Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology

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Objective: To compare obstetric and perinatal outcomes of singleton births after assisted reproductive technology (ART) with blastocyst transfer (days 5 to 6) versus nonblastocyst transfer (days 2 to 4).
Design: Retrospective cohort study.
Setting: Monash IVF.
Patient(s): 4,202 women who conceived using in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) between 2004 and 2009.
Intervention(s): Records analysis of fresh and frozen-thawed embryo transfers resulting in singleton births of at least 20 weeks' gestation.
Main Outcome Measure(s): Perinatal outcomes: preterm birth, low birthweight, very low birthweight, small for gestational age, large for gestational age, preeclampsia, antepartum hemorrhage, placental abruption, placenta previa, and postpartum hemorrhage; and covariates: maternal age, year of birth of the baby, private health insurance status, maternal body mass index, smoking status, parity, gender of baby, and variations in treatment procedures.
Result(s): Multivariate analysis found no statistically significant difference between transfers on days 5 and 6 and days 2 and 4 for all maternal and perinatal outcomes. There were modest increases in the adjusted odds ratios for preeclampsia (adjusted odds ratio 1.72, 99% confidence interval 0.93–3.20) and placenta previa (1.65, 0.92–2.98).
Conclusion(s): Obstetric and perinatal outcomes after blastocyst transfer on days 5 to 6 are similar when compared with embryo cleavage-stage transfers on days 2 to 4. (Fertil Steril® 2012;97:579–84. ©2012 by American Society for Reproductive Medicine.)
Key Words: Blastocyst, cleavage stage embryo, frozen-thawed embryo transfer, ICSI, IVF, morula

Blastocyst culture is a relatively new technique and its long-term health implications have not yet been extensively researched. In a natural pregnancy, the cleavage-stage embryo remains in the ampulla of the uterine tube for 3 days, descending into the uterine cavity after compaction, which typically occurs after day 4 (1, 2). The nutritional environment and metabolite composition of the tubal and the uterine fluids are different (3), and the recognition of this, with the creation of stage-specific media by Gardner, has enabled improved blastocyst culture with better survival and implantation rates (4). Schoolcraft et al. (5) reported blastocyst formation rates of up to 48.8% using stage-specific G1/G2 media.

Implantation of the human embryo, which involves the attachment and penetration of the endometrium by the blastocyst, occurs over an "implantation window" (6), a period of time beginning at approximately 5 to 7 days after fertilization. Studies have shown that, although successful implantation can occur with up to 3 days asynchrony, the optimal time for initiation of implantation is within a day of embryo-endometrial asynchrony (6, 7). This suggests that cleavage-stage embryos may still achieve successful implantation, but blastocysts are more synchronized to the uterine environment, giving them greater implantation potential (4, 8).

Embryos make the transition from the maternal to the embryonic genome only after the eight-cell stage (9). In vitro culture of the embryo to the blastocyst stage, where the embryonic genome has experienced complete activation, allows selection based on more objective criteria than the limited and inconsistent assessment of morphologic criteria at the cleavage stage (10, 11). Furthermore, day-3 embryos with particular types of aneuploidy are associated with slow rates of cleavage and developmental arrest (12). The occurrence of chromosomal abnormalities in the embryos of women over

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36 years decreases from 59% at day 3 to 35% by day 5 (13). Extending in vitro culture to day 5 thus allows identification of the embryos that are fated to arrest and selects in favor of more developmentally competent embryos with a greater likelihood of implantation (12, 14).

The timing of embryo transfers to align with periods of less frequent uterine contractions is also an important consideration in embryo transfer. Uterine contractions at transfer are of concern due to the risk of expulsion of the replaced embryo. A significant decrease in the level of uterine contractions coinciding with the development of the embryo to the blastocyst stage in fresh transfers therefore favors blastocysts over cleavage-stage embryos from being expelled (15).

Blastocyst embryo transfer has a significantly higher rate of pregnancy and live births than cleavage-stage transfer when equal numbers of embryos are transferred (8, 14, 16). In a Cochrane review, Blake described a significantly higher live-birth rate with blastocyst transfer than with cleavage-stage transfer (odds ratio [OR] 1.35; 95% confidence interval [CI], 1.05–1.74) (16). Eighteen studies were accepted into this review, totalling 2,616 women and 395 blastocyst implantations. The total number of births in this study was unclear, and there were variations in the trials reviewed, so generalizing these results to the entire population seeking assisted reproductive technology (ART) is difficult (17). Nonetheless, a meta-analysis similarly demonstrated that live-birth rates were higher after blastocyst transfer (OR 1.39; 95% CI, 1.10–1.76, $P=.005$) when compared with cleavage-stage transfer (8). We can find no detailed analysis of obstetric outcomes after blastocyst transfer and birth. We compared obstetric and perinatal outcomes of singleton births after ART and embryo transfer on culture days 5 to 6 versus transfer on days 2 to 4.

MATERIALS AND METHODS

Study Design

The design of this analysis was a retrospective cohort study of 4,202 women who conceived with intracytoplasmic sperm injection (ICSI) or IVF between 2004 and 2009, and delivered a singleton baby of at least 20 weeks' gestation. Human research ethics approval and permission to access patient histories for the purpose of this study was obtained from the Monash University Human Research Ethics Committee, Monash Surgical Private Human Research Ethics Committee, and Epworth Healthcare Human Research Ethics Committee.

All data were obtained from the Monash IVF patient database. It is a legal requirement in Australia that detailed data are recorded for mandated reporting to the Australian and New Zealand Assisted Reproduction Database. Where information was incomplete, patient history files were manually accessed to complete the record. Patient records were entered into an Excel spreadsheet, and the identifying information was removed.

Study Population

Typically, cleavage-stage transfers are performed on days 2 to 3 and blastocyst-stage transfers on days 5 to 6. There were 2,486 singleton births after embryo transfer on days 2 to 4. Of these transfer procedures on days 2 to 4, 70.8% were cleavage-stage embryos, 27.6% were morula-stage embryos, and 2.6% were blastocysts. There were 1,716 transfers on days 5 to 6, of which 95.3% were blastocysts. Cook Series Media was used in all our patients for embryo culture during the timeframe of this study.

Exclusions

Pregnancies after embryo transfers where the patients had undergone assisted reproductive procedures other than IVF or ICSI were excluded. These procedures were not performed frequently enough to be analyzed as a separate entity. Pregnancies resulting from donor oocytes or embryos were also excluded from this study. Assisted hatching was not used at Monash IVF and was not used in this study. Uncommon stimulation protocols, multiple births, or pregnancies that did not reach 20 weeks' gestation were excluded. Furthermore, where a woman had more than one singleton birth in the study period, only the first listed birth on the database was selected for analysis.

Data Collection

The Monash IVF database was used to obtain information on maternal age at embryo transfer, year of birth of baby, private health insurance status (PHI), body mass index (BMI), smoking status, parity, gender of baby, and number of embryos transferred. Information regarding the etiology of infertility, type of assisted conception treatment undertaken, stimulation of cycle, cryopreservation of embryos, whether ICSI was used, and method of delivery were also obtained.

Outcomes of Interest

The primary obstetric outcomes of interest were placenta previa (PP), placental abruption (PA), preeclampsia, antepartum hemorrhage (APH), and postpartum hemorrhage (PPH). The primary perinatal outcomes were very preterm birth (<32 weeks' gestation), preterm birth (<37 weeks' gestation), very low birth weight (VLBW; <1,500 g at birth), low birth weight (LBW; <2,500 g at birth), small for gestational age (SGA; <10th percentile on intrauterine growth chart), and large for gestational age (LGA; >90th percentile).

Statistical Analysis

We used SPSS software (Statistical Package for the Social Sciences) version 17.0 to compare the obstetric and perinatal outcomes of the singleton IVF and ICSI pregnancies. For univariate analysis of categorical variables, chi-square tests were used. Where the analysis involved a 2×2 table, the Yates' correction for continuity was performed to compensate for the overestimation of the chi-square value when used with a 2×2 table. Where the chi-square analysis of a 2×2 table did not meet the assumption that less than 20% of expected values were less than 5, the Fisher's exact test was used. Independent *t*-tests were used for continuous variables.

Covariates were regarded as known risk factors for the adverse birth outcomes, based on published findings.