Successful elective and medically indicated oocyte vitrification and warming for autologous in vitro fertilization, with predicted birth probabilities for fertility preservation according to number of cryopreserved oocytes and age at retrieval

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Objective: To evaluate a single treatment center's experience with autologous IVF using vitrified and warmed oocytes, including fertilization, embryonic development, pregnancy, and birth outcomes, and to estimate the likelihood of live birth of at least one, two, or three children according to the number of mature oocytes cryopreserved by elective fertility preservation patients.
Design: Retrospective cohort study.
Setting: Private practice clinic.
Patient(s): Women undergoing autologous IVF treatment using vitrified and warmed oocytes. Indications for oocyte vitrification included elective fertility preservation, desire to limit the number of oocytes inseminated and embryos created, and lack of available sperm on the day of oocyte retrieval.
Intervention(s): Oocyte vitrification, warming, and subsequent IVF treatment.
Main Outcome Measure(s): Post-warming survival, fertilization, implantation, clinical pregnancy, and live birth rates.
Result(s): A total of 1,283 vitrified oocytes were warmed for 128 autologous IVF treatment cycles. Postthaw survival, fertilization, implantation, and birth rates were all comparable for the different oocyte cryopreservation indications; fertilization rates were also comparable to fresh autologous intracytoplasmic sperm injection cycles (70% vs. 72%). Implantation rates per embryo transferred (43% vs. 35%) and clinical pregnancy rates per transfer (57% vs. 44%) were significantly higher with vitrified–warmed compared with fresh oocytes. However, there was no statistically significant difference in live birth/ongoing pregnancy (39% vs. 35%). The overall vitrified–warmed oocyte to live born child efficiency was 6.4%.
Conclusion(s): Treatment outcomes using autologous oocyte vitrification and warming are as good as cycles using fresh oocytes. These results are especially reassuring for infertile patients who must cryopreserve oocytes owing to unavailability of sperm or who wish to limit the number of oocytes inseminated. Age-associated estimates of oocyte to live-born child efficiencies are particularly useful in providing more explicit expectations regarding potential births for elective oocyte cryopreservation. (Fertil Steril® 2016;105:459–66. ©2016 by American Society for Reproductive Medicine.)
Key Words: Autologous oocyte vitrification, fertility preservation, live birth, warming

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Until recently, clinical use of oocyte cryopreservation as part of IVF treatment was rare. Poor success rates associated with the slow freeze protocols that were used almost exclusively until 2003 resulted in limiting the use of oocyte cryopreservation to nonelective "emergency" cases (e.g., medically indicated fertility preservation preceding gonadotoxic cancer therapies, or the unavailability of sperm on the day of oocyte retrieval). The advent of oocyte vitrification, which is reported to more than double the percentage of children that can be born from cryopreserved oocytes compared with slow freezing (1), dramatically changes the utility of this treatment option.

Oocyte cryopreservation is receiving increasing promotion and public acceptance since removal of the "experimental" designation by the American Society of Reproductive Medicine and the Society for Assisted Reproductive Technology in October 2012 (2). Demographic trends and increased social and educational awareness point to continued growth in the population that utilizes this treatment option, particularly for elective reasons. Insurance companies and employers are both finding it necessary to consider these factors in their benefits.

As with any emerging technology, it is critical to continuously evaluate the efficacy of oocyte cryopreservation as outcome data accumulate. Reports of oocyte vitrification and warming have thus far been encouraging. Well-controlled studies of donor oocyte IVF cycles have demonstrated clinical outcomes with vitrified oocytes that are comparable to those of freshly retrieved oocytes (3–5). Two small studies of a combined 62 autologous IVF patients compared sibling oocytes inseminated while fresh vs. after vitrification and warming, and reported comparable fertilization rates and embryonic development (6, 7). A third study of sibling oocytes from 44 patients noted reduced rates of fertilization, cleavage, and blastocyst formation after oocyte vitrification, but no increase in aneuploidy or decrease in implantation compared with fresh oocytes (8). A study conducted in Italy during the first 2 years of the legally imposed limit of three inseminated oocytes per cycle reported similar implantation rates (13% vs. 10%) and pregnancy rates (32% vs. 29%) for 120 autologous IVF cycles using vitrified oocytes compared with 251 cycles using freshly retrieved oocytes (9).

The goal of this study was to add to the very limited information yet available on the clinical use of vitrified oocytes, particularly nondonor oocytes, by reporting on our relatively large experience with autologous IVF using vitrified oocytes and comparing with our fresh autologous IVF results using otherwise identical treatment protocols. Comparisons of patient and cycle characteristics and treatment outcomes are also made among different indications for autologous oocyte cryopreservation, including elective fertility preservation, unavailability of sperm at retrieval, and patients' desires to limit the numbers of embryos created by limiting the number of oocytes inseminated from a retrieved cohort and vitrifying the remainder.

An accurate understanding of the efficacy of oocyte vitrification is especially important in the context of elective fertility cryopreservation, because these women are undergoing a medical procedure only as a form of insurance against future declines in their fertility potential. Information on treatment outcomes for this elective patient population is particularly difficult to obtain, because the nature of the treatment inherently involves a potentially long delay between oocyte cryopreservation and subsequent use. To provide clearer guidance for considerations of elective oocyte vitrification for fertility preservation, we model expectations regarding the probabilities of having at least one, two, or three live-born children according to the numbers of oocytes cryopreserved and age-stratified efficiencies with which oocytes result in live-born children.

MATERIALS AND METHODS

All autologous IVF cycles performed from August 2009 through January 2015 using oocytes that had been vitrified were identified through a review of the clinical database. This retrospective review of clinical data was approved by Schulman Associates institutional review board. Women in this cohort were undergoing medically indicated IVF, with cryopreservation of oocytes due to either unavailability of sperm on the day of oocyte retrieval (male partner unable to produce a sample or failed surgical sperm retrieval attempt) or to limit the number of embryos initially created. The cohort also included women who electively cryopreserved oocytes for non–medically indicated fertility preservation. Controlled ovarian hyperstimulation was performed using a mixed protocol of purified or recombinant FSH and purified hMG. Either GnRH antagonist or GnRH agonist pituitary suppression protocols were used, as previously described (10). Final oocyte maturation was triggered with either IM injection of 10,000 U hCG or subcutaneous administration of 4 mg GnRH agonist when three or more follicles reached ≥ 18 mm in diameter. Ultrasound-guided transvaginal oocyte retrieval was performed 36 hours later.

Oocyte Vitrification and Warming

Oocyte vitrification and warming was performed as described by Kuwayama et al. (11). After collection, oocytes were equilibrated in culture medium for 1 hour before they were denuded using hyaluronidase (40 IU/mL in modified human tubal fluid). Vitrification was performed 2 hours after retrieval. Oocytes were first placed into base vitrification solution (M-199 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffered medium + 20% dextran serum substitute; Irvine Scientific) at room temperature (approximately 25°C). Oocytes were then sequentially transferred through 7.5% ethylene glycol (EG) and dimethyl sulfoxide (DMSO) in M-199 medium with 20% synthetic serum substitute (SSS) for 16 minutes for equilibration, followed by 15% EG and 15% DMSO with 0.5 M sucrose for 45–60 seconds. Oocytes were then loaded onto the Cryolock system (BioDiseno) and plunged directly into liquid nitrogen.

To warm vitrified oocytes, the Cryolock device was plunged into a 1-mL droplet of 37°C 1.0 M sucrose solution. Oocytes were identified and passed through decreasing concentrations of sucrose solution (1.0 M–0.25 M) over

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