Freeze-all, oocyte vitrification, or fresh embryo transfer? Lessons from an egg-sharing donation program

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Objective: To compare the outcomes of ETs using cryopreserved embryos, cryopreserved oocytes, or fresh embryos.
Design: Observational, cohort study.
Setting: Private university-affiliated fertility center.
Patient(s): This study included 8,210 mature oocytes obtained from 425 oocyte donors. Of those, 5,440 were used for the donors' own cycles (Fresh Oocyte Cycles Group), and 2,770 were cryobanked for 425 recipients (Banked Donor Egg Group). All of the oocytes were sperm injected, resulting in 4,585 embryos from the donors' own cycles and 2,128 embryos from the recipients' cycles. For the donor cycles, embryos were either cryopreserved and transferred during a subsequent cycle (Thaw Cycles Group, 3,209 embryos), or they were transferred during a fresh cycle (Fresh Cycles Group, 1,307 embryos). For the recipient cycles, embryos derived from vitrified oocytes were transferred (Vitrified Oocytes Group, n = 425 cycles, 2,128 embryos).
Intervention(s): Oocyte/embryo vitrification and intracytoplasmic sperm injection.
Main Outcome Measure(s): Embryo quality, pregnancy, and implantation rates.
Result(s): Decreased embryo quality and lower rates of blastocyst formation were observed among embryos derived from vitrified oocytes. The highest pregnancy and implantation rates were noted for the Thaw Cycles Group, followed by the Banked Donor Egg Group; the Fresh Cycles Group had the lowest rates.
Conclusion(s): Oocyte vitrification followed by intracytoplasmic sperm injection leads to lower embryo developmental competence compared with when fresh insemination methods are used. However, pregnancy and implantation rates are higher when embryos are transferred into a "more receptive" endometrium, free of the adverse effects of gonadotropin. Moreover, the freeze-all method leads to exceptional clinical outcomes. (Fertil Steril® 2016;106:615–22. ©2016 by American Society for Reproductive Medicine.)
Key Words: Cryobanking, embryo cryopreservation, freeze-all, oocyte vitrification

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Since the development of cryopreservation, this technique has been a vital instrument of increasing importance in assisted reproductive technology (ART) (1). The cryopreservation of cleavage-stage embryos ensures the best use of the fertility potential of an oocyte-collection cycle. Cryopreservation eliminates the need to transfer multiple embryos, thereby significantly reducing the rate of multiple pregnancies (2–4).

Driven by an increasing interest in preserving the fertility potential of young women undergoing gonadotoxic treatments or even those who wish to postpone maternity, the cryopreservation of oocytes has become more and more popular and has found other applications in areas such as oocyte donation (5). Oocyte donation, in which the oocyte and subsequent embryo qualities are optimized by oocytes donated from young women, is quite a well-established treatment for female infertility (6). With the introduction of vitrification, it is now possible to vitrify and warm unfertilized eggs at near maximal efficiency, resulting in high cell survival rates (7). The use of frozen donor eggs avoids some difficulties derived from the use of fresh oocyte donations,

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such as the synchronization between the donor cycle and the recipient endometrium (8). In addition, the use of donor oocyte cryobanking may provide patients with more choices when selecting oocytes, more flexibility in timing pregnancy, and potential reductions in the cost of treatments (1, 9).

It has been reported that controlled ovarian stimulation (COS) is associated with impaired endometrial receptivity in IVF cycles (10–13). This may be explained by an embryo–endometrium asynchrony: there have been reports of a positive correlation between embryo developmental pace and implantation in fresh autologous IVF cycles. This asynchrony has not, however, been detected in oocyte donation cycles or cycles using frozen–thawed embryos (14, 15).

In frozen–thawed cycles, when COS is not performed, pregnancy rates have been reported to be higher than in fresh autologous cycles (16). Considering the adverse effects of COS on endometrial receptivity, the freeze-all policy has emerged as an alternative to fresh embryo transfer to improve IVF outcomes (17, 18).

With the freeze-all policy, an entire cohort of embryos is cryopreserved to be transferred later during a natural cycle, or during a cycle with hormonal replacement for endometrial priming. This provides a more physiologic environment for ET (18).

It has previously been suggested that oocyte cryopreservation could result in disruption of the oolema molecular machinery required for normal segregation of chromosomes (19, 20). However, other studies have suggested that embryo quality is not affected by the oocyte vitrification procedure. Similar to embryos derived from fresh oocytes, it has been shown that vitrified oocytes preserve the potential to be fertilized and to develop into high-quality blastocysts (21). Moreover, in a paired randomized, controlled trial, Forman et al (22) have demonstrated that oocyte vitrification does not increase the rate of aneuploidy or diminish the implantation potential of viable blastocysts.

Likewise, embryos can be consistently cryopreserved and warmed to yield pregnancy outcomes similar to those achieved after the use of fresh embryos (23). To date, however, little is known about the optimal oocyte/embryo stage that should be used for vitrification.

Although it has been strongly suggested that ET during a natural or endometrium-prepared cycle is a better approach than transfer during a COS cycle, the question about the vitrification of oocytes or embryos remains under debate.

Therefore, the goal of the present study was to compare the outcomes of ETs using cryopreserved embryos or cryopreserved oocytes in endometrial primed cycles by making use of well-controlled oocyte sampling (i.e., derived from oocyte donor patients). A secondary objective was to compare the quality of embryos derived from fresh or vitrified oocytes. Last, this study aimed to confirm the superiority of the freeze-all protocol over fresh embryo transfer during COS cycles.

MATERIALS AND METHODS

Study Design

This study was performed in a privately assisted fertilization center in Brazil and included 8,210 viable mature oocytes obtained from 425 oocyte donors: 5,440 were used for the donors' own cycles (Fresh Oocyte Cycles Group) and 2,770 were cryobanked for 425 oocyte recipients (Banked Donor Egg Group). After warming, 2,635 oocytes survived. All oocytes were submitted to sperm injection, resulting in 4,585 embryos obtained from the Fresh Oocyte Cycles Group and 2,128 embryos obtained from the Banked Donor Egg Group. Concerning the Fresh Oocyte Cycles Group, the embryos were either cryopreserved and transferred in a subsequent cycle (Thaw Cycles Group, n = 297 cycles, 3,209 embryos) or transferred during a fresh cycle (Fresh Cycles Group, n = 128 cycles, 1,307 embryos). Therefore the Fresh Oocyte Cycles Group was split into two other groups: the Thaw Cycles Group and the Fresh Cycles Group (Fig. 1A).

For the recipient cycles, embryos derived from vitrified oocytes were transferred (Banked Donor Egg Group, n = 425 cycles, 2,128 embryos; Fig. 1A).

All of the embryos were evaluated 16–18 hours after intracytoplasmic sperm injection (ICSI) on days 2, 3, and 5 of development when ET was performed.

To evaluate the efficiency of the use of fresh or vitrified oocytes, the groups Fresh Oocyte Cycles and Banked Donor Egg Group were compared for fertilization rate, embryo quality on days 2 and 3, and blastocyst formation rate (Fig. 1B).

The oocyte/embryo survival rate, total usable embryos rate, implantation rate, pregnancy rate, and miscarriage rate were compared among the Thaw Cycles Group, Fresh Cycles Group, and Banked Donor Egg Group (Fig. 1B).

The oocyte/embryo survival rate was calculated by the number of surviving oocytes or embryos after warming divided by the number of vitrified oocytes or embryos. Total usable embryos was calculated by the number of transferred embryo plus the number of supernumerary cryopreserved embryos for future use dived by the total number of obtained embryos. The implantation rate was defined as the total number of gestational sacs divided by the total number of embryos that were transferred. Clinical pregnancy was defined as the presence of a gestational sac that could be visualized using ultrasound 4–6 weeks after ET, and miscarriage was defined as pregnancy with a total loss of gestational sacs before 20 weeks' gestation.

All of the cases with severe spermatogenic alterations, including frozen and surgically retrieved sperm, were excluded from the study.

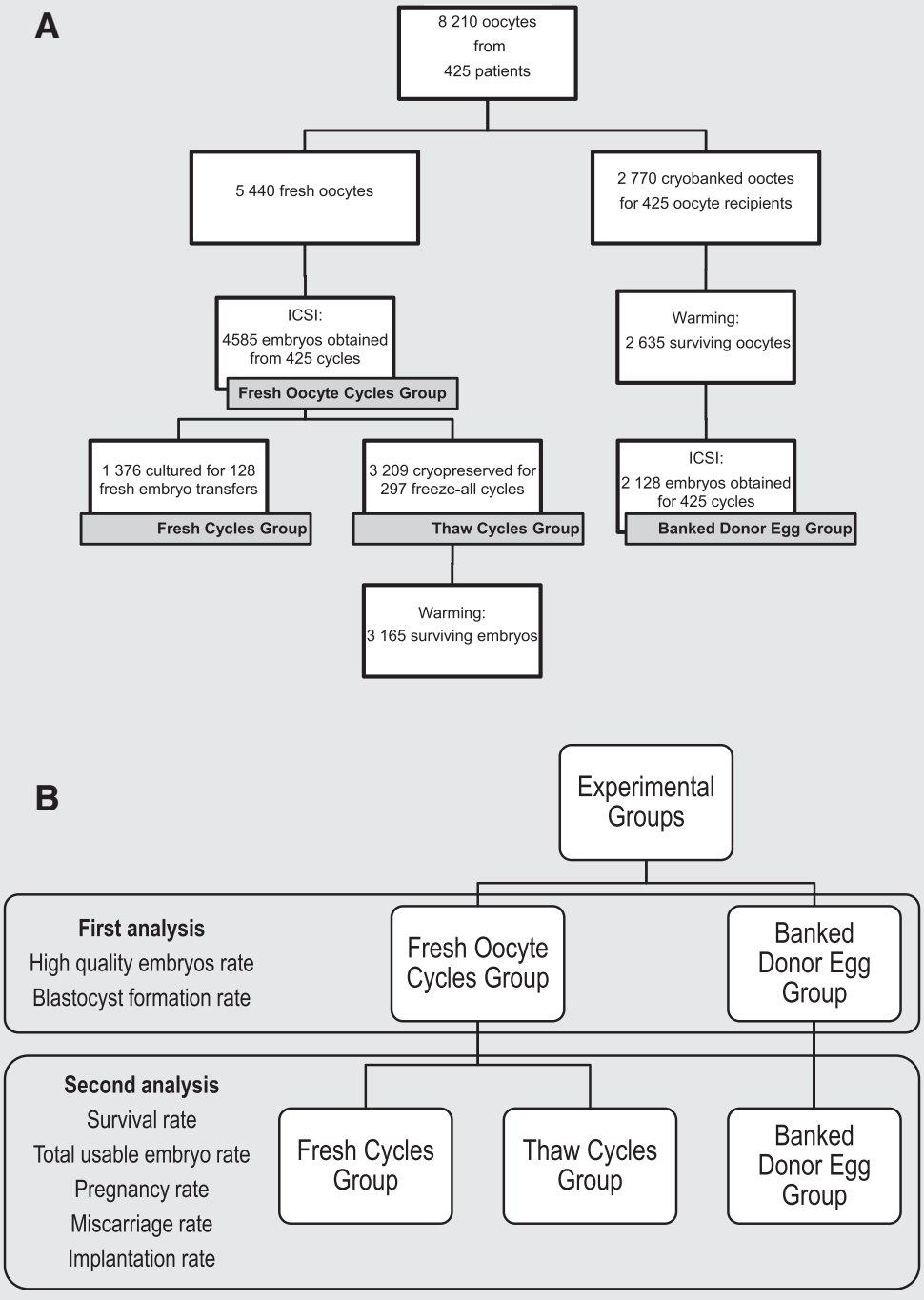
Written informed consent, in which patients agreed to share the outcomes of their cycles for research purposes, was obtained; the study was approved by the local institutional review board.

Controlled Ovarian Stimulation and Laboratory Procedures

Controlled ovarian stimulation was achieved by pituitary blockage using a GnRH antagonist (Cetrotide, Serono); ovarian stimulation was performed using recombinant FSH (Gonal-F, Serono).

Follicular growth was monitored using transvaginal ultrasound examination starting on day 4 of gonadotropin administration. When adequate follicular growth and serum E_2 levels were observed, recombinant hCG

FIGURE 1



Description of **(A)** experimental groups and **(B)** comparison.

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(Ovidrel, Serono) was administered to trigger the final follicular maturation. The oocytes were collected 35 hours after hCG administration through transvaginal ultrasound ovum pick-up.

The recovered oocytes were assessed to determine their nuclear status, and those in metaphase II were submitted to ICSI following routine procedures (24).

Endometrial Preparation

After menses, endometrial development was followed by ultrasound examination, and the patients receive 200 μg of transdermal 17β-E_2 every 3 days (Estradot, Noven Pharmaceuticals). Approximately 14 days after initiation of E_2 administration, serum E_2 levels and endometrial thickness

were determined. When endometrium showed proliferative morphology and thickness of at least 7.5 mm, 600 mg of P was vaginally administered per day. The administration of P was divided into 3 doses that were started (Utrogestan, Farmoquímica) and maintained until ET. Both 17β-E_2 and P were administered concomitantly for 10 days after ET.

The 17β-E_2 and P treatments were suspended in the presence of a negative β-hCG test. In the presence of a positive β-hCG test, the 17β-E_2 and P treatments were maintained until weeks 6 and 12 of gestation, respectively.

For COS cycles and fresh embryo transfers, on the day of ovum pick-up, patients received 600 mg of P vaginally per day divided in 3 doses, until ET. Progesterone was suspended in the presence of a negative β-hCG test or maintained until 6 to 12 weeks of gestation in the presence of a positive β-hCG test.

Vitrification and Warming

Mature oocytes were vitrified within 3 hours after collection and cryo-stored. Embryos were vitrified on day 3 of development. Both vitrification and the warming procedures were performed using the Cryotop method (25).

Briefly, vitrification was achieved by exposure of oocytes/embryos initially to the equilibration solution, followed by a 30-second exposure to the vitrification solution. Individual oocytes were then picked up in an extremely small volume (<0.1 μL) of vitrification solution, to facilitate rapid cooling, and placed on top of a very fine polypropylene strip attached to a hard plastic handle. As soon as the oocyte was placed onto the thin polypropylene strip of the Cryotop, it was immediately submerged vertically into liquid nitrogen. Then the thin strip was covered with a hard plastic cover on top of the Cryotop sheet.

For warming, the protective cover was removed from the Cryotop while it was still submerged in liquid nitrogen, and the polypropylene strip of the Cryotop was immersed directly into the thawing solution at 37°C for 1 minute. Oocytes or embryos were retrieved and transferred into dilution solution for 3 minutes and then washed twice in the washing solution for 5 minutes each.

Tools and solutions required for vitrification and warming processes were obtained from Kitazato.

Three hours after warming, intact oocytes were sperm injected, and embryos were cultured until the blastocyst stage. Cleavage-stage embryos were warmed and evaluated; embryos with more than 50% of the cells intact were considered viable. The embryos were incubated until the blastocyst stage when ET was performed.

Embryo Morphology Evaluation

Embryo morphology was assessed 16–18 hours after ICSI and on the mornings of days 2, 3, and 5 of embryo development using an inverted Nikon Diaphot microscope (Eclipse TE 300, Nikon) with a Hoffmann modulation contrast system under 400× magnification.

To evaluate the cleavage-stage morphology, the following parameters were recorded: number of blastomeres, percentage of fragmentation, variation in blastomere symmetry, presence of multinucleation, and defects in the zona pellucida and cytoplasm. High-quality cleavage-stage embryos were defined as those with all of the following characteristics: four cells on day 2 or 8–10 cells on day 3, $<15\%$ fragmentation, symmetric blastomeres, absence of multinucleation, colorless cytoplasm with moderate granulation and no inclusions, absence of perivitelline space granularity, and absence of zona pellucida dysmorphism. Embryos lacking any of these characteristics were considered to be of low quality.

To evaluate blastocyst formation, embryos were given a numerical score from 1 to 6 according to their degree of expansion and hatching status, as follows: 1, an early blastocyst with a blastocoel that was less than half of the volume of the embryo; 2, a blastocyst with a blastocoel that was greater than half of the volume of the embryo; 3, a full blastocyst with a blastocoel that completely filled the embryo; 4, an expanded blastocyst; 5, a hatching blastocyst; and 6, a hatched blastocyst. Full, expanded, hatching, and hatched blastocysts were classified as complete blastocysts.

Statistical Analyses

Chi-squared analyses were used to compare embryo quality at the cleavage stage, the chance of blastocyst formation, pregnancy rate, and miscarriage rate among the experimental groups. The fertilization and implantation rates were compared using Student's t tests, and results were expressed as the average $\pm$ SD with the corresponding P values.

Logistic and linear regression models were performed to confirm the clinical results. All these analyses were adjusted for endometrial thickness, serum P level on hCG trigger, serum E_2 level on hCG trigger, total dose of FSH used for ovarian stimulation, and the quality of transferred embryos (when at least one embryo was transferred it was considered high quality, and when only low-quality embryos were transferred it was considered low quality), because these variables were considered potential confounders in the association between the factors evaluated and clinical ICSI outcomes.

Results were expressed as odds ratios, regression coefficients, 95% confidence intervals, and P values.

Results were considered to be significant at the 5% critical level ($P<.05$). The data analysis was performed using the Minitab Statistical Program (version 14).

RESULTS

The characteristics of the patients and of the Fresh Oocyte Cycles and Banked Donor Egg Group are described in Table 1. Compared with the Fresh Oocyte Cycles, the ages of men and women and the number of transferred embryos were increased and the fertilization rate was decreased in the Banked Donor Egg Group.

Concerning the embryo developmental competence, a decreased quality on days 2 and 3 and a lower blastocyst formation rate was observed among embryos derived from vitrified oocytes (the Banked Donor Egg Group) compared with embryos derived from fresh oocytes (the Fresh Oocyte Cycles Group; Table 1).

TABLE 1

Comparison of the characteristics of patients and cycles, embryo quality on days 2 and 3, and blastocyst formation rate of the Fresh Oocyte Cycles Group and the Banked Donor Egg Group.

	Group		
Variable	**Fresh oocyte cycles**	**Banked donor egg**	***P* value**
Cycles (n)	425	425	
Embryos (n)	4,585	2,128	
Female age (y)	31.3 ± 3.3	41.4 ± 5.5	< .001
Male age (y)	35.1 ± 5.5	41.2 ± 7.2	< .001
No. of follicles	33.7 ± 12.9	–	NC
Sperm concentration ($\times 10^6$/mL)	46.5 ± 51.0	44.3 ± 39.9	.477
Sperm progressive motility (%)	44.0 ± 15.5	45.4 ± 16.8	.212
Sperm morphology (%)	1.5 ± 2.3	1.4 ± 1.8	.484
No. of oocytes	25.8 ± 9.7	–	NC
No. of metaphase II oocytes	19.4 ± 7.8	–	NC
Oocyte survival rate (%)	–	94.4%	NC
Injected oocytes	12.8 ± 4.8	4.3 ± 1.3	NC
Embryos	10.8 ± 4.3	3.4 ± 3.0	NC
Fertilization rate	85.4 ± 14.4	80.2 ± 18.2	< .001
Transferred embryos	1.6 ± 1.0	1.8 ± 1.1	< .001
High-quality embryos on D2, % (n)	43.2 (1,984/4,585)	31.5 (670/2,128)	< .001
High-quality embryos on D3, % (n)	38.6 (1,770/4,585)	30.7 (655/2,128)	< .001
Blastocyst formation, % (n)	41.1 (1,885/4,585)	36.6 (779/2,128)	< .001

Note: Values are mean ± SD, unless otherwise noted. D2 = second day of embryo development; D3 = third day of embryo development; NC = not comparable.

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Subsequently, the Fresh Oocyte Cycles Group was split into the Thaw Cycles Group and the Fresh Cycles Group. The Thaw Cycles Group and Fresh Cycles Group were then compared with the Banked Donor Egg Group.

The embryo survival rate was superior to the oocyte survival rate when the Fresh Cycles Group was compared with the Thaw Cycles Group. A significant increase in the total usable embryos rate was noted for the Fresh Cycles Group when compared with the Banked Donor Egg Group (Table 2).

In addition, a significant difference in the clinical outcomes was found: the Thaw Cycles Group had the highest pregnancy and implantation rates, followed by the Banked Donor Egg Group, whereas the Fresh Cycles Group had the lowest rates of pregnancy and implantation (Table 2). These findings were confirmed by binary logistic regression or linear regression analyses, for pregnancy and implantation respectively, adjusted for endometrial thickness, serum P level on hCG trigger, serum E_2 level on hCG trigger, total dose of FSH used for ovarian stimulation, and the quality of transferred embryos, using binary logistic regression or linear regression analyses (Table 3).

DISCUSSION

The data from the present study show that embryo vitrification leads to better clinical outcomes when compared with outcomes resulting from oocyte vitrification. The data presented here also indicate that cryopreservation cycles using oocytes or embryos result in higher rates of implantation than when fresh embryos are transferred in COS cycles.

Although cryopreservation has been established as a useful tool in ART, the slow-freezing method has been shown to be limited in terms of allowing for successful implantation. As an alternative to slow freezing, the vitrification procedure has been widely applied to human oocytes and embryos, allowing for improvements in cell survival, fertilization, embryo development, and clinical outcomes (26–30).

It has been more than two decades since the first successful report of oocyte cryopreservation (31). Because of the length of time since development, and because chromosomal abnormalities, birth defects, and developmental defects are no higher for fresh or vitrified oocytes, this technology is no longer considered experimental by the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology (32).

Although our results have demonstrated that warmed oocytes transferred in endometrial prepared cycles yield better clinical outcomes than fresh embryo transfers in COS cycles, the fertilization rate, embryo quality, and developmental competence was decreased in embryos derived from vitrified oocytes. Moreover, the total usable embryos rate and the pregnancy and implantation rates were lower than that observed when frozen embryos derived from fresh oocytes were transferred. Conversely, previous studies have suggested that oocyte vitrification followed by ICSI is not inferior to fresh insemination with regard to fertilization (33), embryo developmental competence (8, 33), pregnancy rates (8, 26, 33), and live birth (29).

In Brazil egg donation may not be conducted for profitable or commercial purposes. Therefore, in Brazil surplus oocytes are only obtained from patients undergoing IVF treatments. Thus, the oocytes available for donation originate from infertile couples. Subsequently, this creates an interesting situation in which, by using an oocyte donor/recipient program, vitrified and nonvitrified oocytes from the same

TABLE 2

Comparison of total usable embryo rate and clinical outcomes considering the status of transferred embryos: [1] fresh oocytes and fresh embryos (Fresh Cycles Group), [2] fresh oocytes and vitrified embryos (Thaw Cycles Group), and [3] vitrified oocytes (Banked Donor Egg Group).

	Group			
Variable	**Fresh cycles**	**Thaw cycles**	**Banked donor egg**	***P* value**
n	128	297	425	
Total usable embryo rate, % (n)	36.4 (501/1,376)[a]	NA	39.7 (846/2,128)[b]	.047
Pregnancy rate, % (n)	39.8 (51/128)[c]	71.4 (212/297)[d]	49.6 (211/425)[e]	< .001
Miscarriage rate, % (n)	9.4 (5/53)	10.8 (23/212)	12.8 (21/164)	.679
Implantation rate (%), mean ± SD	37.2 ± 41.1[f]	67.3 ±38.4[g]	43.0 ± 41.0[h]	< .001

Note: Regarding superscript letters, a ≠ b, c ≠ d ≠ e, and f ≠ g ≠ h. NA = not applicable.

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cohort can be compared. Another advantage of using samples from a Brazilian donor/recipient program is that the impact of vitrification on the functionality of oocytes derived from infertile couples can be analyzed. This is different from most other published studies, in which data come from oocyte donor populations.

The low rates of embryo development observed after oocyte cryopreservation could be explained by possible injury caused by the chilling process. Many organelles, such as meiotic spindles (34), cortical granules (35, 36), and cytoskeleton components (37), are highly sensitive to cryoinjuries. Nevertheless, with the advent of vitrification, which combines ultra-rapid cryopreservation of minimum volumes of sample with a high concentration of cryoprotectants (38), the efficiency and security of many cryopreservation programs has dramatically increased.

Another issue to be discussed is the high paternal age of the Recipients Group (Banked Donor Egg Group). High paternal age could explain the low results obtained for this group. Previous studies have suggested that the level of steroid hormones decreases with advanced age (39). A decreased chance of conception within 12 months (40) and an increased risk of miscarriage were also noted (41). However, in a systematic review of the literature, Dain et al. (42) concluded that advanced paternal age is not associated with decreased ART outcomes, including pregnancy rate, miscarriage rate, and live birth rate. Moreover, in the study presented here, lower seminal parameters were not detected for samples originating from older patients.

An interesting point found in this study is that even with lower embryo developmental quality, warmed oocytes transferred in endometrial prepared cycles result in higher pregnancy and implantation rates compared with transfer in fresh COS cycles. This finding strongly suggests that COS impacts endometrial receptivity, which may be a possible cause of implantation failure after ovarian stimulation.

Indeed, some studies have suggested that the pregnancy rate is inversely related to serum P levels on the day of hCG administration (43–46). Venetis et al. (47) report that during COS and in the presence of GnRH analogues, elevated P levels might be attributed to an excess number of follicles, with each one producing a normal amount of P consistent with the late follicular phase. Consistent with this, higher serum P levels have been related to greater FSH administration in both GnRH (48) agonist and GnRH antagonist COS protocols (49, 50).

It has been demonstrated that elevated P levels on hCG trigger day negatively influence the pregnancy, regardless of the oocyte quality (43). Raised concentrations of P in the late follicular phase are likely to influence the secretory changes of the endometrium, leading to an asynchrony between embryo and endometrial dialogue, which may result in reduced implantation (51).

TABLE 3

Comparison of oocyte/embryo survival rate, total usable embryos rate, and clinical outcomes considering the status of transferred embryos: [1] fresh oocytes and fresh embryos (Fresh Cycles Group), [2] fresh oocytes and vitrified embryos (Thaw Cycles Group), and [3] vitrified oocytes (Banked Donor Egg Group).

	Group			
Variable	**Fresh cycles**	**Thaw cycles**	**Banked donor egg**	***P* value**
n	128	297	425	
Oocyte/embryo survival rate, % (n)	98.6 (3,165/3,209)[a]	95.1 (2,128/2,635)[b]	NA	< .001
Total usable embryos rate, % (n)	36.4 (501/1,376)[c]	NA	39.7 (846/2,128)[d]	.047
Pregnancy rate, % (n)	39.8 (51/128)[e]	71.4 (212/297)[f]	49.6 (211/425)[g]	< .001
Miscarriage rate, % (n)	9.4 (5/53)	10.8 (23/212)	12.8 (21/164)	.679
Implantation rate (%), mean ± SD	37.2 ± 41.1[h]	67.3 ±38.4[i]	43.0 ± 41.0[j]	< .001

Note: Regarding superscript letters, a ≠ b, c ≠ d, e ≠ f ≠ g, and h ≠ i ≠ j. NA = Not applicable.

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On the other hand, patients who respond adequately to COS are more likely to produce better oocytes. Xu et al. (43) reported that the consequences of elevated P levels on IVF cycles outcome in patients with good response are likely to result from a balance between two antagonistic factors: the good embryo quality associated with a good ovarian response, and the impaired receptivity of the endometrium resulting from premature endometrial exposure to P.

The oocyte survival rate was slightly lower than the embryo survival rate, possibly because in this study oocytes from infertile patients were used. Even then the oocyte rate was quite satisfactory (95.1%).

A therapeutic option for patients with a history of fresh implantation failure is to cryopreserve an entire cohort of embryos and later transfer them into a "more receptive" endometrium (13, 17, 52). Other applications of the freeze-all policy include fertility preservation, reducing the risk of ovarian hyperstimulation syndrome, avoiding the effects of premature P elevation, or awaiting the results of preimplantation genetic screening or preimplantation genetic diagnosis (52, 53). In this investigation the embryo survival rate was extremely high, and the clinical outcomes after embryo vitrification and warming are encouraging.

In conclusion, our results indicate that oocyte vitrification followed by ICSI leads to lower embryo developmental competence when compared with fresh insemination procedures. However, pregnancy and implantation rates are higher when embryos are transferred into a "more receptive" endometrium free of the adverse effects of gonadotropin. Therefore, the use of this technology for egg donation and cryobanking programs is a good alternative. In addition, our findings demonstrate that the embryo freeze-all policy leads to exceptional clinical outcomes and is an excellent alternative to fresh embryo transfer.

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