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 $\geq$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

1-minute intervals until transfer to modified human tubal fluid with 20% dextran serum substitute.

Oocyte Fertilization and Embryo Culture, Transfer, and Cryopreservation

Intracytoplasmic sperm injection (ICSI) was performed on all mature (metaphase II, MII) oocytes after 3 hours of re-equilibration in culture medium with 20% SSS. Embryos were cultured in a continuous single culture medium with gentamicin + 10% SSS for up to 6 days (Irvine Scientific). Ultrasound-guided ETs were performed at the cleavage stage on day 3 or at the blastocyst stage on day 5 (occasionally day 6) of embryo culture. The uterine lining was prepared using intramuscular estrogen (delestrogen, 4 mg IM every third day; JHP Pharmaceutical) until the endometrium thickness reached >7 mm. Progesterone in oil (50 mg/d IM; Watson Pharmaceuticals) was then started and ET occurred on the 4th (day-3 transfers) or 6th (day-5 transfers) day of P.

Any blastocysts of adequate quality (minimum inner cell mass/trophectoderm grade of BB) according to a previously described grading system (12, 13) that were not transferred were cryopreserved on day 5 or 6 of culture using the Cryolock vitrification carrier system. Blastocyst cryopreservation was performed by placing an embryo into 7.5% EG and DMSO for 9 minutes, followed by 15% EG and DMSO + 0.5 M sucrose for 60 seconds, and finally plunging the embryos directly into liquid nitrogen for storage.

Data Analysis

Quantitative patient and cycle characteristics and treatment outcomes were compared among the three primary indications for autologous oocyte vitrification by analysis of variance followed by post hoc Tukey-Kramer Honest Significant Difference tests to evaluate pairwise differences. Qualitative characteristics and outcomes were compared by Fisher's exact or χ^2 analysis, as appropriate. Comparisons between cycles using vitrified and freshly retrieved oocytes were conducted by t test (for quantitative variables) or Fisher's exact or χ^2 (for qualitative variables). In comparisons of implantation, pregnancy and birth rates between vitrified and fresh oocytes, generalized estimating equations analysis was used to control for repeated cycles by individual patients and adjust for potential confounders including age, body mass index (BMI), diagnoses, stage of ET (cleavage or blastocyst), and numbers of embryos transferred. Statistical analyses were performed using JMP version 11 (SAS Institute Inc.) and SPSS version 22 (IBM Corporation). Expected probabilities of achieving at least one, two, or three live births according to numbers of warmed vitrified oocytes were modeled assuming binomial distributions of observed age-associated efficiencies of children born per oocyte at our center. Outcomes of oncofertility cases are noted separately because the very small sample for this indication prevented meaningful statistical evaluation or comparisons with other indications.

RESULTS AND DISCUSSION

Through December 2014, 1,171 cycles of oocyte vitrification were performed for 875 women intending to use these vitrified oocytes for future autologous IVF treatment. Through January 2015, 117 of these women returned to undergo 128 autologous IVF cycles, using a total of 1,283 vitrified and warmed oocytes for the following indications: infertility patients who vitrified oocytes either because of unavailability of sperm on the day of retrieval (52 warming cycles by 51 patients) or who opted for limited insemination of only a subset of the retrieved oocyte cohort (44 warming cycles by 35 patients); and 32 warming cycles among 31 women who had electively cryopreserved oocytes for non–medically indicated fertility preservation. Specific reasons for sperm unavailability at oocyte retrieval, and the sources of sperm used to inseminate the vitrified oocyte cohorts, are detailed in Table 1. Including both fresh embryo transfers and transfers of cryopreserved embryos, these warming cycles have thus far resulted in 51 live births or normal pregnancies ongoing past the first trimester (n = 7) and 55 live-born children and 8 additional healthy fetuses currently ongoing (63 children total). Twelve of the 51 births/ongoing pregnancies were twins, with no higher-order multiples. Sixty-two good-quality blastocysts still remain in cryostorage from these warming cycles.

Oocyte Maturity Status and Survival

Among the oocytes that were vitrified and warmed, 91.4% were vitrified as mature MII oocytes, 4.8% were vitrified as

TABLE 1

Reasons for lack of sperm availability on the day of oocyte retrieval, and eventual source of sperm used for subsequent insemination of the vitrified oocyte cohort.

Reason no sperm available at fresh retrieval	No. of cycles	Sperm source used for subsequent insemination of vitrified oocytes
No usable sperm from surgical sperm extraction	20	Donor (17) Partner (2 ejaculate, 1 surgical)
No motile sperm in ejaculated sample	16	Donor (4) Partner (9 ejaculate, 3 surgical)
Unable to provide ejaculated sample through masturbation	8	Partner ejaculate (1 fresh, 4 cryo) Partner surgical sperm extraction (3)
Unexpected unavailability of partner/specimen	6	Partner ejaculate
Donor sperm intended for use was not provided	2	Donor

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immature metaphase I (MI) oocytes, and 3.8% were vitrified as germinal vesicles (GV). Vitrification and warming survival rates were slightly higher for MII compared with MI and GV oocytes, but not significantly so (86.1% vs. 74.6% and 80.9%, respectively). However, only 44% of the oocytes vitrified at the MI stage subsequently matured to the MII stage in vitro after warming, and only 4% of the oocytes vitrified at the GV stage did so.

These data suggest a limited role for oocytes vitrified before the MII stage, particularly those vitrified at the GV stage. In vitro maturation of vitrified and warmed immature oocytes produced few oocytes of clinical utility. Others have also documented relatively poor in vitro maturation rates for embryos vitrified at the GV or MI stages (14, 15). The pooling of in vitro–matured oocytes with other MII oocytes in this series prevents comment on subsequent embryonic development, although in both mouse models and human IVF it has been previously demonstrated that in vitro matured oocytes vitrified either before or after maturation suffer from reduced fertilization and poor embryologic development, although outcomes are somewhat better if matured before vitrification (14, 16). Therefore, except in circumstances where ovarian function is expected to be otherwise extremely limited or exhausted (including oncofertility patients), to enhance clinical outcomes, effort is better spent accumulating additional in vivo–matured oocytes. This may change with future improvements in in vitro maturation techniques.

Variation among Indications for Oocyte Cryopreservation

There were several statistically significant, although unsurprising, differences among the three indications for autologous oocyte vitrification.

Compared with the two groups of infertility patients making use of oocyte cryopreservation, women cryopreserving oocytes for fertility preservation were significantly older at the times of both vitrification (by 4.3 years, $P<.0001$) and warming (by 5.7 years, $P<.0001$). This difference was expected, because fertility preservation was specifically directed toward women in their mid- 30s (slightly older than the average infertility patient), who were believed to have the greatest potential to benefit from elective oocyte cryopreservation. The oocytes of elective fertility preservation patients were also cryopreserved for a longer duration than those of infertility patients (means of >2 years vs. 8 months, $P<.0001$). Again, this was expected given the different reasons for oocyte cryopreservation.

Compared with the other indications for autologous oocyte cryopreservation, for obvious reasons those patients requesting oocyte cryopreservation out of a desire to limit the number of oocytes inseminated (and embryos created) per treatment cycle had less than half as many MII oocytes inseminated per warming cycle (4.7 vs. 9.7, $P<.0001$). Predictably, this resulted in a greater likelihood of having no embryos of suitable quality for either transfer or cryopreservation (11.4% vs. 1.2%, $P=.018$). The smaller embryo cohort associated with limited insemination may also lead to some reduction in implantation, pregnancy, and birth rates, although these outcomes did not differ significantly among indications for oocyte vitrification in our sample. Patients desiring limited insemination should be appropriately counseled that success rates per insemination cycle may be somewhat lower, potentially increasing the time and expense needed to reach their family building goals. However, the long-term cumulative success rates per oocyte retrieval should not be diminished, because oocytes vitrified for later use retain their viability.

It should also be noted that although our analysis did not reveal any significant differences in implantation or birth outcomes among the different indications for oocyte vitrification, patients undergoing elective fertility preservation are not necessarily infertile, and therefore their results may differ from those of patients being treated for infertility.

Receiver operating characteristic analysis of the relationship between patient age at the time of oocyte vitrification and the probability of achieving a clinical pregnancy per oocyte warming procedure suggested that the clearest threshold between better and worse outcomes was <38 years vs. ≥38 years (sensitivity = 74%, specificity = 41%). The clinical pregnancy rate for patients aged <38 years at the time of oocyte cryopreservation was 60.2%, compared with 43.9% for patients aged ≥38 years at the time of oocyte cryopreservation ($P=.085$, χ^2).

Vitrified vs. Fresh Oocytes

The 128 cycles of autologous vitrified oocyte IVF were compared with all 2,963 fresh autologous ICSI cycles performed at our center during 2013 (Table 2). Patient age at retrieval did not differ significantly between the two groups. Body mass index was slightly, but significantly, higher for the fresh cycles compared with the vitrified cycles. Patients undergoing fresh cycles were more likely to be diagnosed with diminished ovarian reserve, ovulation disorders, or tubal factor infertility.

The average number of MII oocytes inseminated per cycle was significantly higher for fresh vs. vitrified oocyte cycles. Fertilization rates did not differ between fresh and vitrified oocytes, nor did the proportion of cycles without embryos of sufficiently good quality to transfer or cryopreserve. Blastocyst-stage ET was significantly less common for the vitrified oocyte cycles compared with the fresh cycles.

Unadjusted comparisons between cycles using vitrified vs. fresh oocytes suggested an insignificant trend toward higher implantation, and significantly higher clinical pregnancy rates per transfer, for the vitrified oocytes. Using generalized estimating equations (GEE) analysis to account for repeated cycles by individual patients and adjust for age, BMI, diagnoses, stage of ET, and number of embryos transferred, both implantation rates and clinical pregnancy rates were significantly higher for the vitrified compared with fresh oocyte cycles. However, live birth outcomes did not differ significantly in either the unadjusted or adjusted analyses. These results, indicating no apparent reduction in success rates with the use of vitrified oocytes, are very reassuring and consistent with several previous studies of both donor oocyte IVF (3–5) and autologous IVF (6, 7, 9), indicating

TABLE 2

Patient and cycle characteristics and treatment outcomes of autologous ICSI cycles compared between vitrified and freshly retrieved oocytes.

Characteristic	Vitrified oocytes	Fresh oocytes	*P* value
Cycles (n)	128	2,963	
Age (y) at oocyte retrieval	34.9	35.5	NS
BMI (kg/m^2)	24.6	25.9	.006
Ethnicity (%)			
European	64.1	62.0	NS
African	17.2	13.6	NS
Asian	10.9	15.8	NS
Hispanic	3.1	4.7	NS
Other/unknown	4.7	3.9	NS
Diagnoses (%)			
Diminished ovarian reserve	8.6	16.9	.014
Endometriosis	5.5	4.2	NS
Male factor	43.8	44.5	NS
Ovulation disorders/PCOS	4.7	10.6	.031
Tubal factor	4.7	10.7	.029
Uterine factor	0.8	2.5	NS
No. of MII oocytes inseminated	8.0	10.1	.0002
Fertilization per MII inseminated (%)	69.5	71.7	NS
Cycles without ET or cryopreservation (%)	4.7	4.2	NS
Blastocyst-stage ET (%)	50.9	66.1	.001
Embryos per transfer	1.8	1.9	NS
Implantation per embryo transferred (%)	41.2	35.4	NS
Implantation per embryo transferred (adjusted) (%)	43.1[a]	35.3[a]	.046
Clinical pregnancy per transfer cycle (%)	54.4	45.1	.050
Clinical pregnancy per transfer cycle (adjusted) (%)	57.1[a]	44.4[a]	.011
Pregnancy loss per clinical pregnancy (%)	29.0	20.1	NS
Pregnancy loss per clinical pregnancy (adjusted) (%)	29.9[a]	19.0[a]	.048
Live birth/ongoing pregnancy per transfer cycle (%)	38.6	36.0	NS
Live birth/ongoing pregnancy per transfer cycle (adjusted) (%)	38.6[a]	34.7[a]	NS

Note: P values according to *t* test, χ^2, or Fisher's exact, as appropriate. NS = not significant; PCOS = polycystic ovarian syndrome.
[a] Estimated marginal means according to GEE analysis accounting for repeat cycles by individual patients and adjusting for age at retrieval, BMI, diagnoses, stage of ET, and number of embryos transferred.

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comparable outcomes between freshly retrieved and vitrified oocytes.

Better success rates with the use of vitrified rather than freshly retrieved oocytes are theoretically possible, as has been reported for cryopreserved embryos (17–19). The use of vitrified oocytes allows for ET in a cycle that has not been subjected to the supraphysiologic ovarian hyperstimulation that is required to retrieve cohorts of multiple oocytes. Ovarian hyperstimulation is known to adversely affect endometrial receptivity and endometrium–embryo developmental synchrony, thereby reducing implantation and pregnancy (17, 20, 21). Alternatively, patients electing to cryopreserve oocytes may have been a better prognosis cohort than the average IVF patient. Adjustments for age, BMI, diagnoses, ET stage, and number of embryos transferred, performed in the GEE analyses, provide some degree of control over such potentially confounding bias. It is also possible that vitrification survival may introduce a selection bias on oocyte quality, because surviving oocytes may be more robust and have a better prognosis.

Vitrified Oocyte to Live-born Child Efficiency

The efficiency with which vitrified and warmed oocytes translate into live-born children is of great clinical importance, particularly in the context of elective oocyte cryopreservation for fertility preservation. Accurate estimates of these efficiencies are vital for appropriately informed decisions regarding how many oocytes to cryopreserve as insurance against future age-related declines in fertility. Cryopreservation of too few would limit the potential for success, whereas cryopreservation of excessive numbers of oocytes might cause unnecessary expense and diminishing returns for additional retrieval procedures.

Efficiency per warmed oocyte was calculated for this sample of 128 vitrified oocyte warming cycles according to all actual births from both freshly transferred and cryopreserved embryos derived from the oocyte warming cycles, and assuming eventual transfer of all remaining cryopreserved blastocysts. Outcomes for the remaining cryopreserved blastocysts were assumed to be the same as our past experience with blastocyst vitrification, warming, and transfer. In support of this assumption it has been previously shown that delivery rates for cryopreserved embryos developed from vitrified oocytes are equivalent to cryopreserved embryos developed from freshly retrieved oocytes (22).

All autologous vitrified blastocyst transfers (n = 1,755) performed at our center between January 2009 and April 2012 were reviewed, for which a total of 2,994 vitrified blastocysts were warmed for transfer. The percentages of live-born children per warmed blastocyst, according to

patient age at the time of oocyte retrieval and blastocyst cryopreservation, ranged from 35.9% for women aged <30 years to 13.3% for women aged 43–44 years (Supplemental Table 1). Applying these figures to the 62 remaining cryopreserved blastocysts derived from oocyte warming cycles predicted the birth of 20 additional children. In addition to the 63 children from completed transfers, the result would be 83 total children born from 1,283 warmed vitrified oocytes, for an efficiency of 6.5% per warmed oocyte. The estimated efficiencies per warmed oocyte by age group were as follows: 7.4% for women aged <30 years at the time of oocyte cryopreservation; 7.0% for women aged 30–34 years; 6.5% for women aged 35–37 years; and 5.2% for women aged ≥38 years. The observed efficiency for women aged 41–42 years was high at 6.8%, but the sample size for women in this age range was very small (n = 5 children from 73 warmed oocytes). The calculated efficiencies per cycle, adjusted for age, were not significantly associated with either the number of oocytes thawed per cycle ($P=.26$) or the total number of oocytes retrieved in the originating cycle ($P=.66$).

To derive more accurate and precise estimates of oocyte to child efficiencies than possible with this relatively small sample of vitrified and warmed oocytes, we also evaluated age-associated efficiency rates among our much larger experience with IVF treatment using freshly retrieved oocytes. All oocyte retrievals for autologous IVF conducted from 2009 through 2013 were reviewed, excluding cycles in which not all oocytes were inseminated and those with cryopreservation of non-blastocysts (i.e., unfertilized or two-pronuclei oocytes). Among the 18,746 retrieval cycles reviewed, 243,892 oocytes were retrieved, 8,712 children were born as a result of fresh embryo transfer, and 23,850 blastocysts were cryopreserved for later transfer. Calculating as described above, this methodology indicated age-associated oocyte to child efficiencies ranging from 8.7% for women aged <30 years to 1.1% for women aged 43–44 years (Supplemental Table 2), and an overall oocyte to child efficiency of 6.7%.

These estimates from freshly retrieved oocytes are similar to the rough estimates derived from our preliminary experience with vitrified and warmed oocytes, as expected on the basis of our own analysis and previous reports (3–7, 9) indicating comparable outcomes for freshly retrieved and vitrified oocytes. The much larger sample size available for fresh IVF allowed for much greater precision, and produced more plausible results (e.g., the 6.8% efficiency observed for oocyte vitrification among women aged 41–42 years is clearly overoptimistic). Calculations from fresh cycles included all oocytes retrieved, of which approximately 20% are immature, and may therefore underestimate achievable efficiencies of selective cryopreservation of MII oocytes for fertility preservation. In addition, oocyte viability may tend to be higher for fertility preservation patients who are not seeking treatment for infertility-related issues. We therefore considered the efficiencies calculated from fresh transfers to provide the better available, and likely conservative, guidance for oocyte cryopreservation for fertility preservation. These efficiency estimates were used to derive age-related probabilities of having at least one, two, or three live born children according to the number of MII oocytes cryopreserved, assuming a binomial distribution (Fig. 1).

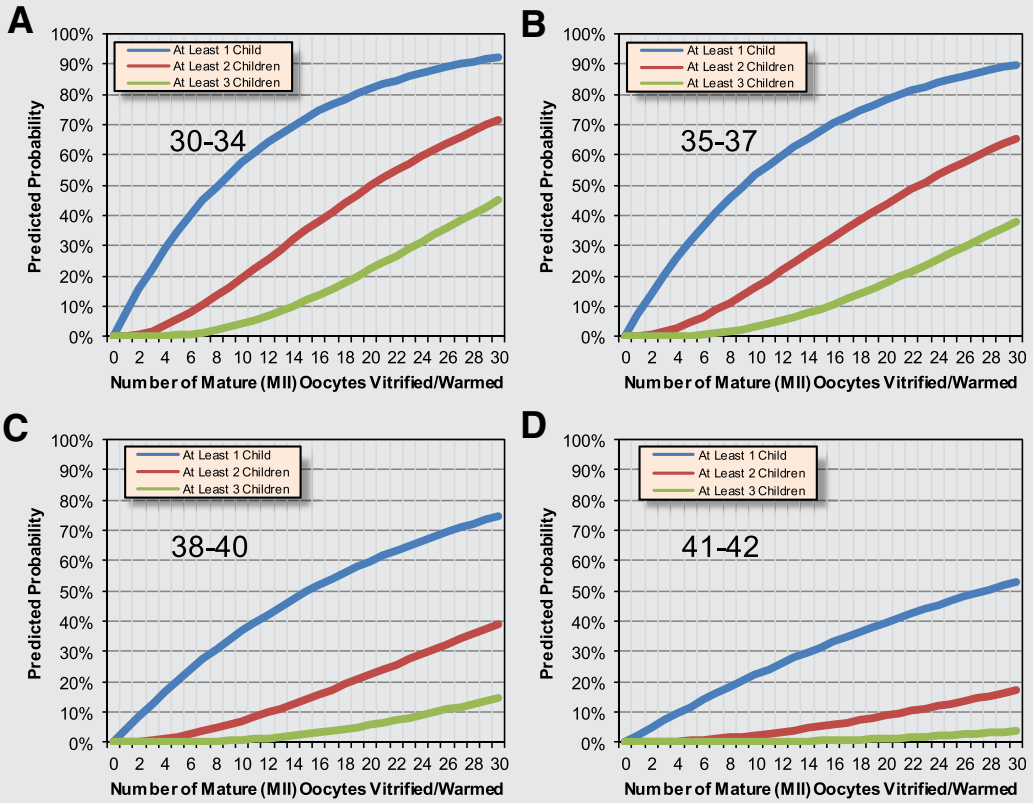
Our typical recommendations are to cryopreserve 15–20 MII oocytes for women aged <38 years (giving them a roughly 70%–80% chance of at least one live birth) and 25–30 MII oocytes for women aged 38–40 years (giving them a roughly 65%–75% chance of at least one live birth). These general recommendations can be individualized according to the specific family building goals of the patients and their ability and desire to invest in this insurance against declining future fertility.

In determining reasonable and optimal timing for elective oocytes cryopreservation, it is important to balance the considerations of potential costs and benefits. Vitrifying oocytes at an earlier age may benefit in terms of maximizing oocyte quality and minimizing the number of cycles necessary to accumulate the recommended number of oocytes, but the cryopreserved oocytes are less likely to be needed. On the other hand, cryopreserving at a later age requires that more oocytes of a lower quality be stored to achieve comparable pregnancy rates. Declining oocyte yields per treatment cycle with increasing age will make recovery of an adequate number of oocytes for a reasonable chance of success increasingly costly for older women. As is clear from Figure 1D, attempting fertility preservation after the age of 40 years is unlikely to be successful. The relative stability of fertility potential in the healthy population through the early to mid-thirties suggests this would be a reasonable span in which to consider oocyte cryopreservation, as consistent with the data herein. One recent analysis of the efficacy and cost effectiveness of elective fertility preservation for women wishing to delay childbearing until 40 years of age supports this proposal, concluding that oocyte cryopreservation at age 35 years would result in a relative increase of 48% in the probability of achieving a live birth and a relative decrease of 27% in the cost per live birth, and that oocyte cryopreservation remained a cost-effective strategy through 37 years of age (23). Another recent study reached a similar conclusion, finding that there was little benefit to oocyte cryopreservation at ages 25–30 years, with maximum benefits achieved with cryopreservation at ages 32–37 years (24). Oocyte cryopreservation might be advisable at a younger age in select women with an ovarian reserve assessment or familial history suggesting an increased risk of early decline in fertility, or personal circumstances likely to preclude pregnancy for a prolonged period. Physicians should consider the responsibility to promote awareness of these age-related changes in fertility potential and how they impact expected birth rates and treatment costs if elective oocyte cryopreservation is being considered.

Oncology Patients

In addition to our primary analysis above, 102 of the 1,171 cycles of oocyte vitrification were performed for women about to undergo chemotherapy for diagnosed cancer, three of whom have since returned to undergo IVF using their cryopreserved oocytes. Two of these three treatment cycles were successful, with one patient achieving a live singleton

FIGURE 1



Predicted probabilities of having at least one, two, and three live-born children according to the number of mature oocytes cryopreserved for elective fertility preservation, according to age at oocyte retrieval and the associated oocyte to live-born child efficiency estimates: (**A**) 30–34 years, 8.2% efficiency; (**B**) 35–37 years, 7.3% efficiency; (**C**) 38–40 years, 4.5% efficiency; (**D**) 41–42 years, 2.5% efficiency.

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birth (with three vitrified blastocysts remaining) and another having a currently ongoing singleton pregnancy (with 14 vitrified oocytes remaining). Other groups have also reported a small number of healthy births among oncologic patients who returned to use their vitrified oocytes (25, 26).

CONCLUSION

This study is among the largest evaluations yet reporting on the clinical use of oocyte vitrification and warming for autologous IVF treatment, with the exception of a report on the national experience of Italy, where legal restrictions enacted in 2004 limiting the numbers of oocytes inseminated resulted in widespread surplus oocyte cryopreservation (27). As such, it provides valuable information regarding the clinical utility of this new and rapidly expanding treatment option. In our evaluation of 128 warming cycles, we observed overall rates of 41% implantation per transferred embryo and 39% live birth per ET procedure, clearly demonstrating the efficacy of autologous IVF using vitrified oocytes. The data in this article provide substantial evidence supporting the efficacy of oocyte vitrification as an adjunct to standard IVF treatment of infertility as indicated, as well as for elective fertility preservation. Age-specific probabilities of live births according to the numbers of oocytes cryopreserved are derived to provide individualized quantitative guidance for women considering fertility preservation. As with any newer treatment modality receiving appropriate scrutiny, continued surveillance is required, though it is reassuring to see the accumulation of additional information demonstrates encouraging outcomes.

It is important to consider that these data represent the experience of a single practice, limiting the generalizability of the conclusions. Variation in both vitrification and warming protocols and the technical experience of laboratory personnel are variables known to be important to pregnancy outcomes. Ideally, as clinical data accumulate at a specific center, center-specific data can be used to provide more directly applicable guidance on the recommended number of oocytes to both vitrify and warm and expectations regarding pregnancy and birth. Continued protocol optimization and limitation of process variability is also needed to improve outcomes and consistency within and among treatment centers. This optimization and consistency in turn will allow greater standardization of recommendations related to the vitrification and warming processes, and limit the total number of oocytes that need to be collected to achieve high standards of live-birth outcomes.

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SUPPLEMENTAL TABLE 1

Children born per warmed vitrified blastocyst according to patient age at oocyte retrieval and embryo cryopreservation, for all autologous vitrified blastocyst transfers performed between January 2009 and April 2012.

Age at cryopreservation (y)	Blastocysts warmed (n)	Children born (n)	Children per blastocyst (%)
<30	451	162	35.9
30–34	1211	419	34.6
35–37	762	260	34.1
38–40	437	76	17.4
41–42	103	16	15.5
43–44	30	4	13.3

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SUPPLEMENTAL TABLE 2

Retrieved oocyte to live-born child efficiency calculations according to patient age at retrieval for all fresh autologous IVF cycles performed between 2009 and 2013.

Age (y)	Cycles	Oocytes	Children (fresh ET)	Vitrified blastocysts	Children (cryo ET)[a]	Total children	Children per oocyte (%)
<30	2,085	34,017	1,339	4,484	1,611	2,950	8.67
30–34	5,715	83,597	3,257	10,409	3,601	6,858	8.20
35–37	4,189	53,794	2,097	5,404	1,844	3,941	7.33
38–40	4,148	46,582	1,573	2,928	509	2,082	4.47
41–42	1,831	18,584	379	544	85	464	2.49
43–44	778	7,318	67	81	11	78	1.06

Note: Values are number, except where noted otherwise.
[a] Estimated assuming transfer of all cryopreserved blastocysts and observed birth rates per vitrified/warmed blastocyst from Supplemental Table 1.

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