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ASSISTED REPRODUCTION TECHNOLOGIES

Achieving the "ideal" family size at advanced reproductive ages through oocyte cryopreservation

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Introduction

Women are increasingly delaying pregnancy, and in 2014 the mean age at first birth in the USA had risen from 25.2 years in 2000 to 26.3 years [1]. Despite advances in IVF technology, advanced reproductive age remains a significant impediment to success with a live birth rate of 3.6% per oocyte retrieval in women over 42 years [2]. Concurrent with the trend of delayed childbearing has been an increase in the use of donor oocytes [3] to overcome age-related fertility decline and diminished ovarian reserve. Oocyte cryopreservation (OC), no longer considered experimental for women with medical indications but widely utilized to defer reproduction, is allowing women to conceive using autologous oocytes at ages when donor oocytes would have previously been required. The success of the technology has been demonstrated as women increasingly return for oocyte thaw/warming and embryo transfer [4–7].

Findings from a 2013 Gallup poll indicate that Americans view the ideal family size as two to three children. Among Gallup poll respondents aged 30–49 years, 47% viewed two children as the ideal number of children and 25% viewed three children as ideal [8]. Our experience shows that most women using their cryopreserved oocytes have previously exhausted other conventional methods of ART, suggesting that their cryopreserved oocytes may represent a last resort for pregnancy with autologous oocytes. If patients returning to use their cryopreserved oocytes are among the 72% of Americans 30–49 years who view two to three children as an ideal family size, OC success would thus be defined by aiding patients in achieving their *complete* family-building goals.

Reports of successful deliveries after OC and thaw/warming have certainly been described in the literature; however, to our knowledge there are no published reports of women achieving more than one singleton live birth with previously cryopreserved autologous oocytes. In this case series, we present six women who underwent OC for fertility preservation and subsequently achieved more than one singleton live birth after oocyte thaw/warming and embryo transfer. These representative patients highlight the use of OC technology to build multi-child families through singleton gestations at advanced reproductive ages.

Materials and methods

A retrospective chart review (IRB S13-00389) identified six patients who underwent OC for fertility preservation and achieved more than one singleton gestation using autologous cryopreserved oocytes. Patients in this series underwent cryopreservation cycles between 2009 and 2012. They returned for oocyte thaw/warming between 2013 and 2016, with a mean duration of cryopreservation 3.9 ± 1.3 years (range 0.8–5.3). The mean age at time of cryopreservation was 38.8 ± 1.6 (range 35–41), thaw/warming 42.9 ± 1.9 (range 40–45), first transfer resulting in a live birth 42.4 ± 1.6 (range 40–44), and second transfer resulting in a live birth 44.5 ± 1.6 (range 41–46). One patient has an ongoing pregnancy after a third successful transfer at age 46.8. Most had undergone preimplantation genetic testing for aneuploidy (PGT-A) with either next-generation sequencing (NGS) or array comparative genomic hybridization (aCGH) and subsequent single euploid embryo transfer for at least one of their transfers. Only

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one patient thawed oocytes without PGT-A for all of her transfers.

Oocyte cryopreservation, oocyte thaw/warming, and embryo transfer cycles

On cycle day 2, a baseline ultrasound was performed and serum estradiol as well as follicle-stimulating hormone (FSH) levels recorded. Patients with acceptable parameters began ovarian stimulation using a standard gonadotropin-releasing hormone antagonist protocol. Oocytes were either slow-frozen or vitrified depending on the time in which the patient presented for cryopreservation. Initially, both slow-freezing and vitrification were utilized in our embryology laboratory, with many patients' oocyte cohorts divided equally between the two methodologies, until oocyte vitrification became our exclusive method of cryopreservation. Oocytes were cryopreserved, thawed/warmed, and cultured using techniques previously described [5]. ICSI was performed as is standard in oocyte thaw/warming cycles.

Of the 17 embryo transfers, 15 were programmed embryo transfer cycles; patients took sequentially increasing doses of oral estradiol until the endometrium reached at least 7 mm in greatest diameter, and progesterone in oil (50 mg/day; Watson Pharmaceuticals) was added such that transfer occurred on the sixth day of progesterone. One patient completed two natural frozen embryo transfer (FET) cycles; she was monitored for ovulation and supplemented with vaginal progesterone (Crinone 8% BID) once confirmed. Again, transfer occurred on the sixth day of progesterone.

Cases

Case 1

The patient underwent OC in 2010 at age 35 with 21 oocytes retrieved and 18 cryopreserved (18 MII). Eight were vitrified and 10 slow-frozen. She returned at age 39 with a partner and attempted spontaneous conception resulting in one biochemical pregnancy and a 9-week missed abortion which was chromosomally abnormal on microarray following dilation and curettage (D&C). In 2015 (age 40), she opted to thaw her cryopreserved oocytes. All 18 were thawed: 17 survived, 14 demonstrated normal two pro-nuclei (2PN) fertilization with one morula and seven blastocysts resulting. All seven blastocysts underwent trophectoderm biopsy (TEBX) and PGT-A with NGS. Two euploid embryos resulted. The first was transferred in 2015 at age 40.5 via a programmed FET cycle. At age 41.2, she delivered a term live born female via cesarean without any complications. The second euploid embryo was transferred in 2016 at age 41.8 again via programmed FET cycle. At age 42.5, she delivered a term live born female weighing 3540 g via cesarean without any complications. Both live births were derived from previously slow-frozen oocytes.

Case 2

The patient and her partner presented to our center in 2012 (age 41) with primary infertility due to diminished ovarian reserve. She had previously vitrified 44 oocytes (41 MII, 3 MI) in 2009 at age 37 in a different state. Upon presenting with infertility in 2012, she attempted five IVF cycles, only successfully completing one cycle resulting in five oocytes retrieved, three fertilized 2PN zygotes, two blastocysts transferred, and a singleton gestation resulting in missed abortion at 8 weeks. In 2013, her oocytes were transferred to our center for warming and fertilization. Forty oocytes survived resulting in 27 fertilized 2PN zygotes and 19 blastocysts which underwent TEBX and PGT-A with aCGH. Eight euploid embryos resulted. The first was transferred in 2013 in a programmed FET cycle resulting in an early spontaneous abortion. The second was transferred in a programmed FET cycle in 2014. At 6 weeks gestation, she presented to the emergency room with shortness of breath and was noted to have a right lobar pulmonary embolism. She was started on therapeutic enoxaparin and followed by hematology as well as maternal fetal medicine (MFM) for the duration of her pregnancy. Thrombophilia workup was negative. At age 43.6, she delivered a term live born female weighing 4400 g via cesarean without any complications. After hematology and MFM clearance, the third euploid embryo was transferred in a natural FET cycle in 2015. She had an uncomplicated pregnancy and was maintained on prophylactic enoxaparin. At age 45.3, she had a term vaginal birth after cesarean of a male neonate weighing 4200 g. The delivery was complicated by a postpartum hemorrhage due to a retained placenta requiring a D&C. The fourth euploid embryo was transferred in a letrozole-stimulated FET cycle in 2017, and at 47 years her pregnancy is currently ongoing. She is again on prophylactic enoxaparin. She has four euploid embryos remaining in storage.

Case 3

The patient underwent OC in 2009 at age 38 with 11 oocytes retrieved and slow-frozen (10 MII, one MI). She did an additional cycle in 2010 (age 40) with 13 oocytes cryopreserved (seven MII, six MI). Six were slow-frozen and seven vitrified. She returned at age 42 with a partner and was counseled regarding options. She conceived spontaneously resulting in a miscarriage and subsequently opted to thaw her cryopreserved oocytes in 2014 at age 44. All 24 were thawed: 19 survived, 13 demonstrated 2PN fertilization with seven blastocysts resulting. Five blastocysts underwent TEBX and rush PGT-A with aCGH. Three euploid embryos resulted, one of which

was transferred on day 6 of a coordinated oocyte warming/endometrial preparation cycle. At age 45.1, she delivered a term live born via cesarean without complications. The second euploid embryo was transferred in a programmed FET cycle in 2017 (age 46.5) and the pregnancy resulted in a live born female. Both pregnancies were derived from previously slow-frozen oocytes. She has one euploid embryo remaining in storage.

Case 4

The patient underwent OC in 2010 at age 41 with 11 oocytes retrieved and 10 cryopreserved (eight MII, two MI), equally split between vitrification and slow-freezing. She completed an additional cycle 2 months later with 11 oocytes cryopreserved (eight MII, three MI). Seven were slow-frozen and four vitrified. She returned in 2013 at age 43 for oocyte thaw/warming with donor sperm. Eight MII oocytes and two MI oocytes were thawed: one blastocyst and two morulae were transferred. She delivered a term live born via cesarean without any complications. She returned in 2014 at age 45 for a second oocyte thaw using the same donor sperm. She thawed 11 oocytes (three from the first cycle, eight from the second cycle): 10 survived, eight demonstrated 2PN fertilization, two blastocysts, and two morulae developed. All four embryos were transferred, and at 46.2 years she delivered a term live born female weighing 3430 g via cesarean without complications. Both transfers included a mix of embryos derived from vitrified and slow-frozen oocytes and thus the origin of the live-born infants is not known.

Case 5

The patient underwent OC in 2012 at age 39 with 25 oocytes retrieved and 20 vitrified (19 MII, one MI). She re-presented at age 41 in a same-sex relationship planning to use donor sperm to achieve pregnancy. She completed four IUI cycles (three monitored natural cycles and one clomiphene cycle) without success at which time she opted to utilize her cryopreserved oocytes. She thawed six MII oocytes, with five demonstrating 2PN fertilization and three resulting blastocysts. One blastocyst was transferred in a coordinated endometrial preparation cycle and the remaining two were cryopreserved. There was no resulting pregnancy. The following month, she underwent a programmed FET cycle and transferred the two cryopreserved embryos with no resulting pregnancy. The 14 remaining oocytes were thawed with 100% survival resulting in five 2PN zygotes and seven blastocysts, six of which underwent TEBX for PGT-A with aCGH. Four euploid embryos resulted. In 2014, one euploid embryo was transferred via a programmed FET cycle. At age 41.6, she delivered a term live born female weighing 3317 g without complications. In 2016, she transferred a second euploid embryo via a programmed FET cycle and had a term live born female weighing 3402 g without complications at age 44.3. She has two euploid embryos remaining in storage.

Case 6

The patient underwent eight cycles of OC from 2010 to 2011 (age 38–39), accumulating 82 MII oocytes and 20 MI oocytes. She returned at age 41 in 2013 to complete an oocyte thaw/warming cycle using donor sperm. She thawed/warmed 18 MII oocytes from three cryopreservation cycles (12 vitrified, six slow frozen). Sixteen survived, 11 demonstrated 2PN fertilization, and six blastocysts developed. Three blastocysts were deemed usable; one was cryopreserved and two were transferred via a programmed embryo transfer cycle. Both embryos were derived from previously vitrified oocytes. One embryo implanted and she went on to deliver a live born female weighing 3090 g without complications at age 42.6. In 2015 (age 44), she underwent an oocyte thaw cycle with PGT-A. She thawed 40 MII oocytes from four cryopreservation cycles (20 vitrified, 20 slow frozen). Thirty-eight survived, 28 demonstrated 2PN fertilization, and 17 blastocysts developed. Fourteen blastocysts underwent TEBX and PGT-A with NGS, with five euploid embryos resulting. One euploid blastocyst was transferred the following month via a programmed FET cycle with no resulting pregnancy. A second euploid blastocyst was transferred the following month, resulting in delivery of a live born female child weighing 3260 g without complications at age 45.4. The embryo transferred was derived from a previously slow-frozen oocyte. She donated her remaining oocytes and embryos to research.

Tables 1 and 2 show the OC, thaw/warming, and embryo transfer data for each patient described.

Discussion

The series described above exemplifies the reproductive autonomy accessible to women through oocyte cryopreservation. These six women achieved multi-child families using autologous cryopreserved oocytes through singleton gestations at advanced reproductive ages. A number of patients achieved their second or third pregnancy at 46–47 years using their cryopreserved oocytes, in essence serving as their own oocyte donor at an age when donated oocytes would have likely been their only ART option. While any live birth following OC is considered a success, offering patients the opportunity to achieve their complete family-building goals via OC is an exciting possibility. We additionally identified nine patients who delivered twins and one set of triplets resulting from OC; however, in this series we opted to focus on patients who underwent sequential embryo transfers with the intent to create siblings. Many were single-embryo transfers, aligning

Table 1 OC and thaw/warming outcomes

Pt	Mean age at OC (y)	Total OC cycles	Total Oocytes Cryo	Total thaw cycles	Thawed/ warmed	Survived	2PN	Mor	Blast	TEBX	Euploid
1	35	1	18 MII	1	18 MII	17	14	1	7	7	2
2	37	1	41 MII 3 MI	1	41 MII 3 MI	40	27	2	20	19	8
3	39	2	17 MII 7 MI	1	17 MII 7 MI	19	13	1	6	5	3
4	41	2	16 MII 5 MI	2	8 MII 2 MI	10	6	2	1	–	–
					8 MII 3 MI	10	8	2	2	–	–
5	39	1	19 MII 1 MI	2	6 MII	6	5	0	3	–	–
					13 MII 1 MI	14	5	0	7	6	4
6	39	8	82 MII 20 MI	2	18 MII	16	11	2	6	–	–
					40 MII	38	28	4	17	14	5

Pt, patient; *y*, years; *Total Oocytes Cryo*, total number of oocytes cryopreserved; *Mor*, morulae; *Blast*, blastocyst; *TEBX*, number of blastocysts that underwent TEBX for PGT-A; –, fresh embryo transfer performed and supernumerary blastocysts cryopreserved

with the trend in our field toward reduction of multiple gestations. For all of these patients, the possibility for family building through OC represents a significant achievement in reproductive medicine.

During the study period, our embryology laboratory moved from using both slow-freezing and vitrification technologies, with many patients' oocytes equally split between the methodologies, to exclusive vitrification. Four patients had a combination of slow-frozen and vitrified oocytes, and two patients' oocytes were exclusively vitrified. While the pregnancies were derived from a relatively even breakdown of previously slow-frozen and vitrified oocytes, this series merely describes outcomes and is not intended to compare the technologies.

The obstetric risks at advanced reproductive ages cannot be overstated as women increasingly delay childbearing. Current

Table 2 Embryo transfer data and outcomes

Pt	Total transfer cycles	Age at transfer (y)	Type of transfer cycle	Embryos transferred	Type of embryo	Outcome	Embryos remaining
1	2	40.5	Programmed	1	Euploid Blast	LB	0
		41.8	Programmed	1	Euploid Blast	LB	
2	4	42.5	Programmed	1	Euploid Blast	SAb	4 euploid
		42.9	Programmed	1	Euploid Blast	LB	
		44.6	Natural	1	Euploid Blast	LB	
		46.8	Natural (Letrozole)	1	Euploid Blast	Ong	
3	2	44.3	Programmed	1	Euploid Blast	LB	1 euploid
		46.5	Programmed	1	Euploid Blast	LB	
4	2	43.8	Programmed	3	Untested Blast	LB	0
					Morula		
					Morula		
		45.5	Programmed	4	Untested Blast	LB	
					Untested Blast		
					Morula		
					Morula		
5	4	40.3	Programmed	1	Untested Blast	hCG < 1	2 euploid
		40.4	Programmed	2	Untested Blast	hCG < 1	
					Untested Blast		
		40.9	Programmed	1	Euploid Blast	LB	
		43.6	Programmed	1	Euploid Blast	LB	
6	3	41.9	Programmed	2	Untested Blast	LB	Oocytes and embryos donated to research
					Untested Blast		
		44.6	Programmed	1	Euploid Blast	hCG < 1	
		44.7	Programmed	1	Euploid Blast	LB	

Pt, patient; *y*, years; *Blast*, blastocyst; *LB*, live birth; *SAb*, spontaneous abortion; *Ong*, ongoing

guidelines from the American Society for Reproductive Medicine (ASRM) recommend an age limit of 55 among recipients utilizing oocyte or embryo donation [9]. As women increasingly return to utilize their cryopreserved autologous oocytes, boundaries will undoubtedly be challenged. Medical risks and longevity must be weighed against reproductive autonomy, and these conversations will likely become increasingly necessary as women return to use their cryopreserved oocytes. Regardless of the gamete source, patients considering pregnancy at advanced reproductive ages should undergo medical evaluation as well as counseling regarding risks including hypertensive disorders, diabetes, operative deliveries, overall morbidity, and mortality [10–12]. The women described in this case series were healthy without pre-existing medical conditions, having received extensive counseling from their reproductive endocrinologist and in most cases MFM specialists and primary care physicians.

Four of the patients in this series returned with a partner (one used donor sperm in a same-sex relationship) and two used donor sperm without a partner. In a survey of women who underwent OC at our center, 72% stated that since completing their cycle they thought more highly of parenthood, had made it more of a priority, and had been made more open to other methods of having children [13]. It is possible that parenthood becomes more salient as women take an active role in their fertility through the process of OC. Initial fertility preservation consultation should include options counseling regarding immediate conception with donor sperm, but many women who will ultimately pursue this route may not be ready to do so at the time of the initial visit. The cornerstone of reproductive autonomy is the ability to decide the time and setting in which parenthood is ideal for that particular individual.

Given the combination of delayed childbearing, advanced cryopreservation techniques, and complex social relationships, gamete disposition should be clearly defined prior to oocyte cryopreservation. In the present series, two patients used all of their cryopreserved oocytes, one patient had remaining oocytes and embryos which were donated to research, and the other three patients still have euploid embryos available. Discussion of patient wishes and precise delineation of embryo/gamete disposition in the event of unexpected life situations such as death or divorce are essential to ART.

It is important to note that the six patients described in this case series do not represent the norm in terms of OC outcomes. All except one underwent PGT-A, which is often reserved for oocyte thaw/warming patients expected to have supernumerary embryos. Of the five who underwent PGT-A, all had more than one euploid embryo available for transfer which also may not be a typical outcome. Indeed, at our center, about 40% of oocyte thaw patients in their age group who intend to do PGT-A do not have any euploid embryos available. It is important that women understand this distinction as many studies have demonstrated the tendency to overestimate success of ART procedures [14–16], and it would be a disservice to offer patients a false sense of security. Recent data suggests a complex relationship with decision regret among women cryopreserving oocytes, further supporting the importance of accurate counseling with regard to typical patient outcomes [17, 18].

It is particularly important to note that the mean age of the patients in this series was 38.8 years, and logic would indicate that such success would occur among patients at younger ages. The mean age in this series is consistent with the mean patient age at OC in our center during that period, but over time we have observed a linear decrease in age at OC from 40.0 years in 2005 to 37.9 years in 2013 [19]. In our experience, only about 10% of women who have undergone oocyte cryopreservation have yet returned for thaw/warming. It is possible that women who cryopreserved oocytes at earlier ages have been able to conceive spontaneously and thus may not yet have returned to attempt pregnancy with cryopreserved oocytes [13]. Patients who cryopreserve oocytes at older ages are by definition not attempting pregnancy until more advanced reproductive ages and are thus more likely to require the use of their cryopreserved oocytes for their first pregnancy and especially for subsequent gestations.

Several studies have attempted to characterize the number of oocytes needed to achieve a live birth with probability modeling based on donor oocyte as well as IVF data [20] and center-specific oocyte thaw statistics [4]. While age is clearly predictive of success in all aspects of ART, the number of oocytes cryopreserved is also a major determinant and is one underlying commonality among the six women described. In this group of patients achieving more than one singleton gestation with cryopreserved oocytes, on average 32 MII oocytes were cryopreserved over 2.5 cycles at an average age of 38.8. The process of OC, as with all of ART, is relatively inefficient. Cryopreserved oocytes are subject to attrition at the stage of thaw/warming, fertilization, impaired progression to blastocyst compared to IVF with fresh oocytes, age-related aneuploidy, and implantation [5]. Because of this serial attrition, one's total number of cryopreserved oocytes is critical to success. These predictive probabilities may offer helpful information which can be used as an adjunct in individualized counseling about expected outcomes and possible need for multiple cycles. As more oocyte thaw data is published, the strength of these counseling tools will be improved.

Fertility awareness is of paramount importance as women increasingly delay childbearing. OC as a means of fertility preservation has made significant advances in recent years, and as this case series demonstrates, may help women and couples achieve multi-child families at advanced reproductive ages.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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