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