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ORIGINAL ARTICLE

Non-medical oocyte cryopreservation at a single center in Japan: 8 years of experience

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Abstract

Purpose: Increasing numbers of women are opting to undergo non-medical oocyte cryopreservation (NMOC). In this report, we present experience at our clinic and discuss NMOC in Japan.

Methods: We followed the progress of 403 women who underwent NMOC at our clinic between 2014 and 2021, totaling 592 reproductive cycles.

Results: In total, 61 women underwent oocyte warming and fertility treatment. Of these, 13 women gave birth to 14 children. The median age at first oocyte cryopreservation was 38.3 years, and the oldest pregnant woman was 42 years. Most clients (60%) were in their late 30s. The median time between first oocyte cryopreservation and warming was 3.0 years. One woman was able to achieve a live birth with four vitrified oocytes.

Conclusions: This is the first report in Japan documenting pregnancies and childbirths resulting from NMOC. Ideally, women hope to achieve natural pregnancy between 20 and 32 years of age. NMOC is an option for individuals who are unable to pursue pregnancy during optimal reproductive years and wish to preserve their fertility for future attempts. NMOC is recommended in cases with few indications, and it is necessary to continue accumulating data on its long-term safety and effectiveness.

KEYWORDS
clinical outcome, egg freezing, fertility preservation, non-medical oocyte cryopreservation, vitrification

1 | INTRODUCTION

Since around 2005, the vitrification method for oocyte cryopreservation (OC) has been established, and pregnancies and births were reported by Kuwayama et al.[1] and Kyono et al.[2] Initially, medical oocyte cryopreservation (MOC) was conducted for patients with malignant tumors; on the other hand, non-medical oocyte cryopreservation (NMOC) was also started for women who were concerned about declining ovarian function due to aging.

With the development of reliable OC techniques and confirmation of the health of resulting offspring,[3] recommendations of NMOC were issued by the European Society of Human Reproduction and Embryology (ESHRE) in 2012,[4] and guidelines were published by the American Society for Reproductive Medicine

(ASRM) in 2013.[5] In Japan, guidelines were presented by the Japan Society of Reproductive Medicine (JSRM)[6] in the same year as the ASRM announcement, which regulated MOC and NMOC, as well as requirements for facilities performing OC and storage.

In recent years, NMOC has become increasingly widespread, and the number of cases has risen in Japan. The advantages of NMOC include the opportunity to pursue self-fulfillment and personal goals first and to protect the future possibility of giving birth from age-related declines in fertility. However, there are some concerns about NMOC such as high cost, uncertainty about future pregnancy and childbirth, and unknown long-term prognosis of offspring.

At our clinic, as of the end of 2021, we had assisted over 400 patients with NMOC. Here, we report the results of NMOC and oocyte warming conducted at our clinic and discuss the practice of NMOC in Japan.

2 | MATERIALS AND METHODS

For non-medical reasons, 403 women underwent cryopreservation of 592 oocytes at Kyono ART Clinic Takanawa between 2014 and 2021. Ovarian stimulation protocol and the dose were determined on the basis of age and ovarian reserve (AMH levels and antral follicle count (AFC)). The GnRH antagonist protocol was the primary ovarian stimulation protocol employed, and the starting dose of FSH was maintained at 150–225 IU. Mild stimulation or natural cycles were used in cases when age is >40 years, AMH is <1.0 ng/mL, AFC is <5, or in poor responders. The starting FSH dosage was modified to 75–225 IU. When considering the patient's preferences to minimize the use of injections, we used oral medication, such as clomiphene (Clomid; Fuji Pharma, Japan) or letrozole (Femara; Novartis, Switzerland). We have expended great effort to obtain the maximum number of oocytes to prevent moderate to severe ovarian hyperstimulation Syndrome (OHSS). In cases of PCOS patients or when blood estrogen level exceeds 3000 pg/mL, only GnRH agonist was used as a trigger.

All retrieved oocytes were cryopreserved using the Cryotop method for vitrification. The post-warm survival rate when using Cryotop in our clinic was 94%. When oocytes were warmed, intracytoplasmic sperm injection (ICSI) was used to fertilize all oocytes. After ICSI, fresh single embryo transfer was generally conducted within 2–3 days. If cryopreservation was chosen, embryos were vitrified at the blastocyst stage, and a single embryo was transferred. For fresh early-stage embryo transfers of warmed, autologous oocytes, a programmed or hormone-replaced protocol was used. For vitrified-warmed blastocyst transfer, a programmed, natural cycle, or modified natural cycle (with the letrozole protocol) was used. In programmed cycles, patients received hormone therapy with a 6-mg daily dose of oral estrogen (Progynova, Bayer, Germany) until the endometrium thickened to 8 mm. After that, estrogen with progestin (a 30-mg daily dose of oral tablet (Duphaston; Abbott Japan LLC, Japan) or a 300-mg daily dose of vaginal tablet (Lutinus; Ferring Pharmaceuticals, Switzerland) were continued until a negative pregnancy test or until 10 weeks of gestation. In natural cycles, follicular growth and hormone titers were monitored until the endometrium reached 8 mm in thickness and the dominant follicle measured 18 mm. Ovulation was then triggered with human chorionic gonadotropin. After ovulation, progestin was supplemented and vitrified-warmed transfer was performed on day 6 of progestin supplementation.

3 | RESULTS

A total of four hundred three women (592 cycles) underwent NMOC. The average age at first oocyte cryopreservation was 38.3 years. One hundred five women with their partners returned to our clinic with a desire for children by the end of 2021. Sixty-one women underwent oocyte warming and ICSI with their partners. The average age at oocyte warming was 42.1 years. The median time between the first cryopreservation and warming was 3.0 years. The return rate was 26% (105/403) and the utilization rate was 15% (61/403) (Figure 1).

The most prevalent age group was women in their late 30s, and this age group comprised 200 women, accounting for approximately half of all clients (Figure 2). The age at a first cryopreservation tends to become younger year by year, indicating a trend toward NMOC at younger ages (Figure 3). As for NMOC, 25–67 cases per year have been implemented since 2014, with a remarkable increase after 2021.

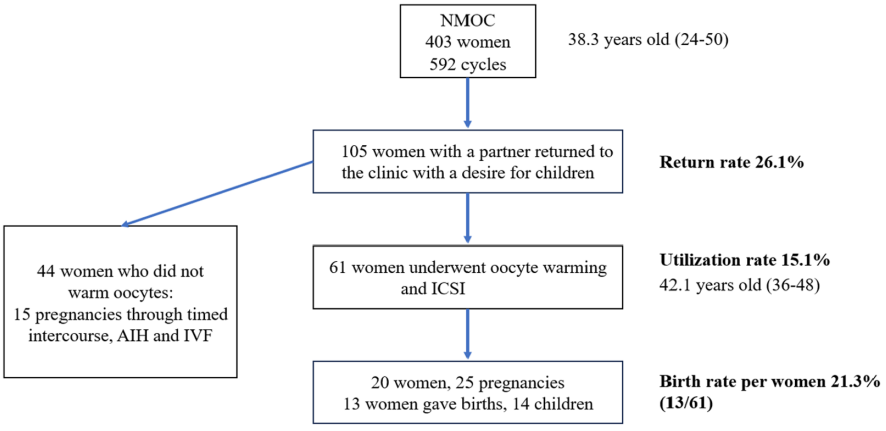

FIGURE 1 Flowchart showing women who underwent non-medical oocyte cryopreservation (NMOC, oocyte warming, and ICSI treatment).

The average number of retrieved oocytes per cycle was 9.2 for women in their early 30s, 7.4 in the late 30s, and 5.4 in the early 40s. The average number of cycles for ovum pick up (OPU) was 2 (1–5), with 1.3 for patients in their early 30s, 1.4 in the late 30s, and 1.6 in the early 40s. The average number of oocytes per woman was 8.5, with 6.3 for women under 30, 11.8 in the early 30s, 9.5 in the late 30s, and 6.6 over 40. There was a tendency for more oocytes to be retrieved at younger patient age, resulting in fewer ovarian stimulation cycles and a higher number of vitrified oocytes.

The frequency of OHSS was 27.9% (17/61) and symptoms were all mild. Patients who underwent OPU several times desired as many oocytes as possible to have more chances to become pregnant.

Among women who underwent oocyte cryopreservation at our clinic, 105 found a partner, and 61 patients underwent treatment with warmed oocytes. The median age at first cryopreservation of those 61 patients was 38.3, and median age at oocyte warming was 42.1 years (Figure 4). The median time between first oocyte cryopreservation and warming was 3.0 (1.0–4.7) years (Figure 5).

The remaining 44 cases did not undergo treatment with warmed oocytes for various reasons, including patient preference to start with conventional infertility treatments, unavailability of partner consent, or others.

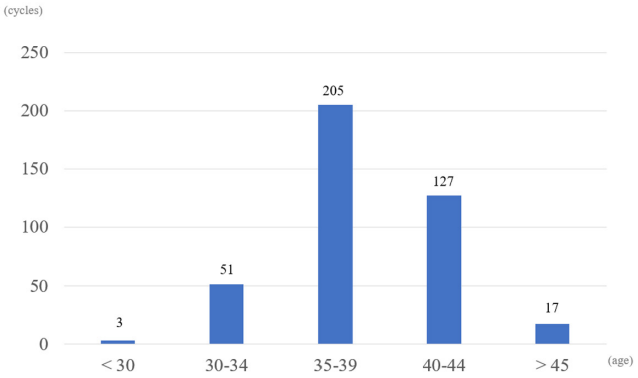

FIGURE 2 Numbers of women who underwent NMOC by age group.

In a total of 61 cases, ICSI was performed on 518 oocytes that survived after warming, resulting in 342 fertilized embryos. The overall survival rate per patient of warmed oocytes was 94% (range 79%–100%). The fertilization rate per patient under ICSI was 75% (38%–100%). Of the 61 cases using warmed oocytes, 49 cases underwent fresh early-stage embryo transfers, while 50 cases were cultured until the blastocyst stage. Of the 50 cases cultured to the blastocyst stage with 275 embryos, 40 cases yielded 120 blastocysts. The rate of reaching the blastocyst stage was 43.6%. The primary reasons for mainly choosing fresh early-stage embryo transfers included a shorter time to pregnancy and consideration of the risk of being unable to preserve embryos at the blastocyst stage.

Among the 61 women who underwent treatment, 20 became pregnant and 13 gave birth (14 babies: one woman delivered one child twice). The live birth rate was 21.3% (13/61). Figure 6 shows the pregnancy and birth rate of each age group. Table 1 shows the details of 13 deliveries from NMOC. The age of the oldest pregnant women at a first oocyte cryopreservation and at embryo transfer following ICSI with warming oocytes was 42 years and 46 years, respectively. Of the 44 women who did not warm oocytes, 15 achieved pregnancies through methods such as intercourse timing, artificial insemination, or in vitro fertilization (Figure 1).

4 | DISCUSSION

Thirteen women delivered 14 children from NMOC. The return rate was 26% and the utilization rate was 15%. Here, we discuss safety, ovarian stimulation, the ideal number of vitrified MII oocytes, age at cryopreservation, and future challenges.

4.1 | Safety

As mentioned above, the frequency of OHSS was 27.9% (17/61) and symptoms were all mild. Erica Velthuis et al., reported 130 of the

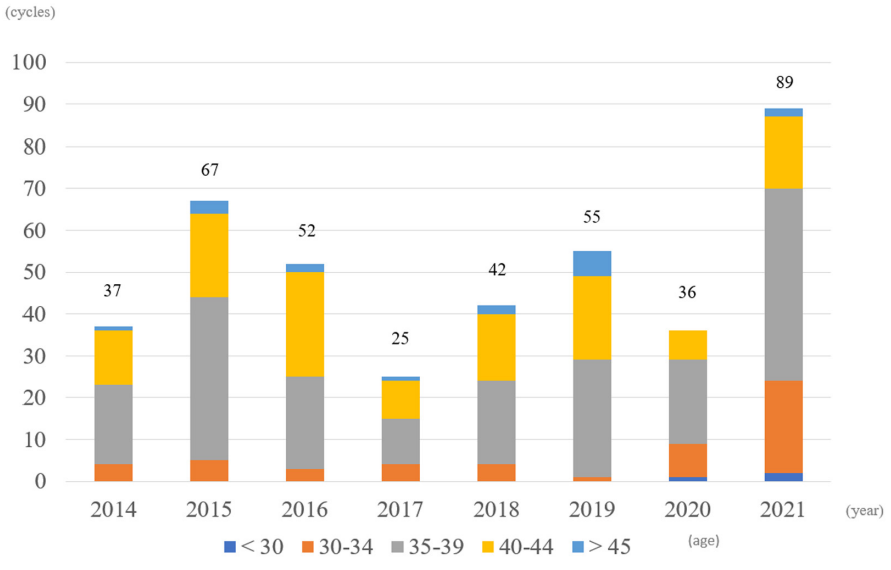

FIGURE 3 Numbers of women who underwent NMOC by year.

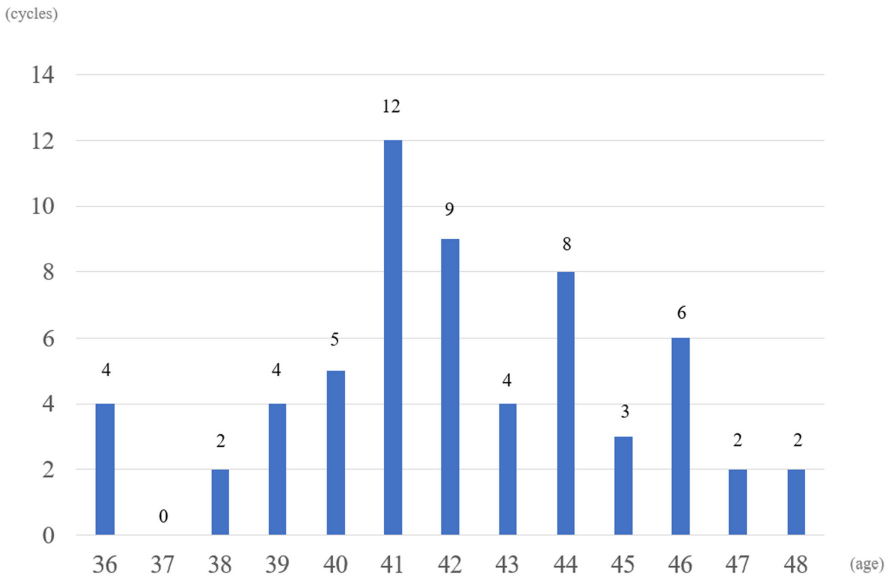

FIGURE 4 The age at warming of oocytes.

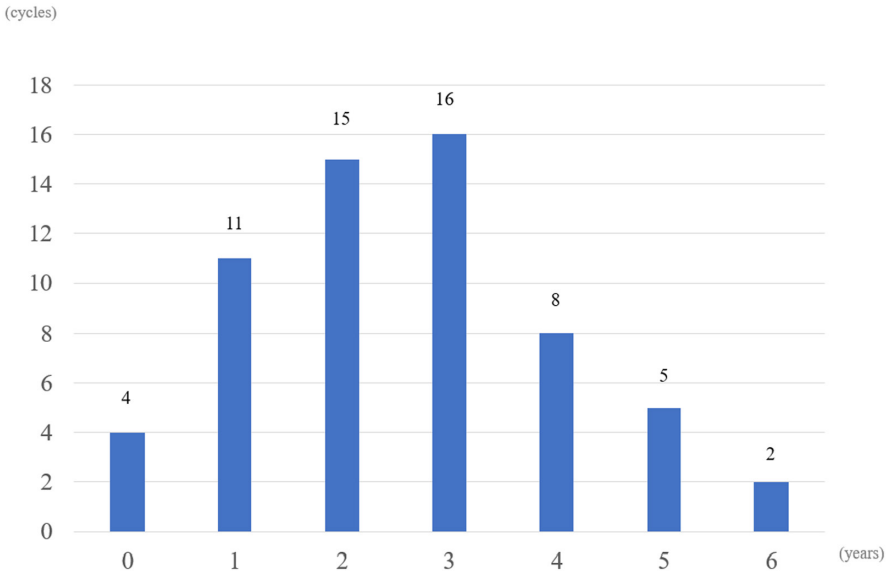

FIGURE 5 The period between first oocyte cryopreservation and warming.

1110 OHSS cases in the Global Safety Database of Merck KGaA, Darmstadt, Germany, were classified as severe,[7] which is 11.7%. Therefore, our incidence rate is considered not significant. In terms of OHSS reduction, it has been effective to use GnRH agonist alone as the trigger, and since 2022, we have added cabergoline (Cabaser, Pfizer, USA), letrozole, and relugolix (Relumina, Aska pharma, Japan) after oocyte retrieval to reduce the risk of OHSS. We are continuously mindful of the balance between the number of retrieved oocytes and frequency of OHSS.

4.2 | Ovarian stimulation

The GnRH antagonist protocol is excellent, which allows for flexibility in adjusting the number of retrieved oocytes and in reducing OHSS based on trigger selection. This protocol is the best one as of this moment. Since 2022, we have routinely used the Progestin-Primed Ovarian Stimulation (PPOS) method. A progestin medication, a 2-mg daily dose of oral tablet chlormadinone acetate (Lutral, Fuji Pharma, Japan), was used with FSH stimulation. The PPOS method can effectively suppress LH surges through the oral intake of progestin, and, in many cases, it prevents severe OHSS, and allows to obtain a number of oocytes close to the target.[8]

4.3 | Ideal number of oocytes to vitrify to achieve more successful pregnancies in the future

We compiled the number of cryopreserved oocytes by age for individuals who achieved pregnancy at our clinic. Focusing on the group age 38 and above, more successful pregnancies were observed with 5 to 9 cryopreserved oocytes. Considering the risk of OHSS, we believe that cryopreservation of 10–15 oocytes per retrieval is a reasonable target number.

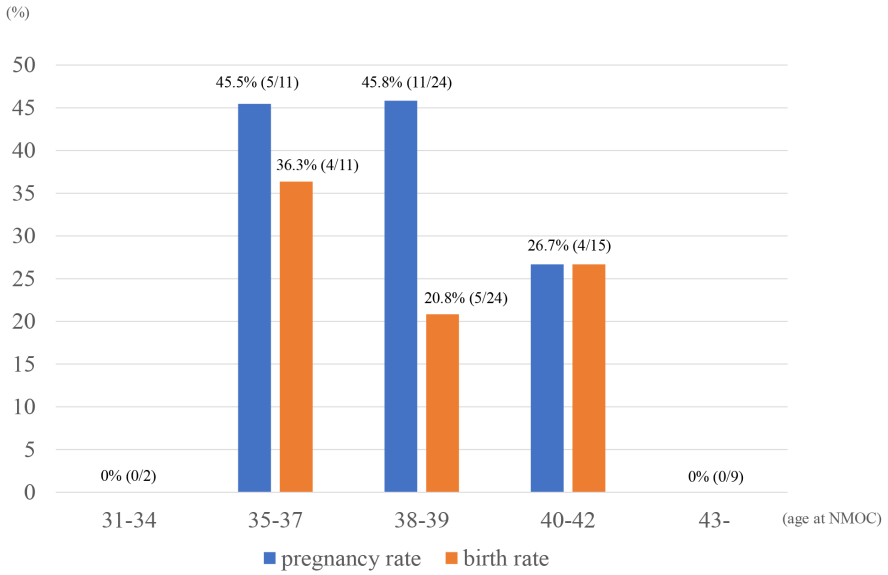

FIGURE 6 Pregnancy and birth rate: no. of pregnancy and delivery per women.

According to a report from New York University, one of the factors contributing to successful childbirth was having "20 or more mature (MII) oocytes".[9] Cobo et al. demonstrated that in cases of prospective mothers under 35 years of age, the cumulative pregnancy rate increased with the number of vitrified oocytes. When ≧15 oocytes were vitrified, the cumulative pregnancy rate reached 85.2%.[10]

4.4 | Whether older women can become pregnant by NMOC

Japan Society of Obstetrics and Gynecology has reported that the oocyte retrieval rate for NMOC was 89.9%–96.9%, the survival rate after vitrification and warming was 86.0%–96.8%, the fertilization rate was 71%–79%, the implantation rate was 17%–41%, and the birth rate per oocyte was 4.5%–12.0%.[11]

At our clinic, three patients gave birth at ages 43–45 (vitrified at 39–42) and one at 46 (vitrified at 41). We have observed several cases of women who began with conventional ART, but were unsuccessful, who later became pregnant after treatment using vitrified-warmed oocytes. This suggests that when vitrifying oocytes at younger ages, patients are more likely to become pregnant and obtain live births, even if embryo transfer was conducted at an older age. However, it is well known that younger oocytes at the time of vitrifying result in a higher fertility rate. At our clinic, 21.3% of women successfully gave birth from the treatment, and most of them had undergone oocyte cryopreservation in their late 30s.

In the report of New York University, one of the factors contributing to successful childbirth was "(oocyte) freezing age below 38 years."[9] Gruhn et al. showed the higher rate of chromosomal normalcy of oocytes at between the ages of 20 to 32,[12] and the guidelines of the JSRM (as of 2023) wrote that collection of unfertilized oocytes from women aged 35 or above is not recommended. Additionally, use of vitrified oocytes beyond maternal age 45 is also not recommended, due to increased risk of pregnancy complications. Based on these reports, from a medical perspective, it is recommended to consider NMOC before a significant increase in chromosomal abnormalities begins, around the age of 35–36 years, and for pregnancy before the age of 40 to reduce the risk of pregnancy complications. On the other hand, considering cost efficiency, including storage fees until transplantation, some reports described that the most appropriate time for vitrification is age 38.[13] Our clinic data also showed that the most common age at NMOC was around 38 years.

4.5 | Future challenges

There appears to be a significant difference in the utilization rate of vitrified oocytes at our clinic (15%) and New York University (61%) (Table 2).[9,14,15] There are several reasons for this. First, younger women have fewer opportunities to use their vitrified oocytes, since they are more likely to achieve results through conventional infertility treatments. Additionally, some women may face challenges in finding a partner, which can hinder them in starting oocyte warming treatment. In some countries, where single motherhood is more accepted, there is a significant increase in success rates for infertility treatments using donor sperm, especially for women in their late 30s. Findings from the report by New York University support this observation. Currently in Japan, only married couples or couples in legally recognized partnerships are allowed to undergo fertility treatments using vitrified oocytes. To utilize vitrified oocytes in Japan, women need to find a partner, agree on pregnancy, childbirth, and parenting, and facilitate their careers. It is indeed a challenging situation with significant hurdles.

TABLE 1 Details of 13 women who delivered 14 children using non-medical oocyte cryopreservation.

Age at NMOC	Number of vitrified oocytes	Age at warming	Period until warming (years)	Pregnancies from fresh transfer	Pregnancies from FBT	Births from fresh transfer	Births from FBT	Miscarriage
35	6	36	1		1		1	
35	4	38	2.2	1	0	1		
35	16	39	4.7	1	0	1		
37	18	41	3.9		1		1	
38	24	40	1.8	1	1	1		1
38	8	41	2.6	0	2	0	2	
38	7	41	2.6	0	2	0	1	1
39	6	42	2.4	0	1		1	
39	17	43	4.3		1		1	
40	23	41	1.3		1		1	
41	19	44	3.7	1	1		1	1
41	13	46	4.6	0	1		1	
42	6	44	2.4	1	0	1		

TABLE 2 Comparison of three centers using non-medical oocyte cryopreservation.

	Hashimoto (2022)[14]	Cascante et al. (2022)[9]	
	Kyono ART Clinic (unpublished)	New York University	Doyle et al. (2016)[15]
Usage rate (%)	15	61	
Age at a first OC (years old)	38.3 (24–50)	38.3 (27–44)	34.9
Period: 2014–2021	April 2014–December 2021	2006–December 31, 2020	August 2009–January 2015
Age at warming (years)	42.1 (36–48)	42.6 (41–44.3)	>2 years vs. 8 months
Survival rate (%)	94	79	86.1
Fertilization rate (%)	75	66	69.5
Number of cryopreserved oocytes per person	8.5	>20	8
	Vitrification	Vitrification: 72%, Slow: 4%, Both: 24%	Vitrification
Live birth rate (%)	21.3 per patient	39 per patient	38.6 per transfer
Remarks	A birth following four MII oocytes	60% Euploid embryo transfer	Two group: fertility preservation vs. infertility
Frequency of OHSS	27.9% (17/61): all mild	<38 years or MII > 20	15–20: <38 years
Recommendations of numbers of MII oocytes giving them 70% chance of at least one live birth	10 ~ 15		25–30: 38–40 years

Based on results and the current situation described above, natural conception and childbirth in their early twenties are ideal. However, for many modern women, the reality is different from the ideal, and pregnancy and childbirth after the age of 35 years have become commonplace. Furthermore, there is a growing emphasis on diversity, such as not desiring marriage, desiring to remain single, and wanting children independently. For unmarried individuals, aged 25 and above, who plan to have children, oocyte cryopreservation is indicated. We also hope that donor sperm can be accepted as one of the options for child-bearing in Japan like other countries that value diversity. At the same time, we believe that education about fertility and age should be provided to both girls and boys starting from elementary school.

5 | CONCLUSIONS

This paper is the first report in Japan documenting pregnancies and childbirths resulting from NMOC. Ideally, it is recommended that women try to achieve natural pregnancy between the ages of 20 and 32, when oocyte chromosomal normalcy is greatest. NMOC can be an effective option for individuals who are unable to pursue

pregnancy during optimal reproductive years and wish to preserve their fertility for future attempts. Further research and reports are awaited to determine the optimal age for oocyte cryopreservation in terms of success rates and economic considerations. We have previously reported two live births after 6 and 13 years from 10 mature oocytes vitrified at age 20[16] as well as follow-up of children born from vitrified-warmed oocytes.[17] We intend to continue our research on the use of vitrified oocytes. In conclusion, 13 women who underwent non-medical oocyte cryopreservation (NMOC) at a single center delivered 14 children.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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