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## Chapter

# Efficiency of Autologous Egg Cryopreservation: Eight Years' Experiences and Clinical Outcomes

*Cassie T. Wang, Xiangli Niu, Qiuyan Ruan  
and Wei-Hua Wang*

## Abstract

Oocyte cryopreservation is one of the state-of-art technologies in human reproductive medicine, which brings opportunities for women to preserve their fertility. In the present study, we analyzed the efficiency and outcomes of 8 years' autologous egg cryopreservation: Frozen oocytes were warmed from 120 cycles and oocyte survival, fertilization, blastocyst development, clinical pregnancy, embryo implantation, live birth rates and birth weights were collected based on the patients' ages of <35, 35–37 and > 37 years old. The details of oocyte cryopreservation and the efficiency were further analyzed based on different patient categories. During the study period, 849 oocytes from 120 cycles were warmed. Oocyte survival, fertilization, and blastocyst development were not affected by women's ages at the time of cryopreservation. However, number of patients without blastocyst formation was significantly ( $P < 0.05$ ) higher in patients >37 years old (31.2%) than that in patients <35 years old (13.1%). Higher live birth rates were observed in patients <35 (51.1%) and 35–37 (46.7%) years old than in patients >37 years old (28.6%) after fresh embryo transfer. Some patients did not have blastocysts mainly due to low fertilization by poor sperm or small number of oocytes warmed. These results indicate that the efficiency of oocyte cryopreservation, evaluated by live birth and embryo implantation rates is affected by women's age, number of oocytes warmed and sperm quality.

**Keywords:** Oocyte cryopreservation, Fertility preservation, Fresh embryo transfer, Frozen embryo transfer, Implantation

## 1. Introduction

Oocyte cryopreservation is one of the state-of-art technologies in human assisted reproductive technologies (ART), which provides opportunities for women to preserve their fertility. Recently, the demand for oocyte cryopreservation has increased significantly, especially in women who want to delay childbearing for medical or no medical indications [1–9].

It has been reported that survival, fertilization, embryo development and pregnancy rates of cryopreserved/warmed human oocytes are similar to those of fresh oocytes, especially in young women and oocyte donors [10, 11]. The rates

of chromosomal abnormalities (embryonic aneuploidies), birth defects, or developmental deficits in offspring born from cryopreserved oocyte in vitro fertilization (IVF) were similar with those from fresh oocyte IVF [12]. However, fewer blastocysts were observed when cryopreserved oocytes were used for IVF as compared with fresh oocytes in patients who used autologous oocytes [13]. Furthermore, large clinical data reported by the Society for Assisted Reproductive Technology (SART) in USA indicated that fresh donor oocytes produced significantly higher live birth rate than cryopreserved donor oocytes [14] and the equivalency between fresh and cryopreserved oocytes still need more data to support [15].

As a new technology in human IVF, oocyte cryopreservation is still a challenge for IVF laboratories as it adds more laboratory manipulations on oocytes including cryopreservation and warming. Furthermore, the optimal time for oocytes to be cryopreserved after retrieval and for oocytes to be inseminated after warming may be different between patients. Therefore, differences in laboratory protocols may make the efficiency different and a case-specific protocol may be necessary to obtain the best outcome. More information remains to be collected whether oocyte cryopreservation will be widely offered to healthy women at any age as an approach to preserve fertility and delay childbirth [16, 17].

More women would like to give birth in their late 30s [4, 18]. However, women's fertility dramatically declines when they reach their late 30s, and further declines in their early 40s [4]. This phenomenon increases the demand for women to preserve their fertility by oocyte cryopreservation before their fertility declines [4, 18]. Although fertility preservation could benefit women who have hematologic diseases, breast cancer, some pelvic cancers and systematic diseases requiring chemotherapy, radiotherapy or bone marrow transplantation [2, 3, 19, 20], most users of this technology are healthy women who want to postpone childbearing [2, 3, 7, 8, 17, 18, 21, 22].

On the other hand, oocyte cryopreservation is offered not only to women for fertility preservation [7, 8, 21–23], it has also been offered to donor banks [24, 25], IVF patients as a backup technology. For example, in cases where semen sample may not be available on oocyte retrieval day, no motile sperm found in a semen sample, or there are not enough motile sperm for insemination of all oocytes retrieved. Some patients may produce a high number of oocytes and do not want to inseminate all, and some patients may want a limited number of oocytes to be fertilized [23]. Therefore, oocyte cryopreservation is required under various situations. Accordingly, the analysis of efficiency of clinical outcomes with cryopreserved oocytes becomes difficult.

The efficiency of human oocyte cryopreservation has been widely studied in donor oocytes and most data were collected from fresh embryo transfer [10, 11, 14, 15]. However, as preimplantation genetic testing for aneuploidies (PGT-A) and other genetic testing are very common in human IVF, it is required that biopsied embryos for testing are cryopreserved for later frozen embryo transfer (FET). Although cryopreservation by vitrification of human embryos from fresh oocytes does not affect embryo implantation [26–28], there is still no published evidence to address whether cryopreservation of embryos from frozen/warmed oocytes disturb embryo implantation or these embryos have a similar implantation as fresh embryos. Therefore, in the present study, we compared fresh blastocyst transfer and frozen/warmed blastocyst transfer to examine whether double cryopreservation (both oocyte and blastocyst cryopreservation) has a similar efficiency as single cryopreservation (oocyte cryopreservation) based on women's ages at the time of oocyte cryopreservation.

## **2. Methods**

### **2.1 Ethics approval and consent to participate**

All patients undergoing oocyte cryopreservation, warming for IVF, embryo culture, and embryo transfer signed informed consents for all laboratory and clinical procedures. All procedures were approved by Houston Fertility Institute's research and clinical committee. The data were retrospectively collected from the medical records and patients' information were not included in the data presentation, so the IRB was waived for this study.

### **2.2 Patients and data collection**

Autologous oocyte cryopreservation was assessed in women whose oocyte cryopreservation and warming were performed between 2009 and 2017. Women's age at the time of oocyte cryopreservation was divided into 3 groups, <35, 35–37 and > 37 years old. Based on these age groups, data were compared between fresh blastocyst transfer and frozen/warmed blastocyst transfer.

There were mainly three reasons for patients to cryopreserve their oocytes: 1) all oocytes were cryopreserved because there was no motile sperm or no semen sample being collected at the time of oocyte retrieval; 2) partial oocytes were cryopreserved because no enough motile sperm was found to inseminate all of the oocytes, or patients required to inseminate a portion of oocytes and purposely required to cryopreserve remaining oocytes; and 3) all oocytes were cryopreserved for single women for fertility preservation. Therefore, the data were further analyzed based on these three categories.

### **2.3 Oocyte cryopreservation, warming and insemination**

Oocyte cryopreservation and warming were based on the procedures previously reported [11] by using commercial vitrification and warming kits (Fujifilm-Irvine Scientific, CA, USA). Briefly, for cryopreservation, matured oocytes were vitrified 4–5 hours after retrieval with initial equilibration of the oocytes in equilibration solution (ES) for 9 mins, and then in vitrification solution (VS) for 90 seconds until vitrification in Cryotop.

For warming, Cryotops were removed from liquid nitrogen and the tips with oocytes were quickly placed in 1 ml thawing solution (TS) at 37°C for 1 min. Oocytes were then transferred to 0.5 ml dilution solution (DS) for 3 min and then to a 0.5 ml washing solution (WS) for 10 min with a solution change after 5 min. After warming, oocytes were washed in Global medium (IVFonline, CT, USA) supplemented with 10% serum protein substitute (SPS, IVFonline) and then cultured in the same medium until insemination. Oocyte survival was evaluated based on morphology after completion of the warming.

### **2.4 Insemination, fertilization assessment, embryo culture and fresh blastocyst transfer**

All oocytes were inseminated by intracytoplasmic sperm injection (ICSI) 2–3 hours after warming. We chose this time for ICSI as it has been reported that most functions, such as meiotic spindle recovery, mitochondria activity and ATP level recovery in frozen/warmed oocytes, take about 2–3 hours after warming [29–31] and it has been found that ICSI time ( $9 \pm 2$  h) after oocyte retrieval in the vitrified human oocytes does not affect clinical outcomes [32].

Fertilization was examined 16–18 h after ICSI and normally fertilized zygotes were cultured in Global medium supplemented with 10% SPS at 37°C in a humidified atmosphere of 5.5% CO<sub>2</sub>, 6% O<sub>2</sub> and balanced nitrogen until Day 7 (some patients' embryo culture was extended to Day 7 if morula or early blastocysts were observed at Day 6). On Day 3, embryo cleavage status was examined, and all embryos that divided to two cells and above were considered as cleaved embryos and were transferred to freshly prepared culture medium. On Day 5, embryo development was evaluated and the best 1 or 2 embryos were transferred.

## **2.5 Blastocyst biopsy for PGT-A**

Blastocysts were biopsied at Days 5, 6 and 7 for PGT-A in some patients based on patients' requests and FET were performed in these patients if there were euploid blastocysts. After biopsy, blastocysts were vitrified individually, and biopsied samples were analyzed with DNA microarray or next generation sequencing by commercial genetic testing companies.

## **2.6 Blastocyst vitrification, warming and transfer**

Blastocysts were vitrified and warmed using commercial vitrification and warming kits (Fujifilm-Irvine Scientific). For vitrification, both ES and VS were warmed in original vials at 37°C for at least 30 min before use. Briefly, collapsed blastocysts by a laser pulse were equilibrated in 100 µl drop (without oil cover) of ES for 2 min, and then 45 seconds in 100 µl drop (without oil cover) of VS (both steps were performed on a 37°C warming stage) before loading to vitrification devices. The devices were then immersed to liquid nitrogen for vitrification and all samples were stored in liquid nitrogen until warming for FET.

For warming, blastocysts were exposed to a TS at 37°C for 1 min, transferred to a DS for 3 min and finally to a WS for 10 min with a solution change after 5 min at room temperature. After completion of the warming process, blastocysts were cultured in Global medium supplemented with 10% SPS for 2–4 h before transfer. For blastocyst transfer, we selected the best quality of blastocyst for transfer regardless of Day 5, 6 or 7. However, if the blastocysts had the same quality, Day 5 blastocyst is preferred than Days 6 and 7 blastocysts. If embryos after PGT-A were transferred, we used same embryo selection criteria but only euploid blastocysts were transferred.

## **2.7 Patient preparation for fresh and frozen/warmed blastocyst transfer**

All patients for embryo transfer received estradiol orally and transvaginally. Intramuscular administration of progesterone oil was initiated at about Day 14 of estradiol treatment. Endometrium thickness was measured on the day of progesterone administration. Embryo transfer occurred on the sixth or seventh day of progesterone administration and progesterone was continued until the first serum β-hCG test two weeks after transfer. Pregnancy was assessed 14 days after embryo transfer by a serum β-hCG assay. When the β-hCG was >5 mIU/ml, the patients were regarded as having a biochemical pregnancy and pregnancies were supported by continued estradiol and progesterone. Four weeks after embryo transfer, when a gestational sac and a heartbeat appeared ultrasonographically, the patients were diagnosed as having a clinical pregnancy. Live birth rates were calculated based on healthy baby delivery per transfer.

## 2.8 Statistical analysis

Interval data was analyzed by one-way analysis of ANOVA. The differences between groups were compared with chi square test. If the P value was less 0.05, the difference was considered to be statistically significant.

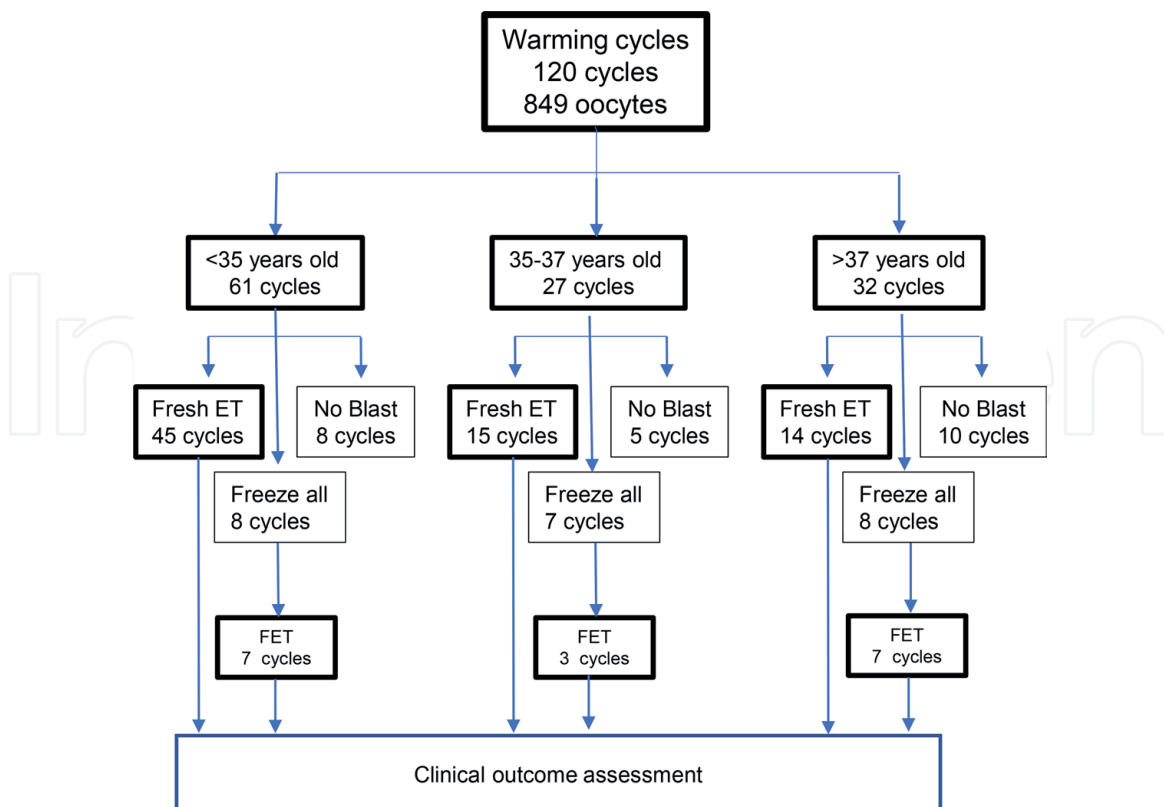
## 3. Results

As shown in **Figure 1**, 849 oocytes from 120 patients were warmed, 61 cycles were from women <35 years old, 27 cycles were from women 35–37 years old, and 32 cycles were from women >37 years old. The resulting blastocysts were either fresh transfer (74 cycles) at Day 5 or cryopreserved at Day 5–7 (23 cycles) for later FET. Twenty-two patients had frozen all blastocysts due to PGT-A, and one patient (45 years old) had plan for fresh blastocyst transfer but did not have blastocyst at Day 5, so blastocyst transfer was canceled. However, one embryo developed to blastocyst at Day 7, so it was frozen and then processed for frozen/warmed blastocyst transfer.

A total of 67 blastocysts from 22 patients were biopsied and 55.2% (37) blastocysts were euploid after PGT-A. Euploid blastocysts in 16 patients and a Day 7 blastocyst without PGT-A in 1 patient were transferred in FET cycles.

Twenty-three cycles did not have embryo transfer due to no blastocyst formation, including no fertilization, no cleavage or arrested embryo development before blastocyst stage.

Women's ages at the time of oocyte cryopreservation on post-warming outcomes were shown in **Table 1**. It was found that average numbers of oocytes warmed



**Figure 1.** Diagram of patient population and cycle information. Patients were grouped based on ages of <35, 35–37 or > 37 years old at the time of oocyte cryopreservation. Only the first embryo transfer (either fresh or FET) was included in the data analysis.

Age	<35	35–37	>37
Cases	61	27	32
No. of oocytes warmed	439	195	215
Mean No. of oocytes	7.2 ± 6.0	7.2 ± 5.5	6.7 ± 3.7
No. of oocytes survived (%)	410 (93.4)	177 (90.8)	204 (94.9)
No. of oocytes fertilized (%)	292 (71.2)	128 (72.3)	149 (73.0)
No. of oocytes cleaved (%)	278 (95.2)	118 (92.2)	140 (94.0)
No. of blastocysts (%)	158 (56.8)	64 (54.2)	69 (49.3)
No. of patients without blastocysts (%) <sup>*</sup>	8 (13.1) <sup>a</sup>	5 (18.5)	10 (31.2) <sup>b</sup>

<sup>\*</sup>No. of patients without blastocysts includes all cases in which oocytes were not fertilized after ICSI, fertilized oocytes did not cleave, and cleaved embryos did not develop to blastocyst stage.  
<sup>ab</sup>Values are significantly different with different superscripts in the same row,  $P < 0.05$ .

**Table 1.**  
*Women’s ages at the time of oocyte cryopreservation on post-warming laboratory outcomes.*

(7.2 ± 6.0, 7.2 ± 5.5 and 6.7 ± 3.7, respectively), proportions of oocytes survived (93.4, 90.8 and 94.9%, respectively), fertilized (71.2, 72.3 and 73.0%, respectively), cleaved (95.2, 92.2 and 94.0%, respectively) and developed to blastocysts (56.8, 54.2 and 49.3%, respectively) were similar ( $P > 0.05$ ) among patients <35, 35–37 and > 37 years old. However, cancelation rates, which were determined by no available blastocyst for transfer were significantly ( $P < 0.05$ ) higher in patients >37 years old (31.2%) than in patients <35 years old (13.1%).

As significant differences in the cancelation rates were present in the three age groups, we further analyzed the detailed reasons of the cancelation. As shown in **Table 2**, it was found that oocyte survival after warming did not cause any cancelation, and all patients had survived oocytes after warming. However, cancelation was observed in 30.4% (7/23) patients without fertilization after ICSI, in 8.7%

Age	<35	35–37	>37	Total
No. of cases with no survived oocyte after warming (%)	0 (0)	0 (0)	0 (0)	0 (0)
No. of cases with no fertilization after ICSI (%)	5 (62.5)	1 (20)	1 (10)	7 (30.4)
No. of cases with no cleavage after fertilization (%)	0 (0)	1 (20)	1 (10)	2 (8.7)
No. of cases with no blastocyst (%)	3 (37.5)	3 (60)	8 (80)	14 (60.9)

<sup>\*</sup>No statistical differences were found between different age groups,  $P > 0.05$ .

**Table 2.**  
*Detailed reason analysis of patients without blastocysts<sup>\*</sup>*

No. of oocytes warmed	Patients’ age			Total
	<35	35–37	>37	
1–3	4 (50)	1 (20)	3 (30)	8 (34.8)
4–8	3 (37.5)	4 (80)	5 (50)	12 (52.1)
>8	1 (12.5)	0 (0)	2 (20)	3 (13.0)

<sup>\*</sup>No statistical differences were found between different age groups,  $P > 0.05$ .

**Table 3.**  
*Relationship between number of oocytes warmed and no blastocyst formation<sup>\*</sup>*

(2/23) patients without embryo cleavage and in 60.9% (14/23) patients without blastocyst development that was the main reason of cancelation. However, no statistical differences were found among three age groups.

Age	<35	35–37	>37
No. of transfer	45	15	14
No. of clinical pregnancy (%)	24 (53.3)	7 (46.7)	6 (42.9)
Mean No. of embryos transferred	1.6 ± 0.6	1.8 ± 0.5	1.71 ± 0.47
Implantation rate (%)	39.2	29.6	25.0
No. of live birth (%)	23 (51.1)	7 (46.7)	4 (28.6)
Mean birth weight (g)	3037 ± 805	3462 ± 674	2856 ± 516

<sup>\*</sup>*P* > 0.05 in all comparison groups within the same age group between transfers (only fresh embryo transfers were compared).

**Table 4.**  
 Women's ages at the time of oocyte cryopreservation on post-warming clinical outcomes<sup>\*</sup>.

Patient categories	All oocyte freezing (No motile sperm & no sperm) <sup>*</sup>	Partial oocyte freezing <sup>**</sup>	All oocyte freezing for fertility preservation <sup>***</sup>
No. of cases	37	67	16
Age	35.5 ± 5.7	33.4 ± 4.5	36.8 ± 3.7
No. of oocytes warmed	363	336	150
Mean No. of oocytes warmed	9.8 ± 5.8 <sup>a</sup>	5.0 ± 3.7 <sup>b</sup>	9.4 ± 4.4 <sup>a</sup>
No. of oocyte survived (%)	339 (93.4)	307 (91.3)	145 (96.7)
No. of oocyte fertilized (%)	251 (74.0) <sup>ab</sup>	207 (67.4) <sup>b</sup>	111 (76.6) <sup>a</sup>
No. of oocyte cleaved (%)	235 (93.6)	194 (93.7)	107 (96.4)
No. of blastocysts (%)	132 (56.2)	112 (57.7)	47 (43.9)
No. of cancelation (%)	5 (13.5)	14 (21.8)	4 (8.7)
No. of transfers	32	50	9
Mean No. embryos transferred	1.4 ± 0.8	1.6 ± 0.5	1.7 ± 0.5
No. of clinical pregnancy (%)	17 (53.1) <sup>ab</sup>	18 (36) <sup>b</sup>	8 (88.9) <sup>a</sup>
No. of live birth (%)	15 (46.9) <sup>ab</sup>	18 (36) <sup>b</sup>	7 (77.8) <sup>a</sup>
Implantation rate (%)	21/53 (39.6) <sup>ab</sup>	21/79 (26.6) <sup>a</sup>	11/15 (73.3) <sup>b</sup>

<sup>ab</sup>*P* < 0.05 at least in the same row with different superscripts.

<sup>\*</sup>Semen samples were not able to collect or samples did not have motile sperm, all oocytes were frozen.

<sup>\*\*</sup>Semen sample had motile sperm but sperm number was not enough to fertilize all of the oocytes or patients wanted to fertilize partial oocytes and to freeze the remaining oocytes.

<sup>\*\*\*</sup>All oocytes were frozen for fertility preservation.

**Table 5.**  
 Patient categories for oocyte cryopreservation and clinical outcomes.



As shown in **Table 3**, when oocyte number (1–3, 4–8 and > 8 oocytes per warming cycle) and cancelation were analyzed, it was found that no blastocyst formation was found in all groups: 34.8% with 1–3 oocytes, 52.1% with 4–8 oocytes and 13.0% with >8 oocytes. There is increased tendency that less cancelation was observed if more than 8 oocytes were warmed. However, no statistical differences were found among three age groups or three oocyte number groups.

As shown in **Table 4**, when fresh blastocyst transfers were compared in terms of clinical pregnancy, mean no. of embryo transferred, embryo implantation, live birth and birth weight in three age groups, there were no statistical difference be observed although the rates were lower in the patients at age of >37. FET cases in each age group were small (7, 3 and 7 cases for age of <35, 35–37 and > 37, respectively), the clinical pregnancies (2, 2 and 2 cases, respectively) and live births (2, 2 and 2 cases, respectively) were not included in the comparisons.

When the data were analyzed based on three categories of patients for oocyte cryopreservation, as shown in **Table 5**, it was found that the number of oocytes warmed and reasons for oocyte cryopreservation had significant impact on clinical outcomes. The patients who had partial oocyte cryopreservation had significantly ( $P < 0.05$ ) fewer oocytes to be warmed ( $5.0 \pm 3.7$ ) as comparing with patients who had all oocyte cryopreservation, including patients for backup oocyte cryopreservation ( $9.8 \pm 5.8$ ) and women for fertility preservation ( $9.4 \pm 4.4$ ). There was no statistical difference in the oocyte survival (91.3–96.7%) among three categories, however, significantly ( $P < 0.05$ ) lower rates in fertilization (67.4 vs. 76.6%), clinical pregnancy (36.0 vs. 88.9%), live birth (36.0 vs. 77.8%) and embryo implantation (26.6 vs. 73.3%) were observed in patients with partial oocyte cryopreservation as compared with women for fertility preservation. Other comparisons, including women's age, cleavage, blastocyst formation, cancelation, and mean no. of embryos transferred in all groups did not show statistical differences.

#### **4. Discussion**

It has been demonstrated that oocyte cryopreservation does not compromise in vitro development and pregnancy rates as compared with fresh oocytes [1, 9–12]. Because of its reliability and efficiency, oocyte cryopreservation allows young cancer patients to have their oocytes collected prior to the initiation of chemo- or radiotherapy for the treatment of various malignant diseases, with the expectation of having their oocytes fertilized after recovery [2, 3, 19, 20]. It also would permit healthy women to have their oocytes collected and preserved for use in the future [4, 7, 8, 16–18] and for donor oocyte bank establishment [24, 25, 33].

It has been found that live birth rate was reduced significantly in women >37 years old after fresh oocyte IVF and the reduced live birth rate was mainly caused by embryonic aneuploidies [34–37]. Therefore, women's age at the time of oocyte cryopreservation is the most important factor affecting live birth rates. Present and previous data [3, 9, 16–18] suggest that women should preserve their oocytes before 37 years old if they plan to rely on oocyte cryopreservation to have a live birth. However, for the women who are more than 37 years old, it is still possible to have their oocytes to be cryopreserved for future use, but success mainly relies on oocyte quality and number [3, 9, 13].

In the present study, when we analyzed the efficiency of oocyte cryopreservation in women at different age groups, we found that live birth rates can reach to 51.1, 46.7 and 28.6% in women <35, 35–37, and > 37 years old, respectively, with their first embryo transfer (fresh), which is comparable to live birth rates with embryo transfer from fresh oocytes in our clinic or other published data [2, 3, 9, 23, 25]. A decreased

tendency in live birth rate was observed in women >37 years as compared with women ≤37 years old. This is true because embryo quality (competence to develop to blastocysts and chromosome status) decreases when women reach >37 years old [37, 38]. Even when high quality blastocysts were transferred, embryo implantation rates also dropped, which eventually reduced live birth rates. Furthermore, morphological assessment of embryos does not always choose chromosomally normal embryos, thus high miscarriage rates were found in this population [35, 36]. In the present study, most patients had fresh blastocyst transfer without PGT-A. Actually frozen/warmed euploid blastocyst transfer after PGT-A did not further increase embryo implantation in all age groups in the present study. Similar outcomes have recently been found when embryos (with or without PGT-A) from fresh oocytes were transferred [39, 40], especially the benefits of PGT-A were not found in patients <37 years old. However, large data analysis of pregnancy outcomes in women aged 35–40 years demonstrated a significant improvement in clinical pregnancy rate and live birth rate with the use of PGT-A per embryo transfer [41]. Thus, it is difficult to explain the differences between reports.

Cancellation is very common in human ART, especially in poorly responding and/or older patients. In the present study, we cultured all embryos to Days 5–7 to allow embryos to develop to blastocyst stage and found that more patients >37 years old had to cancel embryo transfer due to lack of blastocyst development than patients <35 years old, and this indicates that oocyte quality in older patients are poorer than that in young patients. Although number of oocytes is also a reason for cancellation of a cycle, oocyte quality may be the main reason. Other factors should also be considered as the reasons for cancellation. For example, some canceled cycles had previous failed IVF cycles with the same cohort of fresh oocytes or had failed cycles due to severe male factor infertility. From laboratory results, it was found that no fertilization (mainly due to male factor infertility) and no embryo cleavage also caused cycle cancellation. Therefore, the reason(s) for cycle cancellation is complicated and multiple factors should be considered to explain the cause of failed blastocyst development.

Blastocyst transfer has been one of the most practical embryo selection strategies in human ART [42, 43], which could reduce number of embryos to be transferred and multiple pregnancy [43–45]. Our clinic has adopted blastocyst transfer for all patients, even in patients with a limited number of oocytes. However, we still do not know if pregnancy can be improved by early-stage embryo transfer for oocyte warming cycles. As some fertilized oocytes (as high as 10%) did not cleave during the culture, it is unknown whether embryo development arrest can be overcome by transferring early stage of embryos to uterus. It would also be possible that embryo arrest is caused by damages of some intra-oocyte structures during oocyte cryopreservation and warming, as embryo arrest is less than 1% in human IVF with fresh oocytes in our laboratory. Thus, current oocyte cryopreservation and warming technology needs further improving.

In the present study, we also found that average birth weight and proportions of babies with low birth weight after fresh embryo transfer were comparative to average weight of babies from fresh oocytes [42, 45, 46]. However, low birth weight was observed in babies from frozen/warmed blastocyst transfer in the present study although there are no statistical differences as compared with babies from fresh blastocyst transfer. This is certainly different from those with fresh oocyte IVF in which birth weights were higher in babies from FET than babies from fresh embryo transfer [47]. Because this is the first time that we noticed the difference in birth weight between fresh blastocyst transfer and frozen/warmed blastocyst transfer from frozen/warmed oocytes and the case number is also very limited, further data collection is necessary to reveal whether low birth weight after transfer of frozen/warmed blastocysts resulting from frozen/warmed oocytes is a common phenomenon.

Oocyte cryopreservation has been widely provided to women for various purposes. In the present study, we found that the efficiency was different among three different patient categories. Women used oocyte cryopreservation as fertility preservation had higher live birth rate as compared with patients who initially underwent infertility treatment. For infertility patients, live birth rates between partial oocyte cryopreservation/warming and all oocyte cryopreservation/warming were not significantly different although fewer oocytes had less opportunity to have a live birth, which was similar as that predicted by other researchers [3], especially when cumulated live birth rates were calculated [9].

However, for patient own oocytes, there are many factors, such as age and ovarian and hormone status. Some patients with oocyte cryopreservation may be due to lack of (enough) sperm for insemination, thus the time for oocyte cryopreservation may be delayed (after insemination of partial oocytes or after waiting for attempt of sperm collection). Oocyte cryopreservation is usually performed 3–5 h after egg retrieval [2, 9, 11], thus it is still unknown whether delayed cryopreservation affects oocyte survival, fertilization, and embryo development. We did not examine these factors in the present study because the case numbers are very small in each category. For these patients, male factor infertility may also affect oocyte fertilization and embryo development and implantation.

We found that embryo development is slower with frozen oocytes as compared with fresh oocytes. Recently, Cobo et al. used time-lapse scope to track oocyte fertilization and embryo development, they found that pronuclear formation is about 1 h delayed in frozen oocytes as compared with fresh oocytes [48]. We also found that Day 5 blastocyst rates were lower but overall blastocyst rates (Days 5–7) were same between fresh and frozen donor oocytes in a previous study [11, 49].

A comprehensive analysis should be done whether an oocyte warming cycle can eventually result in a live birth. Many factors, such as oocyte quality, numbers of oocytes warmed, previous IVF outcomes, male factor infertility and others, should be carefully evaluated because women will rely on cryopreserved oocyte IVF to have a live birth in the future. It should be realized that if a fresh oocyte IVF cycle fails (no embryo available for transfer after IVF or no live birth after embryo transfer), the patients can attempt the second or more cycles to achieve a live birth. However, if an oocyte warming cycle fails to have a live birth, it may be too late for the patients to attempt the second or more oocyte retrieval cycles, especially when women use oocyte cryopreservation as their fertility preservation.

## **5. Conclusions**

In conclusion, many factors affect the successful application of oocyte cryopreservation for women who want to preserve their fertility. Women's age at the time of oocyte cryopreservation is one of the most important factors to consider. Based on our current and previously published data, we can conclude that oocyte cryopreservation for fertility preservation can be done at any time during a woman's reproductive age. Similar as fresh oocyte IVF, the overall efficiency of oocyte cryopreservation is dependent up on women's age and reproductive health at the time of oocyte cryopreservation.

## **Conflict of interests**

The authors declare that they have no conflict of interests.

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