Optimal euploid embryo transfer strategy, fresh versus frozen, after preimplantation genetic screening with next generation sequencing: a randomized controlled trial

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Objective: To compare two commonly used protocols (fresh vs. vitrified) used to transfer euploid blastocysts after IVF with preimplantation genetic screening.
Design: Randomized controlled trial.
Setting: Private assisted reproduction center.
Patient(s): A total of 179 patients undergoing IVF treatment using preimplantation genetic screening.
Intervention(s): Patients were randomized at the time of hCG administration to either a freeze-all cycle or a fresh day 6 ET during the stimulated cycle.
Main Outcome Measure(s): Implantation rates (sac/embryo transferred), ongoing pregnancy rates (PRs) (beyond 8 weeks), and live birth rate per ET in the primary transfer cycle.
Result(s): Implantation rate per embryo transferred showed an improvement in the frozen group compared with the fresh group, but not significantly (75% vs. 67%). The ongoing PR (80% vs. 61%) and live birth rates (77% vs. 59%) were significantly higher in the frozen group compared with the fresh group.
Conclusion(s): Either treatment protocol investigated in the present study can be a reasonable option for patients. Freezing all embryos allows for inclusion of all blastocysts in the cohort of embryos available for transfer, which also results in a higher proportion of patients reaching ET. These findings suggest a trend toward favoring the freeze-all option as a preferred transfer strategy when using known euploid embryos.
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Key Words: PGS, aneuploidy, transfer, embryos

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Embryo aneuploidy is likely the leading cause of implantation failure in IVF cycles. There is well-documented evidence of increasing maternal age directly correlating with an increase in embryonic aneuploidy rates (1–4). With recent advances in IVF (extended embryo culture, trophectoderm biopsy, and vitrification) along with the combination of new and advanced technology in preimplantation genetic screening (PGS) (the use of array comparative genomic hybridization, quantitative polymerase chain reaction (PCR), and next generation sequencing [NGS] to determine all chromosome copy number), ongoing pregnancy rates (PRs) have improved with the selective transfer of euploid blastocysts (5–8). Preimplantation

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genetic screening is routine in some clinical IVF practices in the United States (5, 9). However, despite ongoing advances in reducing error rates (10) and increasing implantation (11, 12), the optimal ET strategy for euploid embryos still needs to be determined.

The two transfer strategies for euploid embryos currently in clinical practice are to use vitrified/warmed ("freeze-all") or fresh embryos for the first ET. The freeze-all strategy involves cryopreservation of all embryos after biopsy, and then waiting for the PGS results of the whole cohort (day 5 and day 6 embryos) in preparation for a frozen ET. The fresh strategy involves biopsy of expanded blastocysts before 10 AM on day 5 and culture overnight to await PGS results for a fresh ET of euploid embryos before noon on day 6. In this scenario, slower growing embryos may be biopsied on day 6 and frozen for later use.

There are benefits and challenges to each approach. There is evidence that implantation and clinical ongoing PRs may be higher when transferring vitrified/warmed embryos in a nonstimulated cycle compared with fresh transfer in a stimulated cycle (13). The incidence of low birthweight babies and preterm delivery has also been shown to be lower in pregnancies resulting from frozen transfers compared with fresh transfers (14, 15).

However, success with frozen ET requires that a laboratory's embryo vitrification methods have high survival rates. Even with the latest methods, an embryo still has about a 3% chance of being damaged by either the vitrification or the warming process (16).

Although there are an increasing number of studies supporting improved clinical outcomes after frozen ET (17–20), fresh transfer protocols are typically more affordable, require little to no additional medications, and potentially allow the patients immediate transfer. However, a successful fresh day 6 transfer approach necessitates not only that expanded blastocysts be available on the morning of day 5, but also that at least one of these embryos is euploid, thereby also reducing the chance for a transfer. The main aim of the present clinical trial was to identify which ET strategy after PGS by NGS, freeze-all or fresh, would improve implantation and live birth rates or whether the strategies were equally successful.

MATERIALS AND METHODS

Patient Population

Participants were recruited at the Oregon Reproductive Medicine Center between December 2013 and August 2015. Patients between the age of 18 and 42 years, while undergoing IVF and PGS using their own eggs, were eligible to participate in the trial. The exclusion criteria included the following: the need to use surgically retrieved sperm (microsurgical epididymal sperm aspiration [MESA] or testicular sperm aspiration [TESA]), patients using preimplantation genetic diagnosis for a single-gene or chromosomal disorder, egg donor cycles, gender selection cycles, decreased ovarian reserve indicated by early follicular phase serum FSH level >10 IU/L or random serum antimüllerian hormone level <1 ng/mL, and any medical reasons occurring before recruitment that would not allow a patient to undergo a fresh ET such as the need for uterine surgery before transfer. Patients were excluded after recruitment, before randomization, if they were unable to undergo a fresh transfer for medical reasons such as ovarian hyperstimulation syndrome (OHSS) or other medical issues.

Randomization

At the time of hCG administration, patients were randomized to either a freeze-all cycle or a fresh day 6 ET during the stimulated cycle. The stratified block randomization sequence was prepared by a professional third party (sealedenvelope.com). The allocation sequence was stratified for female age (<35, 35–37, 38–40, and 41–42 years) and number of prior assisted reproductive technology (ART) cycles (≤ 2 or ≥ 3). Women were randomized in a 1:1 ratio in blocks of 10 (i.e., of every 10 women in each stratum 5 were allocated to fresh and 5 to frozen transfer in a random order). Principal investigator registered each participant in the designated trial website, and allocation information was disclosed after confirmation of the eligibility criteria.

Stimulation and Embryo Culture Protocols

Oral contraceptive (OC) administration was initiated 2–3 weeks before stimulation. A GnRH antagonist protocol was preferentially used (84/91 [92%] of the frozen group and 78/88 [89%] of the fresh group) unless the patient had previously had a suboptimal response to this protocol. The antagonist was started on day 6 of stimulation. Ovarian stimulation was achieved with both FSH and hMG preparations. When the lead follicle was ≥ 18 mm, 10,000 IU of hCG (Novarel) was used for final oocyte maturation. Serum P levels were obtained on the day of trigger. Oocyte retrieval was performed 36 hours after trigger shot. On completion of the retrieval procedure, oocytes were placed in Quinn's Advantage Fertilization Medium (Origio) supplemented with 5% human serum albumin (HSA) (Irvine Scientific) under oil (Ovoil, Vitrolife), and intracytoplasmic sperm injection (ICSI) or standard insemination performed approximately 4 hours after retrieval (21). Once all eggs had been either inseminated or injected, they were returned to the incubator for overnight culture. All embryos were moved to Quinn's Advantage Cleavage Medium (Sage, Origio) supplemented with 10% HSA (Irvine Scientific) from days 1–3 and subsequently moved to Quinn's Advantage Blastocyst Medium (Sage, Origio) supplemented with 10% HSA from days 3–6.

Assisted hatching was performed on all embryos on day 3 after retrieval using a Hamilton Thorne Zilos laser (Hamilton Thorne) with 1–2,800-μm pulses to breach the inner and outer zona layers. The embryos were transferred back to culture media until day 5 or day 6 of development. Embryos were considered suitable for biopsy before 10 AM on day 5 when at least 10% of the trophectoderm was protruding from the breach in the zona pellucida (ZP) made on day 3. All embryos that were not hatching by day 5 were cultured until day 6 and

then biopsied. Embryos were only biopsied if there was a visible inner cell mass (ICM) and multicelled trophectoderm protruding from the ZP. Embryos that grew to an expanded blastocyst stage had an estimated 3–8 trophectoderm cells excised using a Hamilton Thorne Zilos laser (Hamilton Thorne) with 1–2,800-μm pulses to break apart cell junctions in the trophectoderm layer for tissue removal. Biopsied day 5 embryos in the fresh group were kept in culture overnight to await results of the PGS results before noon of day 6. Biopsied day 5 and day 6 embryos in the freeze-all group and surplus embryos in the fresh group were individually vitrified using Irvine Vitrification media with dimethyl sulfoxide (DMSO; Irvine Scientific) on Cryotops (Kitazawa) and stored in liquid nitrogen for future use.

DNA Analysis

Testing by NGS was processed using Ion Torrent PGM (Ion Torrent) technology as previously described by Kung et al. (22). Embryo biopsies were whole genome amplified using SurePlex (Bluegnome). Libraries were prepared by fragmenting Whole Genome Amplification products with DNA concentrations of 100 ng using Ion Xpress Plus Fragment Library Kit (Life Technologies). Library fragments were selected at 200 bp using E-Gel SizeSelect Gels (Life Technologies) and then were normalized to 100 pM using Ion Library Equalizer kit (Life Technologies). Libraries were subsequently pooled together into a mastermix, and clonal amplified on the Ion One Touch 2 system. The template was then loaded into a 316 V2 chip or a 318 V2 chip (Life Technologies) and sequenced at 200 bp. Aneuploidy data analysis was performed using Ion Reporter software, using Low-coverage Whole-gEnome workflow.

Frozen ET Cycle Uterine Preparation

Frozen ETs were performed in an artificial cycle. A combined OC containing 30 μg ethinyl E_2/0.15 desogestrel (Apri, Teva) was administered for 15–21 days starting from the third day of the menstrual cycle.

Estradiol valerate (4 mg/d; Delestrogen, JHP pharmaceuticals) by IM injection was started 5–7 days after the last OC pill, increasing by 1 mg each injection until dosage of 6 mg twice weekly was reached and the endometrium measured a minimum of 7.5 mm thickness and had a trilaminar pattern visualized on ultrasound using a GE S6 device (General Electric). Then P in oil (Watson) was commenced at a dose of 50 mg/d IM for the initial 2 days and increased to 100 mg/d thereafter. Frozen thawed ET was performed on the seventh day of P injections. Patients who had a positive pregnancy test were also given 100 mg endometrin (Ferring), administered vaginally three times a day. A weaning schedule for E_2 and P in oil was followed until discontinuation of the medication at 11–12 weeks of pregnancy.

Fresh ET Cycle Uterine Preparation

Fresh ETs were carried out during the original egg retrieval cycle. On day 2 after retrieval, supplementary P (endometrin, Ferring) was administered vaginally once per day along with 2 mg oral estrogen (E) supplementation (Estrace, Teva) twice a day. Day 3 after retrieval P increased to twice a day. The transfer occurred on the morning of day 6 of embryo growth. These medications continued up to and beyond the pregnancy test as for the frozen ET luteal support protocol.

Statistical and Ethical Considerations

The primary outcome measure was the live birth rate. Live birth was defined as the delivery of a viable fetus. Ongoing pregnancy was defined as viable pregnancy progressing beyond the eighth week of gestation. Embryo implantation rate was calculated as the number of gestational sacs divided by the number of embryos transferred per group.

Continuous variables were defined with mean (SD) or median (25th–75th percentile) and compared between the groups with *t* test for independent variables or Mann-Whitney *U* test, depending on distribution characteristics. Categorical variables were defined as numbers and percentages and compared between the groups by the χ^2 test or its derivatives as appropriate. Significance was defined as a P value of $< .05$. A logistic regression analysis involving live birth as the dependent variable and female age, number of metaphase two oocytes, and ET cycle (fresh transfer as the reference) was conducted to adjust for possible differences in baseline characteristics in the per protocol analysis including all women who eventually underwent a fresh or frozen ET regardless of initial allocation.

For reference, the study center had a 63% ongoing frozen ET PR with euploid embryos for patients who were younger than 42 years using their own eggs the year before commencing the study. Seventy-four patients would be required to detect an absolute increase of 20% from 63% with an alpha error level of 0.05 and a beta error level of 0.2. To account for possible drop-outs the aim was to recruit up to 186 participants during the study period. Offspring or products of conception were not routinely karyotyped for concurrence with the embryo biopsy results.

The protocol was Institutional Review Board approved and registered with clinicaltrials.gov (NCT02000349). All patients who met inclusion criteria and expressed the desire to participate in the study were consented before ovarian stimulation.

RESULTS

One hundred eighthy-three patients were consented to the study. Four patients were cancelled due to poor response before hCG administration. A total of 179 patients received hCG for egg retrieval and were randomized; 88 patients were allocated to the fresh transfer group and 91 to the frozen transfer group. Patients had either one or two embryos transferred depending on availability of euploid embryos and patient request. Demographics are presented in Table 1.

Outcome of Patients in the Intention to Treat Analysis

The intention-to-treat analysis considered all randomized patients in their original group of allocation regardless of

TABLE 1

Baseline and IVF cycle characteristics of patients randomized to each treatment group.

Characteristics	Fresh ET (n = 88)	Frozen ET (n = 91)	*P* value
Age (y), average (range)	36.6 (25–42)	36.7 (27–42)	.7
AMH, average (range)	3.6 (1.5–4.5)	3.3 (1.4–4.7)	.7
FSH, average (range)	7.4 (0.5–21)	7.9 (1.8–15)	.2
Oocytes collected, n (range)	14 (0–41)	17 (4–44)	.1
Average no. of metaphase II oocytes	11.5	13.3	.3
2PN fertilization rate/mature oocyte, %	78	77	.6
Aneuploidy rate/embryo, %	37	40	.4
Infertility diagnosis, %			
Unexplained	23.6	29.7	.4
Female factors	31.4	30.7	1.0
Male factor	12.4	7.7	.3
AMA ≥40	11.2	16.5	.4
RPL	3.4	2.2	.6
>1 factor	19.1	13.2	.3

Note: AMA = advanced maternal age; AMH = antimüllerian hormone; RPL = recurrent pregnancy loss.

Coates. RCT of euploid embryo transfer strategy. Fertil Steril 2017.

achieving an embryo transfer, fresh or frozen (Table 2). Fourteen patients of the fresh group allocation failed to achieve a fresh day 6 transfer (9 had only day 6 euploid embryos, 2 had to rebiopsy day 5 embryos and freeze, 1 had OHSS, 1 NGS equipment failure, 1 had pulmonary embolism so could not transfer) but did have euploid embryos available that were transferred in a subsequent frozen ET cycle. These 14 patients, although they had their embryos transferred in an frozen ET cycle, were still included in the fresh transfer group according to intention-to-treat protocol, as this was their original randomization allocation. One patient dropped out of the study after randomization and had a day 3 transfer of untested embryos. Eight patients in the freeze-all group did not have embryos available to biopsy on day 5 but had day-6 euploid embryos available and these were transferred in their primary frozen ET cycle. Ongoing PRs (40.9% vs. 62.2%; $P \leq .01$) and live birth rates (39.8 vs. 61.5%; $P \leq .01$) per intended treatment was significantly higher for the freeze-all group compared with the fresh group.

Outcome of Patients Receiving the Intended Transfer Protocol

Only patients who had at least 1 expanded blastocyst to biopsy on day 5 were included in this analysis (Table 3). A total of 46 of 88 patients (52.27%) underwent a fresh euploid blastocyst transfer and 61 of 91 patients (67.03%) underwent a frozen thawed euploid blastocyst transfer (Supplemental Fig. 1, available online).

The mean number of embryos transferred was similar in both fresh and frozen transfer groups (1.4 and 1.5, respectively; $P=.3$). The implantation rate (sac formation/embryo transferred) was higher in the frozen group (75%) compared with the fresh group (67%), but this difference was not significant ($P=.3$) (Table 3). The ongoing PRs and live birth rates were significantly higher for the frozen group compared with the fresh group (ongoing, 80% frozen vs. 61% fresh; $P=.03$; live births, 77% frozen vs. 59% fresh; $P=.04$).

The analysis was further broken down into single ETs and double ETs. In the single ET subset, ongoing pregnancy (52% fresh vs. 64% frozen; $P=.5$), implantation rates (68% fresh vs. 73% frozen; $P=.7$), and live birth rates (52% fresh vs. 64% frozen; $P=.7$) were all trending higher in the frozen group than in the fresh group, but not significantly. In the double ET subset, implantation rates (67% fresh vs. 73% frozen; $P=.5$) and live birth rates (67% fresh vs. 86% frozen; $P=.1$) were trending higher but not significantly; however, the ongoing PR (71% fresh vs. 91% frozen; $P=.04$) was significantly higher in the frozen group compared with the fresh group (Table 4).

Despite a strong trend toward improved live birth rates with frozen transfer, the ET strategy did not have a statistically significant effect on the likelihood of achieving a live

TABLE 2

Outcome in the intention-to-treat population.

Characteristics	Fresh ET (n = 88)	Frozen ET (n = 91)	*P* value
Embryo transfer rate/cycle	61/88 (69.3)	69/91 (75.8)	.40
No. of embryos transferred, mean (SD)	1.4 (0.5)	1.5 (0.5)	.27
Ongoing pregnancy	36/88 (40.9)	57/91 (62.6)	<.01
Live birth	35/88 (39.8)	56/91 (61.5)	<.01
Implantation rate (total no. of gestational sacs/total no. of embryos transferred)	58/86 (67.4)	79/104 (76.0)	.19

Note: Values are n (%), unless stated otherwise.

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TABLE 3

Outcomes for each transfer strategy (only includes patients who had at least one day 5 embryo to biopsy).

Characteristics	Fresh ET (n = 46)	Frozen ET (n = 61)	*P* value
Embryo transfer rate per cycle	46/88 (52%)	61/91 (67%)	.03
Average no. of embryos transferred	1.4	1.5	.3
No. of ongoing clinical pregnancies (%/ET)	28/46 (61)	49/61 (80)	.03
No. of live births (%/ET)	27/46 (59)	47/61 (77)	.04
Live birth rate (babies born per embryo transferred)	55% 37/67	66% 62/94	.1
Implantation rate (% sacs/embryo transferred)	45/67 (67)	72/96 (75)	.2

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birth when adjusted for female age and number of metaphase two oocytes in the logistic regression model. Odds of a live birth was 2.1 (95% confidence interval 0.95–4.68; $P=.68$) with a frozen ET compared with a fresh ET.

Comparison between Fresh and Frozen ET of Only Day-5 Biopsied Euploid Blastocysts

When comparing the use of only day 5 blastocysts, 46 patients underwent a fresh ET and 37 patients underwent a frozen ET (Supplemental Table 1, available online). The average number of embryos transferred in each group was the same at 1.5 ($P=.3$). Live birth, ongoing PRs, and implantation rates were higher in the frozen transfer group, but did not reach statistical significance.

Comparison between Only FET of Euploid Blastocysts That Were Biopsied after Retrieval Day 5 or Day 6

Although 37 patients in the freeze-all group exclusively transferred day 5 biopsied euploid blastocysts, 20 patients in the freeze-all group exclusively transferred day 6 biopsied euploid blastocysts (Supplemental Table 2, available online). Euploidy rates between day 5 and day 6 biopsied blastocysts in the study were similar at 56.5%/embryo for day 5 and 53%/embryo for day 6 ($P=.54$). The average number of embryos transferred in each group was significantly different at 1.5 for day 5 and 1.2 for day 6 ($P=.009$). Despite this, the positive pregnancy, ongoing PRs, and implantation rates for each group were nearly identical.

Progesterone Monitoring at hCG Administration

Progesterone levels in the present study were monitored at hCG administration to capture premature luteinization and therefore possible cancellation of a fresh transfer in the stimulated cycle. Progesterone levels of >1.5 ng/ml have been reported to have a deleterious effect on implantation (23). Three patients in the fresh arm of the present study exhibited an increase before hCG administration and had borderline high *P* values of 1.7ng/ml, 2.2ng/ml, and 2.4ng/ml. They were not canceled because of this premature increase and the patients with levels of 2.2ng/ml and 2.4ng/ml both had a live birth from the fresh cycle. The patient with a level of 1.7ng/ml had a negative pregnancy test. The other patients in the fresh transfer group had a range of P4 of 0.3–1.4ng/ml with an average of 1.

Stimulation Protocols in the Fresh Transfer Group

Of the 46 patients who achieved a fresh transfer, 37 had an antagonist stimulation protocol and 9 had an overlap stimulation protocol. Of 37 in the antagonist group 22 had a live birth and of the 9 in the overlap group 7 had a live birth.

DISCUSSION

This randomized controlled trial has demonstrated that the ongoing PRs and live birth rates were significantly higher in

TABLE 4

Further breakdown of analysis into single (SET) versus double (DET) embryo transfer between the two transfer strategies.

	SET		DET	
Characteristics	Fresh	Frozen	Fresh	Frozen
No. of ET	25	25	21	36
No. of ongoing pregnancies (%/ET)	13 (52)	17 (65)	15 (71)	32 (91)
P value	.4		.04	
No. of live births (%/ET)	13/25 (52)	16/25 (64)	14/21 (67)	31/36 (86)
P value	.5		.1	
No. of live births resulting in twins	0/13	1/15	8/13	15/30
P value	.3		.4	
Implantation rate (sacs/embryo)	68% (17/25)	73% (19/26)	67% (28/42)	73% (51/70)
P value	.7		.5	

Coates. RCT of euploid embryo transfer strategy. Fertil Steril 2017.

the frozen ET group compared with the fresh ET. In addition, a significantly higher proportion of patients are able to attain the desired ET strategy in the frozen ET group compared with the fresh ET group. However, although a higher proportion of embryos transferred implanted in the frozen ET group than in the fresh ET group, the variations were short of statistical significance.

The two transfer strategies, when using known euploid embryos, are very different in their execution and each have their own challenges to take into consideration. There is increasing evidence in favor of transferring embryos created during IVF in an unstimulated uterine environment (18–20, 24–26). This approach, however, necessitates embryo cryopreservation during the IVF cycle. With the adoption of vitrification as a cryopreservation method during the past few years the survival rate of blastocyst embryos has improved significantly, making the transfer of frozen thawed embryos a pragmatic option for patients and practitioners alike. Although there have been studies comparing fresh versus frozen ET outcomes and PGS tested versus non-PGS tested ETs (9, 27), there has not been a study comparing fresh versus frozen/thawed transfer of known euploid embryos.

To confirm that the improvement in implantation and ongoing PRs in the freeze-all group was related to the transfer strategy alone, we compared only embryos that had been biopsied on day 5 in the fresh and frozen groups (Supplemental Table 1). The ongoing PRs and implantation rates all showed positive trends in favor of the frozen ET group, but these did not reach significance, probably due to the smaller sample size.

As part of the study analysis we assessed whether the implantation potential of day 6 blastocysts was different to embryos ready for biopsy on day 5. We found almost identical implantation rates with both groups—78% with day 5 embryos compared with 75% for embryos ready for biopsy by day 6 ($P=.8$; Supplemental Table 2). Therefore to be able to include day 6 embryos in the cohort for transfer is a definite advantage for the freeze-all group and leads to more patients reaching the goal of their intended transfer protocol compared with the fresh transfer strategy.

Transferring fresh embryos on day 6 could be regarded as a disadvantage for the fresh transfer protocol, as usual clinical practice is to transfer blastocysts on day 5 of embryo growth. However, fresh transfer of tested embryos on day 5 is not possible with current NGS technology due to the length of time needed to run the assay (17 hours) before results are obtained. For this reason day 6 transfer is currently the standard procedure for fresh transfer of known euploid embryos when using NGS. Arguably, the ideal comparison for this study may have been to transfer both fresh and frozen embryos on day 6, but the point of the present trial was to compare the two currently available options using NGS technology in daily practice. In addition, the optimal window of implantation where the endometrium is most receptive is not precisely known and may differ from cycle to cycle and from patient to patient (28, 29). Thus we elected to conduct fresh transfers before noon on day 6, as soon as the PGS results became available. A faster methodology for counting chromosomes is real time quantitative PCR where the turnaround time is 4 hours, therefore allowing for same day transfer on day 5. In the present study we were comparing the use of NGS for the two transfer strategies, as this is the technology of choice for many PGS laboratories and one that is in place for clinical testing in the study center laboratory. Other studies are needed to compare fresh versus frozen transfers of euploid embryos using other technologies and this was outside the scope of this trial.

The consequences of transferring embryos in a high estrogenic environment are higher rates of abnormal placentation (30) and possible reduced endometrial receptivity (31, 32) compared with cycles with more physiological levels of E_2. Abnormal placentation includes incorrect placenta placement in the uterus, such as placenta previa and vasa previa, or abnormal cord insertion, such as velamentous or marginal cord insertion. The risk of fetal–maternal hemorrhage is high if the placenta or vessels lie over the cervix or if the cord is easily detached from the placenta during the birthing process. Farhi et al. (30) showed in a retrospective single center study that there seems to be a higher incidence of abnormal placentation in fresh ET cycles associated with E_2 levels $>2{,}724$ pg/mL at the time of hCG administration and in a large Australian multicenter study of singleton pregnancies, Healy et al. (33) found that there is a higher risk of obstetric hemorrhage after transfers in a stimulated IVF transfer cycle than in frozen transfer cycles or naturally conceived pregnancies.

An insufficient placenta can be associated with an increase in the rate of low birthweight at term infants resulting from fresh ET cycles compared with frozen ET cycles (14, 30, 34). In the present study, maximum E_2 levels for the fresh group reached an average of 3,489 pg/mL compared with an average of 1,098 pg/mL in the freeze-all group. Although the frozen ET E_2 levels were not as physiologically normal as during natural cycle conception, they were three times lower than the fresh transfer group and did not exceed the levels above that an increase in abnormal placentation has been observed (30). The present study, however, did not assess placental defects of offspring and the average weight of singleton offspring in each group was similar.

In addition to biologic concerns, infertility is psychologically challenging for patients, and stress related to infertility and undergoing a demanding IVF cycle should be taken into account while making treatment decisions. The uncertainty of possibly having no euploid embryos available for fresh transfer on the morning of day 6 could significantly increase patient's perceived stress. On the other hand, knowing in advance that a fresh transfer will not be attempted, thus allowing time to carefully consider the results of the whole cohort of embryos, may relieve some of that stress for the patient.

It should be noted that fresh transfers also have several advantages. A fresh transfer avoids direct costs associated with cryopreservation and the subsequent frozen thawed embryo transfer. A fresh transfer also saves on future indirect costs of treatment, such as loss of earnings for monitoring visits during the frozen transfer cycle and travel costs for

patients traveling from another state or even another country. The chance of a positive outcome is more immediate compared with a freeze-all cycle where the patients often have to wait weeks or months to even attempt a transfer. Thus, it is also possible that some patients can regard a fresh transfer as less stressful.

Either of the treatment protocols investigated in this study can be a reasonable option for patients using their own eggs. On balance the nonstimulated uterine environment of the frozen ET protocol may result in pregnancies with fewer obstetric complications, as shown by previous studies of fresh versus frozen protocols using untested embryos and IVF versus non-IVF pregnancies (30, 33, 35). Although the present study did reveal a significant improvement in live birth rate per transfer event using frozen thawed euploid embryos compared with fresh, it was unable to demonstrate a statistically significant improvement in live born offspring per transferred embryo between the two transfer strategies. Freezing all embryos allows for inclusion of all blastocysts in the cohort of embryos available for transfer, which also results in a higher proportion of patients reaching their primary ET goal. In conclusion, our findings suggest a trend toward favoring the freeze-all option as a preferred transfer strategy when using known euploid embryos. Further randomized controlled trials using known euploid embryos need to be executed to further substantiate the outcome data described in the present study.

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SUPPLEMENTAL TABLE 1

Comparison of outcome between the two transfer strategies when transferring only day 5 biopsied embryos.

Characteristics	Fresh ET (n = 46)	Frozen ET (n = 37)	*P* value
Average no. of embryos transferred	1.5	1.5	.3
No. of ongoing clinical pregnancies (%/ET)	28/46 (61)	29/37 (78)	.1
No. of live births (%/ET)	27/46 (59)	26/37 (70)	.3
Implantation rate (% sacs/embryo transferred)	45/67 (67)	45/58 (78)	.23

Coates. RCT of euploid embryo transfer strategy. Fertil Steril 2017.

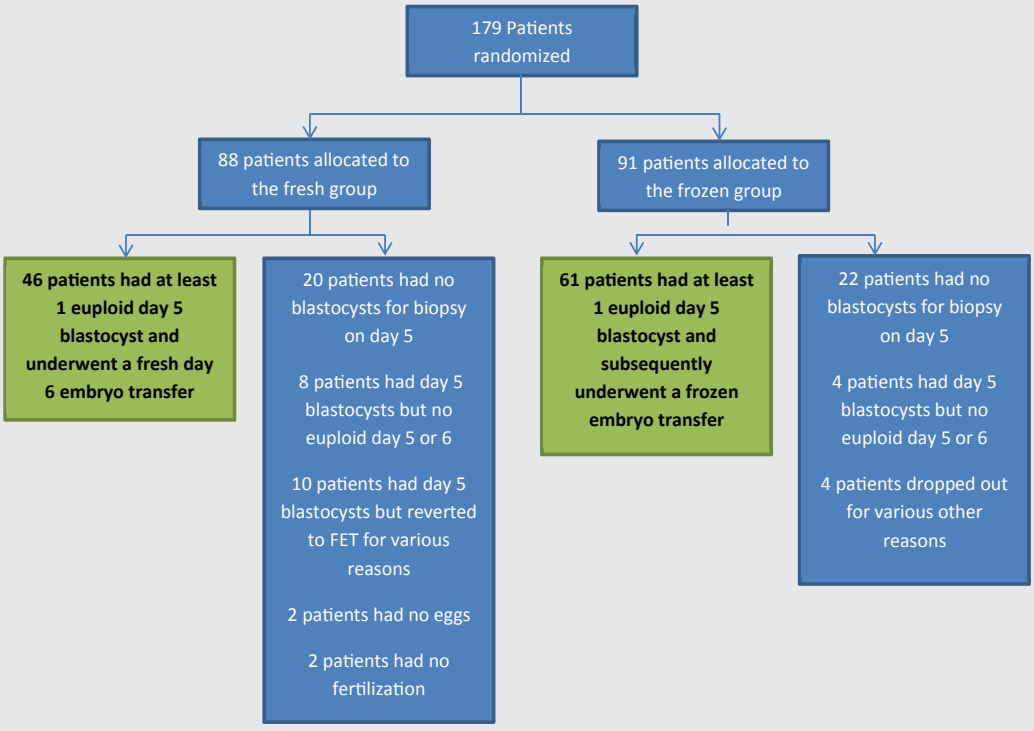
SUPPLEMENTAL TABLE 2

Comparison of outcome within the frozen transfer strategy group between transferring day 5 versus day 6 biopsied embryos.

Characteristics	Frozen day 5 (n = 37)	Frozen day 6 (n = 20)	*P* value
Average age of patient (y, range)	35.7 (27–42)	37.3 (30–41)	.1
Average no. of embryos transferred	1.5	1.2	.009
No. of ongoing clinical pregnancies (%/ET)	29/37 (78)	15/20 (75)	.7
No. of live births (%/ET)	26/37 (70)	14/20 (70)	1.0
Implantation rate (% sacs/embryo transferred)	45/58 (78)	18/24 (75)	.8

Coates. RCT of euploid embryo transfer strategy. Fertil Steril 2017.

SUPPLEMENTAL FIGURE 1



After randomization flow chart showing allocation to each treatment group and final treatment strategy realized. FET = frozen embryo transfer.

Coates. RCT of euploid embryo transfer strategy. Fertil Steril 2017.