OBSTETRICS

Are perinatal outcomes affected by blastocyst vitrification and warming?

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BACKGROUND: Transfer of cryopreserved-warmed embryos into an appropriately prepared uterus unaffected by controlled ovarian hyperstimulation is common in the practice of in vitro fertilization. There is limited information on the effect of blastocyst vitrification and warming on perinatal outcomes.

OBJECTIVE: We sought to determine if perinatal outcomes are affected after the transfer of vitrified-warmed blastocysts compared to the transfer of fresh blastocysts, by comparing preeclampsia rate, birthweight, percentage of low birthweight, and preterm delivery rate between embryo transfer types.

STUDY DESIGN: We performed a retrospective database cohort study of 289 fresh and 109 vitrified-warmed blastocyst transfer cycles at an academic medical center. Cycles were performed from July 2, 2009, through Dec. 8, 2014, and included infants born at ≥20 weeks gestational age, excluding donor egg cycles. We examined the association between transfer type (fresh or vitrified-warmed) and proportion of deliveries complicated by preeclampsia, preterm delivery (gestational age <37 weeks), and low birthweight (<2500 g). We assessed associations using generalized linear models, both unadjusted and adjusted, for maternal age, newborn sex, diabetes status, and parity.

RESULTS: We observed more pregnancies complicated by preeclampsia following vitrified-warmed transfers (7.6%) compared to fresh embryo transfers (2.6%) ($P = .023$) (adjusted odds ratio, 3.1; 95% confidence interval, 1.2–8.4). Newborns resulting from vitrified-warmed embryo transfer cycles were similar to those resulting from fresh embryo transfer cycles with regard to low birthweight (7.4% vs 5.3%, $P = .421$), mean birthweight (3443 vs 3431 g, $P = .865$), and preterm delivery rate (9.2% vs 8.7%, $P = .869$).

CONCLUSION: We conclude that embryo vitrification with warming may affect some perinatal outcomes since preeclampsia is increased compared to fresh blastocyst transfer. However, other perinatal outcomes such as low birthweight and preterm delivery rate are not affected. Fresh blastocyst transfers should be considered when possible as they may reduce the incidence of preeclampsia.

Key words: assisted reproductive technology, embryo cryopreservation, in vitro fertilization, perinatal outcomes, preeclampsia, vitrification

Introduction

Assisted reproductive technology (ART) may have an adverse impact on the health of pregnant patients and their offspring. While most children born following ART are healthy, there are reports of low birthweight, increased preterm birth, and increased preeclampsia in ART cases compared to natural conceptions.[1] It is unclear if this effect is due to the underlying infertility or to the processes associated with ART.

Some reports suggest that perinatal outcomes are better following cryopreservation and warming compared to those following fresh transfer.[2-4] Specifically, a lower preterm delivery rate and a decrease in low birthweight have been reported following cryopreserved-warmed transfers.[3] Despite limited data, it has been proposed that all embryo transfers should occur following cryopreservation and warming rather than in fresh ART cycles to improve perinatal outcomes.[5]

Less information is available with regard to maternal outcomes in ART cycles following fresh vs cryopreserved-warmed transfers. Women undergoing in vitro fertilization (IVF) are reported to have a 1.5-fold increase in preeclampsia compared to those conceiving naturally.[6] We hypothesized that the process of embryo vitrification with warming may have an effect on preeclampsia, as preeclampsia is thought to be related to abnormal trophoblast migration. Our study aims to measure preeclampsia rates, percent of low birthweight, and preterm birth rates, as indicators of perinatal outcomes, following fresh compared to vitrified-warmed embryo transfers.

Materials and Methods

Study details

We performed a retrospective cohort study of all blastocyst transfers performed from July 2, 2009, through Dec. 8, 2014, at Baystate Medical Center that resulted in a singleton delivery (n = 647). Of these, delivery information was available for 427 transfers (66%) that were delivered at our institution. An additional 29 delivered at our institution but had missing data points regarding delivery outcomes, thus were excluded, leaving a total of 398 deliveries for analysis (289 from fresh and 109 from vitrified-warmed transfers).

Infants born at ≥20 weeks' gestational age were included, as infants born at <20 weeks are considered spontaneous abortions. Donor egg cycles and multiple gestations were excluded. We chose to exclude multiple gestation pregnancies because our study sought to determine the risk of embryo cryopreservation with warming on preeclampsia, and multiple gestations are known to increase preeclampsia, which would complicate our analysis.[7] The study was approved by the institutional review board and ethics committee of Baystate Medical Center. Informed consent was not required.

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Treatment protocol

Beginning in July 2009, at Baystate Medical Center we modified our cryopreservation technique and began using vitrification for all embryos. Blastocyst vitrification and warming were carried out using commercially available solutions following manufacturer instructions (Innovative Cryo Enterprises, Rockaway, NJ). Blastocysts were vitrified either in Cryo Bio System straws (July 2, 2009, through Aug. 31, 2010) or in a stripper tip (Sept. 1, 2010, through Dec. 8, 2014). All vitrified-warmed embryos were held in embryo transfer media until transferred into the uterus. During the study period, no other substantial changes were made in the laboratory or in clinical stimulation protocols.

Patient treatment protocols for fresh IVF cycles included pituitary down-regulation with gonadotropin-releasing hormone (GnRH) agonists, diluted GnRH agonist administered after oral contraceptives, or estradiol patch administered before gonadotropins with GnRH antagonist pituitary down-regulation. When leading follicle(s) reached 18-20 mm diameter, human chorionic gonadotropin was administered, and the egg retrieval was carried out 36 hours later. Retrieved oocytes were co-incubated with processed sperm, or metaphase II oocytes were subjected to intracytoplasmic sperm injection. Zygotes were cultured in protein supplemented Quinn Advantage sequential culture media system (Sage Biopharma, Trumbull, CT) until reaching the blastocyst stage.

Fresh blastocysts were transferred into uteri prepared and supported as follows: starting on the day after egg retrieval, patients initiated luteal phase support consisting of 2 estradiol patches (Vivelle Dot 0.1 mg; Novartis Pharmaceuticals Corp) and vaginal progesterone 3 times daily (Prometrium 200 mg; AbbVie Products LLC, Abbott Park, IL). These were continued until 6 weeks' gestational age if pregnant.

For vitrified-warmed embryo transfer cycles, patients began estradiol patches (Vivelle Dot 0.1 mg) on the first day of menses, and increased up to 4 patches daily on day 12. Vaginal progesterone (Prometrium 200 mg) was initiated on day 14. Blastocysts were transferred after 7 days of progesterone. Estradiol patches were continued until 8 weeks' gestational age, and vaginal progesterone was continued until 12 weeks' gestational age.

Outcomes

IVF medical records were linked with hospital discharge diagnoses, billing data, or both through our electronic medical record to obtain outcomes. Our primary outcome was preeclampsia. We hypothesized that vitrification could negatively impact the trophoblast cells, which could lead to placental damage and subsequent adverse pregnancy outcomes. Preeclampsia was defined clinically by accepted guidelines at the time of diagnosis.[8] Patients had a blood pressure >140 mm Hg systolic or >90 mm Hg diastolic with >1+ in a random clean catch urine analysis ≥300 mg/24-hour urine collection, a urine protein to creatinine ratio of ≥0.3, or blood pressure parameters with severe features including headache not resolved with medication, elevated blood concentrations of liver transaminases to twice normal concentration, a serum creatinine doubling baseline or >1.1 mg/dL, or platelets <100,000/μL.[8] No patients in our study had eclampsia, defined as new-onset grand mal seizures in a woman with preeclampsia.[8] The secondary outcome measures were proportion of deliveries complicated by preterm delivery (gestational age <37 weeks), percentage of low birthweight deliveries (<2500 g), and mean birthweight in grams.

Statistical analysis

Descriptive statistics are presented as the mean and SD for continuous data and as percentages for categorical data. The independent sample *t* test was used to compare the means, and the χ^2 or Fisher exact test was used to determine statistical significance between percentages. We assessed associations using generalized linear model regression, with a binomial family and logit link for the outcomes of preeclampsia, low birthweight, and preterm delivery. For the outcomes of birthweight and gestational age, generalized linear models with a Gaussian family and identity link were used. Potential adjustment variables included maternal age, newborn sex, primiparity (yes/no), and diabetes status (yes/no). Model building proceeded by first including transfer type and those variables associated with the outcome in univariable analysis with a *P* value ≤.25. The final model included transfer type and any adjustment variables with a *P* value ≤.05, as well as those thought to be clinically relevant. If no adjustment variables remained significant, we reported the unadjusted regression results. Separate models were developed for each outcome. All *P* values are 2-sided, with a critical significance level of ≤.05.

Results

A total of 398 singleton deliveries following ART from July 2, 2009, through Dec. 18, 2014, were included in this study. Patient demographics of cycles employing vitrified-warmed and fresh embryo transfers were similar with regard to age, body mass index, gestational age at delivery, and race (Table 1). There were differences between groups with respect to parity and numbers of embryos transferred.

We observed more pregnancies complicated by preeclampsia following vitrified-warmed transfer (7.6%) compared to fresh embryo transfer (2.6%) ($P = .023$) (odds ratio, 3.1; 95% confidence interval, 1.2−8.4), adjusted for diabetes status and parity (Figure, A).

Newborns resulting from vitrified-warmed embryo transfer cycles had a similar rate of low birthweight (7.4%) compared to those resulting from fresh embryo transfer cycles (5.3%) ($P = .421$). (Figure, B), results unadjusted. Similarly, infants had similar mean birthweights between embryo transfer types (3443 g for vitrified-warmed vs 3431 g for fresh, $P = .865$), with results adjusted for nulliparity and infant sex.

The preterm delivery rates were similar for vitrified-warmed compared to fresh embryo transfers

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