Freeze-all at the blastocyst or bipronuclear stage: a randomized clinical trial

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Objective: To compare outcomes for patients randomized to have all embryos cryopreserved at the blastocyst stage or at the bipronuclear stage with subsequent post-thaw culture to the blastocyst stage.
Design: Randomized controlled trial.
Setting: Private fertility center.
Patient(s): This study included 140 women, age 18–40 years, with at least eight antral follicles, and day 3 FSH <10 IU/L undergoing IVF.
Intervention(s): After oocyte retrieval, subjects were randomized to have entire embryo cohorts cryopreserved at either the bipronuclear stage (2PN Cryo group) or at the blastocyst stage (Blast Cryo group).
Main Outcome Measure(s): Ongoing pregnancy (viable fetal heart motion at 10 weeks' gestation) per oocyte retrieval through the first transfer attempt.
Result(s): No significant differences were observed between the two study groups in age at retrieval, body mass index, antral follicle count, day 3 FSH level, or IVF cycle parameters. No significant differences were observed in ongoing pregnancy rate per retrieval (62.0%; 95% confidence interval [CI], 50.3%–72.4%) in the 2PN Cryo group; and 55.1%; 95% CI, 42.6%–67.1% in the Blast Cryo group), implantation rate (60.0% vs. 62.7%), ongoing pregnancy rate per thaw (62.0% vs. 59.4%), ongoing pregnancy rate per transfer (67.7% vs. 69.1%), and the cumulative ongoing pregnancy rate per retrieval from all thaws to date of embryos derived from the study retrieval cycle (64.8% vs. 60.9%).
Conclusion(s): Freeze-all at the blastocyst stage or at the bipronuclear stage has similar efficacy and IVF outcomes. The choice between them may depend primarily on logistical factors.
Clinical Trial Registration Number: NCT01247987. (Fertil Steril® 2015;104:1138–44. ©2015 by American Society for Reproductive Medicine.)
Key Words: In vitro fertilization, embryo cryopreservation, frozen embryo transfer, embryo vitrification, blastocyst transfer

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It is becoming increasingly common to freeze all embryos in cycles of IVF (1). Nationally, there were clear trends in increasing use of frozen-thawed transfers and total number of live births in the United States over the period 2006–2012. The United States Centers for Disease Control and Prevention reported 18,591 banking cycles in 2012 and 27,564 in 2013, an increase of 48% in 1 year (2).

Embryo cohort cryopreservation is used for various indications, including fertility preservation, reducing the risk of ovarian hyperstimulation syndrome (OHSS), delaying transfer to obtain a more receptive uterine environment in a subsequent cycle, avoiding the effects of premature P elevation, or awaiting results of genetic tests (3–8). The use of GnRH agonist instead of hCG has become a frequent and standard method for reducing OHSS risk, but cryopreservation of all embryos after such cycles has also become standard (9–11). There is also evidence of improved obstetric and perinatal outcomes after thawed embryo replacement (12–14).

Various embryonic stages have been used for cryopreservation with both conventional slow freezing and with vitrification, and the optimal developmental stage for cohort cryopreservation has not yet been determined. Good

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success rates have been reported with vitrification of oocytes, conventional slow frozen bipronuclear oocytes, slow-frozen blastocysts, and vitrified blastocysts (15). A retrospective comparison of success rates with thawed 2 pronuclear (PN) oocytes and thawed blastocysts concluded that the success rates were greater with frozen blastocysts (16). However, in that study the thawed 2PN oocytes were cultured to the cleavage stage before transfer, while the thawed blastocysts were, obviously, transferred at the blastocyst stage, creating a confounding variable and potentially handicapping the 2PN group.

The current study compares outcomes after cryopreservation at the blastocyst stage with those after cryopreservation at the bipronuclear stage with subsequent culture to the blastocyst stage before transfer. In all cases, the entire cohort was cryopreserved and only blastocysts were transferred. Because blastocyst cryopreservation was performed initially by conventional slow freezing, but was changed to vitrification in midstudy, a further comparison of these two methods for blastocyst cryopreservation is also provided.

MATERIALS AND METHODS

Study Design and Patient Population

This was a prospective randomized equivalence trial with subjects having a 50% chance of being randomized to each study group. Power analysis indicated that a total of 140 subjects were needed to provide an 80% probability (power) of obtaining a significant difference in a two-tailed test if the true ongoing pregnancy rate for each method differed by 25%. The 25% difference was expected based on observed outcomes at this center in the first half of 2010 (79% ongoing pregnancy rate per transfer with 2PN cryopreservation, 52% ongoing pregnancy rate per transfer with blastocyst cryopreservation). The study was approved by an Institutional Review Board (IRB) and registered on ClinicalTrials.gov (trial registration number NCT01247987) before subject recruitment at a single private fertility center. Per IRB instructions, the study was monitored from the outset by an independent monitor who reviewed all records.

Informed consent was obtained from each subject before initiating treatment. Inclusion and exclusion criteria specified that subjects were females 18–40 years of age scheduled for IVF treatment and with at least eight antral follicles and cycle day 3 FSH <10 IU/L. Embryo biopsy was exclusionary. Prior IVF history was allowed. Nonphysician medical staff enrolled and screened subjects.

Treatment Protocols

Subjects underwent conventional ovarian stimulation with gonadotropins (recombinant FSH and hMG), followed by ovulatory trigger with hCG alone, GnRH agonist alone, or both in combination. Oocyte retrieval was performed 34–36 hours after trigger. Immediately after oocyte retrieval, subjects were randomized to have their entire embryo cohorts cryopreserved at either the bipronuclear stage (2PN Cryo group) or at the blastocyst stage (Blast Cryo group). Randomization was performed by nonmedical staff drawing among identical, unmarked, opaque, sealed envelopes.

For subjects in the 2PN Cryo group, all bipronuclear oocytes were frozen by conventional slow freezing as described elsewhere (3). In a subsequent cycle, typically 1–2 months after oocyte retrieval, the entire cohort was thawed and cultured to the blastocyst stage before the best one or two blastocysts were selected for transfer to the uterus.

For subjects in the Blast Cryo group, blastocysts were cryopreserved by a conventional slow freezing technique as described elsewhere (17) before February 2013. The technique included cooling 2°C per minute from room temperature to −6°C, holding at −6°C for 10 minutes, during which time each 0.25 mL straw was seeded; then cooling resumed at 0.3°C per minute to −37°C. Each straw was then plunged into liquid nitrogen. Glycerol was used as a cryoprotectant.

Owing to a general change in the center's laboratory practices, the study protocol was revised in February 2013 (with IRB approval) to allow vitrification of blastocysts in the Blast Cryo group, and this method was used uniformly from that point onward. That method has also been described in detail elsewhere (18). Briefly, the blastocysts were placed into commercial vitrification media (Irvine Scientific VitKit) loaded into 300 μm Cook Flexiplets inserted into prelabeled 0.3 mL CBSembryo straws that were sealed and plunged into liquid nitrogen.

With either blastocyst cryopreservation method, post-thaw survival was defined by observed evidence of reexpansion of the blastocele and visualization of the inner cell mass (ICM).

In both groups, embryos were cultured to the blastocyst stage in sequential media (Quinn's Advantage Protein Plus) before August 2011, and in one-step media (Global) afterward. For each transferred blastocyst, the blastocyst diameter and the length and width of the ICM were measured using an ocular micrometer. The product of the ICM length and width was used to estimate the cross-sectional ICM area. The number of trophectoderm cells were counted in a single plane of focus around an embryonic "equator."

Subjects received oral E_2 (Estrace, 6.0 mg daily, Warner Chilcott) and E_2 patches as needed starting 15–20 days before ET to achieve a target endometrial thickness of at least 8 mm. Daily P injections (typically 100 mg) were started the evening before thaw (5–6 days before transfer) in the 2PN Cryo group and 5 days before transfer in the Blast Cryo group. After pregnancy was established, subjects were transitioned to vaginal P (Crinone, 90 mg twice daily, Actavis Inc.). E_2 and P supplements were continued until early pregnancy loss or until rising serum levels indicated adequate placental production at approximately 10 weeks' gestation.

Each subject was allowed one retrieval, one thaw, and one transfer under this study. However, cumulative pregnancy rates resulting from any additional transfers of thawed embryos remaining after study participation have also been calculated and are described separately.

Outcome Measures and Statistical Methods

Pregnancy was defined by rising serum hCG titers >5 IU/L observed 5–10 days after transfer. Biochemical pregnancy losses were pregnancies without sonographic confirmation