically with embryo freezing. One can glean useful comparisons, however, through results based on nationalized data banks and/or in published studies. In 2007, SART reported an overall delivery rate per embryo transferred as 30.6% for autologous and 31.7% for donor oocytes in frozen/thawed embryos cycles (SART.org, 2007). Results from series of frozen oocytes (reviewed in ref [32]) compare favorably to these data, especially when one takes into account that in many oocyte thaw studies (those performed by the Italian groups) the number of oocytes that could be thawed and inseminated was limited to three, due to federal statute. If one looks at studies using frozen donor oocytes, the data are even more favorable, with ongoing pregnancy and delivery rates exceeding 50% in some reports [29, 33–35]. Thus, although the source of oocytes in the latter studies is from women age <39 years, existing age-appropriate data suggests that there may be no benefit in offering embryo cryopreservation vs. OC, at least from a subsequent thaw success perspective.

Oocyte freezing clearly has one obvious advantage over embryo freezing; in the case of a single woman, oocyte freezing offers future "social choice" where embryos created using donor sperm limit such. Take for example a young, single woman requiring chemotherapy or surgery that will render her sterile. Using current standards, such a patient would be counseled that the only *non-experimental* option to preserve her fertility would be to fertilize her oocytes with donor sperm prior to freezing. This would be in the setting of her new diagnosis including all its tests, treatments and concerns including survival. Certainly, when evaluating the riskiest ART procedures practiced today, multi-embryo transfer resulting in multiple birth (with its associated prematurity sequalae) tops the list. Yet, this practice is not considered *experimental* and IRB approval for its use is not necessary. In addition, IRB approval is not required to perform IVF in women >42 years, a technology associated with significantly lower success rates and the highest risk for aneuploid outcomes. These cycles are instead performed following standard and comprehensive informed consent. We argue OC should be treated likewise.

Focusing back to the single cancer patient that would additionally be requested to designate the disposition of her created embryos given she doesn't survive. And if surviving and subsequently engaging in a male-female relationship, donor sperm embryos would be her only chance for a biologic offspring. OC might be offered, but with the caveat that it is *experimental* and requires special approval by an ethics review board, possibly influencing an already stressed and vulnerable patient to use donor sperm, because it represents the only *acceptable* means to preserve her fertility. In addition, religious and other personal beliefs may contribute to an unwillingness to create embryos, particularly if they may never be used. Recently, Porcu reported a successful case of fertility preservation through OC where twins were born to an oophorectomized ovarian cancer survivor [37].

Another scenario where OC becomes useful relates to supernumerary embryos created during standard IVF. Since the late 1970s, more than 400,000 embryos have been stored in the USA [38] and it is estimated <50% of the embryos currently cryopreserved will ever be thawed for use. In most centers, current standard IVF practice dictates that a patient undergoing IVF will have all retrieved oocytes inseminated (or injected) with sperm, the best embryo(s) will be transferred back to the uterus and any remaining viable embryos will be cryopreserved for later use. For example, a 28 year-old tubal factor patient might have 25 oocytes retrieved in her fresh IVF attempt. Of these, about 20 will fertilize and she would most likely have 1–2 embryos transferred and 8–12 cryopreserved. With her chance of pregnancy >50% using the fresh embryos, there is a likely scenario that none or only a few of the frozen supernumerary embryos would ever be thawed for use. A second example is a 47 year-old woman undergoing an IVF treatment using donor oocytes. Thirty oocytes are retrieved from the donor, 1–2 embryos are transferred to the recipient and the remaining 15–20 viable embryos are frozen. The odds that any or very few of those frozen embryos will ever be used is low. Knopman et al. recently published NYU results (n=444 cycles) showing that only 21% of recipients with supernumerary cryopreserved donor-oocyte embryos returned for transfer if succeeding with their fresh attempt [39].

Both of these situations leave the patient (and partner, if there is one) in the uncomfortable position of deciding the disposition of their extra embryos. While choices such as embryo donation for either research or adoption are

possible, these are difficult decisions for patients. We propose a hybrid approach to freezing where the patient may elect to have, for example, half of her oocytes placed with sperm and the other half frozen unfertilized; this would certainly help alleviate the issue of "excess" frozen embryos, one becoming more prevalent as efficiencies in assisted reproduction continue to increase.

Some have suggested that OC should not be offered because of its lack of efficiency based on comparing the "per-oocyte-frozen" pregnancy rate (i.e. the actual number of embryos that implant and create pregnancy per number of oocytes frozen) [40]. Viewing success in this way is unique to OC. Currently available data suggests that the efficiency of fresh IVF, when calculated as live born infant per oocyte is about 5–6% overall [40, 41], whereas some studies with frozen oocytes report similar per oocyte efficiencies [15, 35]. Efficiency defined in this manner is dependent on a number of variables such as patient age, quality of stimulation, whether there are restrictions on the number of oocytes that can be used, efficiency of embryo transfer, and the like. Given these variables, it should not be surprising that efficiency of IVF and OC varies from program to program and study to study. Similar arguments can be made for FET. Indeed, it is difficult to directly compare national data on efficiency from any given ART procedure on a per-oocyte basis; neither the CDC nor SART databases currently calculate pregnancy rates for either fresh embryo transfer or FET based on the number of oocytes collected or inseminated (injected) per cycle. If efficiency per oocyte thawed is to be used as criteria to judge the adequacy of OC as a clinical method, then it stands to reason that all ART procedures should be held to the same standard.

Governmental regulation

Some have expressed concern that should OC no longer be considered *experimental*, regulative bodies such as the FDA may require that, for donor oocyte cycles, all oocytes be cryopreserved and quarantined prior to use, as is done with donor sperm. The rationale for such an effort would be to decrease the potential risk of infectious disease transmission to the donor egg recipient, and while this is a theoretic possibility, the fact remains that there has never been a recorded incident of infectious disease transmission to an embryo recipient from an affected embryo. Whether mandated quarantine is an inevitable consequence should not affect the decision to label OC *experimental*; that decision should be made independently based on available scientific evidence as to the suitability and safety of the technique and not out of fear of regulation. We make the point, however, that if the FDA wanted to make all donor oocyte cycles frozen cycles, they could do so immediately without concern for whether oocytes should be frozen or not. The FDA could mandate that, for donor oocyte cycles, no fresh embryos be transferred but rather all resultant embryos created after sperm insemination (or injection) be frozen and quarantined until the oocyte donor is rescreened for infectious disease. The fact that the FDA has not done so would suggest that the applicability of OC may not result in a change in federal guidelines. One could argue that semen vs. oocyte quarantine is more important as the former usually contain millions of cells and associated body fluids and has a greater potential for infectivity than do single oocytes.

Exploitation

Concerns have been expressed that women could be exploited by widespread use of OC. For instance, clinics not proficient in the method could provide false hope to patients wishing to achieve fertility preservation through this option. Because there is generally a significant lag between gamete freezing and usage, the technology currently lacks real-time accountability on the part of the provider. While this is a legitimate cause for concern, similar statements could be made about virtually any other ART method, including frozen embryos storage, in that some clinics are better at freezing embryos than others. Despite such, we do not label embryo freezing *experimental*, subject to IRB approval. What about prenatal genetic diagnosis (PGD) and/or screening (PGS)? Many clinics offered PGS before data became available showing that routine use of aneuploidy screening may not provide any benefit, yet this technique was not deemed *experimental* requiring IRB approval. Embryo vitrification, use of lasers for assisted hatching, agonist triggering of ovulation, and the use of sequential embryo culture media, are all fairly routine procedures that escaped *experimental* labeling or IRB approval prior to use. Instead, such techniques are used in conjunction with appropriately worded informed consent statements. The truth is that most results from IVF vary from clinic to clinic, because of factors such as patient specifics, laboratory methodology, clinical expertise, etc.

The demand for use of IRB approval for a new procedure that is not part of a research project is questionable. If legitimate concerns exist about the reliability of any method in the hands of a given clinic, steps can easily be taken to address these issues. Clinics can be audited relative to specific OC outcomes, just as they currently are for general IVF procedures. Additionally, they can be audited relative to the patient consenting process. Regardless of IRB approval, clinics should be directed to offer patients reliable data regarding their own oocyte cryopreservation

experience and outcomes. To facilitate OC advancement, larger ART societies are now in a position to take a leading role by working with clinics to provide central OC registries that can be accessed both by professionals and, more importantly, patients. Such initiatives have been started by industry [42, 43]. The authors feel compiling such data, even if lagging by a year or two, will be invaluable to the advancement of OC technology as a whole.

Summary

OC has made rapid progress in the past decade, most significantly in the past 3 years, with more and more clinics around the world able to achieve OC outcomes rivaling standard ART procedures such as fresh IVF and FET. With fertility preservation now being embraced more routinely by treating oncologists and OC being the best currently available fertility preservation option for young single women diagnosed with a malignancy, we as a profession should strive to advance this field as rapidly as possible. Accordingly, we, like others [41], argue that OC should no longer be considered *experimental*, and are encouraged that the most recent ASRM Practice Committee statement acknowledges that OC offers "great promise for applications in oocyte donation and fertility preservation" [44]. The authors and acknowledgers feel that OC should be treated as any other reproductive technology in that it should be used judiciously and when offered to patients, should be done so with thorough informed consent including an explanation of the methods, clinic outcome, risks and benefits as well as alternative options available. As the demand for OC continues to grow, clinic-specific OC data should be reported to central registries to alert patients and treating physicians as to progress being made as well as provide unbiased, up-to-date outcome data on which to base medical decisions.

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