Cryopreserved Oocyte Banking: Its Prospects and Promise

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11.1 Introduction

It is difficult to imagine in vitro fertilization (IVF) and assisted reproductive technologies (ART) without cryopreservation. The science and craft of freezing cells and tissues with preservation and resumption of their biological functions after thawing results from the research of a host of investigators. Much is owed to their contributions in defining cryopreservative and warming solution formulations and description of cell- and tissue-specific methodologies [1].

Both patients and practitioners of ART have been unique beneficiaries of the ability to cryopreserve reproductive cells. The use of frozen sperm was broached as early as 1950 [2] and ART with both frozen autologous and donor sperm is a long-standing treatment option for infertility. Cryopreservation of zygotes, early cleavage stage embryos, and blastocysts is integral to allowing patients to maximize and optimize a single cycle of ovulation induction for ART.

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The cryopreservation of human eggs, in contrast, has been elusive [3], but significant strides in technique have been made, yielding the desired characteristics of consistently high rates of post-thaw survival, fertilization, embryo development, and implantation. It is a testament to this achievement that the potential for cryopreserved egg banking is addressed in this biennial review. The recent withdrawal of the qualifier, "experimental," from oocyte freezing by the American Society of Reproductive Medicine [4] may hasten the rapid acquisition of this technology by more ART laboratories and augment the range of reproductive options by both fertile and subfertile women and those using third-party reproductive strategies for family building.

11.2 The Unique Challenges of Egg Cryopreservation

Success of oocyte freezing, i.e., implantation and pregnancy, was reported early in the history of ART [5]—only 3 years after the report of the first successful embryo thaw [6]—inspiring the hope that oocytes would lend themselves to the prevailing slow-cooling methods for cryopreservation for cleavage stage embryos. The advantages of being able to freeze the full range of reproductive cells, i.e., *both* types of gametes as well as embryos, were enormous. While very encouraging results followed [7–14], oocyte freezing proved challenging and was not integrated into routine practice at the same trajectory as cleavage-stage

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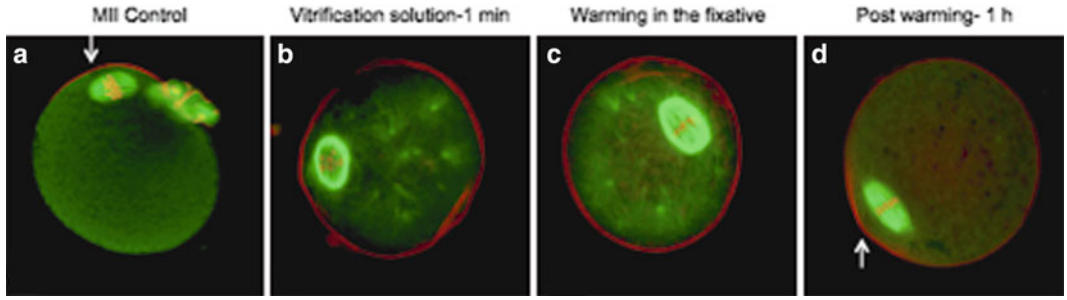

Fig. 11.1 Influence of the oocyte vitrification on cytoskeleton structures of mouse oocytes. Confocal images of microtubules (*Green*), microfilaments (*Red*) with chromatin (*Red*), and merge of representative oocytes before vitrification (**a**), treated with vitrification solution (containing 15 % DMSO and 15 % ethylene glycol, and 0.5 M sucrose) for 1 min at RT (**b**), warmed the vitrified oocyte directly into the fixative (**c**), and an oocyte was cultured for 1 h after warming (**d**). After oocytes warmed, it displays that stabilized MII spindle with chromosomes and the adjacent microfilament-rich domains (*arrow*) resembling to oocytes prior to the vitrification process

embryos and later blastocysts. The most prevailing challenge was at the level of survival, requiring up to 100 oocytes for a single successful pregnancy.

Some unique factors must be surmounted in freezing mature (Metaphase II) oocytes. Human oocytes (a) are large cells presenting the challenge of high intracellular water volume; (b) have a complex intracellular architecture comprised of cortical granules, organelles, and microtubules that must be protected [15–18]; and (c) are arrested in meiosis thereby requiring special care to avoid disruption of the spindle and its chromosomes (Fig. 11.1) [7, 8, 19, 20]. In addition, the membrane properties of an oocyte are significantly different than the similarly sized zygote, possibly attributable in part to aquaporin, a protein channel that can provide transport of water and other solutes through the oolemma.

These translated to the technical hurdles of adequate dehydration, protection from cryopreservative toxicity, and conservation of cellular integrity at warming. Postthaw survival would be measured not only in recovery of an intact, hydrated cell but also an egg that could be fertilized, resuming meiosis without risk of aneuploidy from a disrupted spindle, and capable of normal developmental progression.

Egg cryopreservation required the confluence of two techniques to realize its clinical application: vitrification and ICSI. The adoption of vitrification into ART—brief exposure to high cryoprotectant concentration with the use of "open" cryopreservation carriers that allowed maximal cooling rates—was catalytic to the rapid development of egg cryopreservation methods [21, 22]. Careful formulation of equilibration and vitrification media was coupled with determination of optimal equilibration times to avoid the toxicity from exposure to high concentration of cryoprotectants. Open systems, such as OPS (open pulled straw), CryoTops, CryoLocks, CryoLeafs, CryoLoops, and others, as well as several closed carriers, in conjunction with these carefully designed techniques for warming, yielded the desired high rates of egg recovery and survival [23–26].

To counter any changes in the physical characteristics of the zona pellucida that might impede sperm binding and/or penetration, intracytoplasmic sperm injection (ICSI) has generally been accepted as the optimal approach to insemination [27, 28] although some studies reported normal fertilization of frozen-thawed eggs with conventional insemination [8, 9]. While minimizing the risk of fertilization failure, ICSI also allows close appraisal of the postthaw oocyte as appearance of the ooplasm, membrane resistance and dynamics of the sperm injection can be reliable markers or predictors of oocyte quality [9, 29].

11.3 The Clinical Utility of Cryopreserved Egg Banking

The application of oocyte cryopreservation can fulfill several therapeutic purposes. Two of the most anticipated are autologous fertility preservation and the development of donor oocyte banks [30, 31].

11.3.1 Autologous Oocyte Banking

To forestall the inevitability of declining ovarian reserve and oocyte quality with age, women can elect to undergo one or more cycles of ovulation induction with freezing of the oocytes for later use [32]. Oocyte freezing may thus relieve the pressure of the inexorable advance of the biological clock and ameliorate the disappointment of women in their waning reproductive years who undergo IVF with reduced odds of pregnancy [33].

Fertility preservation may take a more pressing form, as when young women confront loss of ovarian function from cancer treatment. A chance for reproductive potential is preserved through oocyte freezing if ovulation induction and retrieval are not counter-indicated [34].

In advance of the hormonal and surgical interventions for gender reassignment, women can freeze their oocytes, preserving the opportunity reproduction with their genetic material.

With the admission of military women to combat roles, oocyte freezing may provide some insurance against fertility loss from grievous wounds.

For patients who wrestle with the implications of creating more embryos than needed for embryo transfer and cycle completion and the thorny issues of their disposition if these embryos are not required or desired for future transfers, oocyte cryopreservation allows allocation of some oocytes to be used for insemination and others to be stored [35]. In alleviating some of the ethical concerns of cryopreserved embryos, oocyte freezing and banking may be a welcomed adjunct to IVF. Somewhat unexpectedly, the option of cryopreserving "extra" eggs (not used for insemination) and avoidance of excess embryos is currently one of the most frequent applications of oocyte cryopreservation.

11.3.2 Donor Oocyte Banking

One of the many dividends of ART has been the opportunity for individuals to reproduce using donor oocytes, widening the reproductive horizon for women whose fertility was imperiled by diminished ovarian function or loss. IVF with donor oocytes became a well-established treatment but was offered primarily with "fresh" oocytes until recently. While this was a practical treatment model, there were some disadvantages.

Cycle synchronization between oocyte donor and oocyte recipient had to be achieved. Because the schedules of two individuals (donor and recipient) required accommodation, convenience to the recipient was not a hallmark of this approach. Compared to sperm banks, the array of desired characteristics and ethnicities was limited to the donors provided by agencies specializing in their recruitment or individual IVF centers who developed their own donor catalogues. In addition, the safety of fresh oocyte donation, despite rigorous donor screening and testing, may not be at the same level as cryopreserved donor oocytes, in which retesting of donors for infectious agents after 6 months is an option, completely analogous to the standard for sperm donors.

While the clinical efficacy of fresh oocyte donation in yielding pregnancies and live births is evident as reflected in the outcomes published annually by the Society of Assisted Reproductive Technology (SART) and the Centers for Disease Control and Prevention (CDC), some potential patients may be daunted and discouraged by the need for cycle synchronization between recipient and donor that may result in treatment delay, the lack of an appropriate oocyte donor, lapses in donor compliance that may lead to cycle cancellation, and a prolongation of disappointment and frustration.

Donor oocyte banks can provide (a) wide selection of donors with desired phenotypic characteristics from a catalogue; (b) the availability of a relatively rare donor, e.g., of mixed ancestry; and (c) the convenience of commencing IVF treatment once the donor oocytes are selected and obtained by the clinic. In addition, IVF clinics would be relieved of the considerable financial and administrative burdens of recruiting, screening, and maintaining their own donors and may be able to increase the number of donor oocyte cycles using donor oocyte banks.

Access, variety, immediate availability, and comparable pregnancy outcomes to fresh egg donation (Table 11.1): these are all features that would render donor oocyte banks the same successful enterprise that sperm banks have proven

Table 11.1 The IVF treatment outcome of using vitrified donor oocytes for recipients (vitrified donor oocytes provided by My Egg Bank North America and recipients treated at Reproductive Biology Associates, Atlanta, GA)

Outcome	
Donation cycle	119
Age of donors (years)	26.3 ± 2.7
Recipient cycles	436
Age of recipient (years)	41.4 ± 4.4
Total oocyte warmed (per recipient)	2,656 (6.09 ± 1.65)
Total oocyte survived (%)	2,453/2,656 (92.3 %)
Total oocyte fertilized (%)	2,161/2,453 (88.0 %)
Good-quality embryo on day 3 (per fertilized oocyte)[a]	1,501/2,161 (69.4 %)
Blastocyst formation rate (per embryo subjected to extended culture)	1,482/2,089 (70.9 %)
Total number of embryo transferred (per recipient)	592 (1.36 ± 0.48)
Total number of embryo revitrified	1,054 (2.42 ± 1.23)
Clinical pregnancies (%)	285/436 (65.3 %)
Total number of implantation (%)	352/592 (59.4 %)
Total number of ongoing pregnancy	279
Total number of recipient delivered[b]	168
Total number of infants born	219 (103 female and 116 male)

[a]According to SART morphological assessment for embryo grading system

[b]There were still 111 recipients with ongoing pregnancy who have not delivered yet by the time this manuscript was prepared

to be for decades. Additionally, acquiring oocytes from a cryobank is financially more affordable than fresh oocyte donation, mainly because the cost of a single oocyte donor is distributed among several recipients.

11.3.3 Comparison with and Contrast to Sperm Banking

Although donor oocyte banks may now emerge, the considerable difference between oocyte and sperm banking merits attention. Sperm banks have the luxury of evaluating a high number of candidates who, despite normal seminal parameters of sperm concentration, motility, and morphology, may not produce the minimal number of motile sperm post-thaw and are declined. The rate of acceptance to be a sperm donor at a commercial sperm bank can be restrictive without limiting the creation of inventory. Owing to this ability to be selective and eliminate donors whose sperm are cryo-sensitive, many sperm banks are able to offer a warranty for each sample, guaranteeing a minimum of total motile sperm post-thaw, a feature that augments their attractiveness to clients.

Oocyte banking does not easily make this accommodation for donor exclusion. Once candidates are screened and accepted, and reasonable ovarian response to controlled hyperstimulation is achieved with retrieval and freezing of mature eggs, knowledge of the oocytes' quality must be obtained empirically. A "test thaw" will reveal if an egg can be recovered structurally intact, but it will be only after ICSI, appraisal of embryo development and transfer, that the "quality," i.e., the ultimate ability of the frozen oocyte to advance to embryo implantation and clinical pregnancy can be determined. For this reason, donor oocyte banks can only *retrospectively* withdraw a suboptimal donor after appropriate review.

11.4 A Model for a Donor Oocyte Bank

A donor oocyte bank represents not only a scientific and medical resource to assist women and couples in achieving pregnancy and live birth, but it is also a novel business model. As such, an effective and successful oocyte bank demands the appropriate infrastructure, support, and maintenance for its organization and establishment, production of consistent positive outcomes to build a reputation for service, reliability, and quality, and to evolve as patients and the marketplace suggest or dictate. Some of the required elements of a donor oocyte bank are the following:

1. A well-designed and executed donor recruitment program.
2. An efficient and effective screening process for applicants who wish to be oocyte donors.

3. A qualified mental health professional who can administer the appropriate instruments required to assess the donor's understanding of gamete donation and its potential ramifications.
4. A prescribed methodology for ovulation induction of the oocyte donors to achieve consistency in this critical phase of the process.
5. A validated, reproducible method for oocyte vitrification and warming must be applied and the embryologists of the oocyte bank and the recipient laboratories must be carefully and rigorously trained in the vitrification and warming methods, respectively. This will ensure the consistency and quality control leading to optimal outcomes and be an integral part of the foundation for the bank's reputation and success.
6. A vigorous quality control program for the reagents and materials used in the oocyte bank, completely analogous to that of an ART laboratory.
7. A team of administrators to manage and organize the communication with and information from applicants, accepted donors, cycling donors, and their respective recipients.
8. A database that can track donor oocyte acquisition, distribution, clinical use, and clinical outcomes.
9. A database manager who will provide oversight on donor outcomes, e.g., to ensure that maximal cycle number by a given donor is not exceeded or that an underperforming donor is reviewed.
10. Excellent communication and coordination between the oocyte bank and its recipient laboratories.
11. A full understanding of and compliance with all regulations governing reproductive cells and tissue, i.e., those of the Food and Drug Administration (FDA), the Society of Assisted Reproductive Technology (SART), and individual state requirements.
12. A mission statement that includes a commitment to the welfare of both donors and recipients.

11.5 The Future of Oocyte Banking

A reliable method of cryopreserving oocytes allows patients to freeze and store their own oocytes to ameliorate loss of fertility through age, disease, or ovarian loss or injury. An additional application is assisting patients who wish to avoid creation of supernumerary embryos through allocation of some oocytes to IVF and some to freezing. This strategy ensures that every oocyte is clinically used and maximizes the potential of each treatment cycle while avoiding the difficult decisions and controversies that may surround cryopreserved zygotes or embryos.

The ability to offer cryopreserved donor oocytes, i.e., through a donor oocyte bank, is exceedingly attractive from a convenience-to-patient perspective and the ability to initiate therapy rapidly. Donor oocyte banks are a significant venture, requiring medical, scientific, business and administrative skill and strong communication and organization. Their emergence may impart greater urgency to the effort to create a central oocyte donor registry to keep an accounting of how many cycles a specific oocyte donor undergoes and how many offspring result from her donations [36–38].

As cryopreserved oocyte banking becomes established as the newest ART, it may be important to consider how it will evolve. A new generation of potential users of this technique, whether for autologous fertility preservation or as donors or recipients, brings its own expectations and values. This is a generation accustomed to rapidly developing medical technology and fully expectant of virtually instant communication, high levels of social connectivity through electronic media, and robust access to information.

Just as IVF, embryo cryopreservation, assisted hatching, ICSI, and embryo biopsy for preimplantation diagnosis fulfilled the family-building ambitions of patients in the 1980s and 1990s, oocyte cryopreservation and its benefits of fertility preservation and donor oocyte banking brings greater prospects and maybe even the *promise* for family building in the twenty-first century.

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