Trophectoderm grade predicts outcomes of single-blastocyst transfers

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Objective: To estimate the effect of the embryo stage, trophectoderm (TE) morphology grade, and inner cell mass (ICM) morphology grade on live birth in single-blastocyst transfers.
Design: Retrospective cohort study.
Setting: Large private assisted reproductive technologies (ART) practice.
Patient(s): Fresh autologous ART cycles.
Intervention(s): None.
Main Outcome Measure(s): Live birth.
Result(s): A total of 694 single-blastocyst transfers met the inclusion criteria. Univariate regression analysis showed embryo stage and TE score to be correlated with implantation and live birth. Live birth rates were 57%, 40%, and 25% for TE grades A, B, and C, respectively. There was no significant association between ICM grade and implantation or live birth. Live birth rates were 53%, 52%, and 0% for ICM grades A, B, and C respectively. Multiple logistic regression analysis showed that only patient age and TE grade were significantly associated with implantation and live birth, whereas ICM grade was not significantly associated with outcome. The TE score had the strongest correlation with live birth.
Conclusion(s): TE grading, but not ICM grading, significantly correlated with implantation and live birth for single-blastocyst transfers. (Fertil Steril® 2013;99:1283–9. ©2013 by American Society for Reproductive Medicine.)
Key Words: Trophectoderm, inner cell mass, embryo stage, live birth, ART

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The ability to predict assisted reproductive technology (ART) outcomes is important for patient counseling and in the identification of those patients who achieve pregnancy from a single-blastocyst transfer. Recent publications have reported single-blastocyst transfer algorithms that rely on the trophectoderm (TE) and inner cell mass (ICM) grading of the blastocyst (1–3). Mandating single-blastocyst transfers in patients with BB grade blastocysts or better, Csokmay et al. reported a high clinical pregnancy rate with a marked reduction in twin gestations (1). Using similar blastocyst grading criteria to implement a mandatory single-blastocyst policy, van Voorhis et al. showed a 50% reduction in twin gestation while maintaining live birth rates similar to those before the mandatory policy (2, 3).

The assessment of the blastocysts in those studies was based on the morphologic grading system first described by Gardner and Schoolcraft (4). Studies evaluating the validity of this scoring system have shown that transferring two or more high-grade blastocysts resulted in the highest implantation rate (5–8). However, those studies were limited in that the TE and ICM grades were grouped together and multiple embryos were transferred. Therefore, it is difficult to interpret the impact of the separate TE and ICM grades on an individual embryo's implantation and live birth potential. It has been reported that the ICM grade has a positive correlation with pregnancy rate (7, 9–11). However, two recent studies have demonstrated that the TE grade, but not the ICM grade, correlates with ART outcomes in both fresh and frozen embryo transfers (12, 13).

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The purpose of the present study was to evaluate the ability of morphologic assessment of the TE, ICM, and blastocyst expansion to predict implantation and live birth in fresh autologous single-blastocyst transfer cycles.

MATERIALS AND METHODS

Study Design

This was a retrospective cohort analysis of 694 fresh autologous single blastocyst transfer cycles performed during 2010. The study was performed at Shady Grove Fertility Reproductive Science Center, Rockville, Maryland. The retrospective review and analysis of data collected during routine clinical care was Institutional Review Board approved.

Patients

All patients who underwent a fresh autologous single-blastocyst transfer during 2010 were included in the analysis. Exclusion criteria were frozen-thawed embryo transfer and donor oocyte cycles. There were no exclusion criteria based on patient characteristics. The cycles included both elective and nonelective single-embryo transfers.

Stimulation Protocol

Ovarian stimulation occurred primarily with mixed FSH/LH protocols under GnRH antagonist or GnRH agonist pituitary suppression as previously described (14). In general, oral contraceptive treatment was initiated 19 days before stimulation start. For GnRH antagonist cycles, the antagonist (Ganirelix) was initiated when the lead follicle was 14 mm in size. For GnRH agonist cycles, 20 units of leuprolide acetate (Lupron) was initiated during the last 3 days of oral contraceptives. The leuprolide acetate dose was decreased to 5 units when ovarian suppression was confirmed. Ovarian stimulation was typically achieved with a mixed protocol using recombinant FSH and hMG. When the lead follicle was $\geq$ 18 mm, final oocyte maturation was triggered with 5,000–10,000 units of hCG or with 40 units (2–4 mg leuprolide acetate) of GnRH agonist in some of the GnRH antagonist cycles. Oocyte retrieval occurred 36 hours later and insemination was achieved with conventional in vitro fertilization or intracytoplasmic sperm injection as clinically indicated. Ultrasound-guided embryo transfer was performed on day 5 or 6 if an adequate number of high-quality embryos were available. Serum hCG levels were assessed 18 days after hCG trigger injection, and ultrasonographic confirmation of pregnancy was obtained in all pregnant patients.

Embryo Grading

All blastocysts were evaluated by an embryologist using a variation on the grading system of Gardner and Schoolcraft (4, 15). The TE was assigned one of the following grades: A: many cells organized in epithelium; B: several cells organized in loose epithelium; or C: few large cells. The ICM was assigned one of the following grades: A: numerous tightly packed cells; B: several and loosely packed cells; or C: very few cells. Blastocyst expansion was assigned one of the following descriptors: early: blastocele less than half the blastocyst (Gardner grade 2); expanded: blastocele fills the blastocyst with thin zona pellucida (Gardner grade 3–4); or hatched: blastocyst has hatched or is hatching out of the zona pellucida (Gardner grade 5–6). Examples of embryo grading are shown in Figure 1. Day 5 or 6 embryos that had not yet progressed past the morula stage were also included in the analysis of embryo stage, but not in the analysis of ICM or TE grading, because morulas are not assigned these grades.

Outcomes

The primary study outcomes were implantation and live birth. Implantation was defined as a rising serum hCG test and a gestational sac seen in the uterus by transvaginal ultrasound. Live birth was defined as the birth of a live infant after 23 weeks' gestation (16). Miscarriage was a secondary outcome and was defined as an implanted embryo that failed to result in live birth (17).

Statistics

Chi-square analysis with Bonferroni correction for multiple comparisons was used to compare the proportion of ART outcomes within each morphologic grade. Univariate logistic regression and multivariate logistic regression analysis were used to evaluate the effect of age, TE grade, ICM grade, and embryo morphology on implantation and live birth. Statistical analysis was performed with the use of SPSS software (IBM, Armonk, New York) and vassarstats.net. A P value of $<.05$ was considered to be statistically significant.

RESULTS

There were 694 single-blastocyst transfer cycles during the year 2010 included in the analysis. An additional 16 single-morula transfers were included in the analysis of embryo stage. All embryo transfers occurred on day 5 or 6. The mean patient age was 32.0 $\pm$ 3.5 years. The mean number of oocytes was 17.5 $\pm$ 8.2, the mean fertilization rate was 65.0 $\pm$ 17.5%, and the mean number of blastocysts was 4.6 $\pm$ 2.9. The overall live birth rate for the cohort was 52.7%. Because these were single-blastocyst transfers, the majority of embryos were high quality and only 8 patients had an embryo with ICM or TE of grade C.

There were 16 morula transfers, 52 early-blastocyst transfers, 544 expanded-blastocyst transfers, and 98 spontaneously hatched-blastocyst transfers. Implantation rates were significantly higher in expanded and hatched blastocysts (64% and 63%, respectively) than in morulas and early blastocysts (38% and 44% respectively; $P<.05$ for each comparison) by chi-square analysis (Table 1). Univariate regression analysis demonstrated embryo stage to be significantly associated with implantation ($P<.05$). Morula embryos had a significantly lower live birth rate (13%) than all other stages of embryo transfer. Early blastocysts had a live birth rate of 37%, compared with expanded blastocysts at 53% and hatched blastocysts at 58%, although this was not statistically