ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Blastocyst development rate influences implantation and live birth rates of similarly graded euploid blastocysts

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Objective: To determine whether the blastocyst development rate, as assessed by the day of trophectoderm biopsy (day 5 vs. day 6), affects the live birth rate (LBR) of similarly graded euploid blastocysts.
Design: Retrospective cohort study.
Setting: Academic medical center.
Patient(s): Patients who underwent frozen-thawed single euploid blastocyst transfers from 2013 to 2016 were included. Blastocyst morphologic grading was performed on day 5 or day 6 before the biopsy, with embryos designated into the following groups: good (3–6AA, 3–6AB, and 3–6BA), average (2–6BB), and poor (2–6BC and 2–6CB).
Intervention(s): Frozen-thawed embryo transfer.
Main Outcome Measure(s): Implantation rate (IR) and LBR.
Result(s): A total of 701 frozen-thawed single euploid blastocyst transfer cycles were included. Cycles in which day 5 blastocysts were transferred (n = 366) were associated with a significantly higher LBR than those in which day 6 blastocysts were transferred (n = 335; 60.4% vs. 44.8%). The odds ratio remained significant after controlling for all confounders, including the blastocyst grading. Furthermore, there was a significant difference in LBRs between good-quality, average-quality, and poor-quality blastocysts (67.8%, 53.4%, and 29.5%, respectively). Embryos reaching good-quality blastocysts on day 5 yielded significantly higher LBR (72.8% vs. 56.5%) and IR (77.7% vs. 58.7%) compared with those reaching similar quality blastocysts on day 6. Similarly, day 5 average-quality embryos conveyed a significantly higher IR compared with day 6 embryos of the same quality (64.4% vs. 53.4%).
Conclusion(s): In addition to aneuploidy assessment, the speed of embryo development to the blastocyst stage and an evaluation of blastocyst morphology are critical to selecting the best embryo. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)
Key Words: Day of trophectoderm biopsy, timing of blastulation, blastocyst development rate, preimplantation genetic testing for aneuploidy (PGT-A), blastocyst morphologic grading

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Although the widespread use of assisted reproductive technologies has helped many couples to conceive, it has also increased multiple gestation rates (1, 2). Multiple gestations are associated with increased maternal and neonatal complications, including hypertension, diabetes, hemorrhage, anemia, chorioamnionitis, genitourinary infection, preterm delivery, and low birth weight (1, 2). In addition, the delivery of twins or triplets costs ~5–20 times more than that of singletons (1). However, the emotional and financial burdens associated with fertility treatments can persuade many couples, especially those who have tried to conceive for more than a year, to accept the risk of multiple gestations to achieve their dream of parenthood sooner (3). Therefore, a major goal in reproductive medicine over the past three decades has been to improve the outcomes of elective single-embryo transfers while

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minimizing multiple gestation rates. Enhancing embryo selection through embryo morphology, preimplantation genetic testing for aneuploidy (PGT-A), time-lapse microscopy, metabolomics, and proteomic profiles has helped to achieve this goal (4–9).

The benefits of PGT-A with the use of comprehensive chromosome screening technology on trophectoderm (TE) cells have been described in three randomized controlled trials (4, 8, 10). Determining embryo ploidy status decreases the miscarriage rate, especially in older women, and increases the implantation rate (IR) per transfer, leading to fewer transferred embryos and a lower multiple gestation rate (4, 8–10). However, selection based on ploidy alone does not guarantee successful implantation.

Traditionally, standard morphologic assessment has been used to determine embryo competency and predict implantation success (7). Embryos with a better grading of inner cell mass (ICM), TE, and blastocele expansion are associated with a higher euploidy rate and thus a higher IR compared with embryos with lower morphology scores (11). Even among euploid embryos, morphologic grading has been shown to select blastocysts of superior implantation potential (12).

Moreover, among similarly graded blastocysts, the speed at which each embryo reaches this grade, i.e., by day 5 versus day 6, may further predict competence. Several studies have shown that blastocysts transferred on day 5 of fresh IVF cycles have a significantly higher IR than embryos transferred on day 6, a finding that might indicate a higher endometrium/embryo synchrony or that day 5 blastocysts are of better quality (13–16). However, similar improvements in the IR of day 5 versus day 6 transfers have been reported from studies on frozen-thawed embryo transfers (FETs), which mitigate the effect of endometrial receptivity (17–19). Although blastocyst morphology and the speed of blastulation correlate with embryo aneuploidy rates (11), it is not clear whether the speed of embryo development correlates with the implantation potential of euploid blastocysts. Therefore, the aim of the present study was to establish whether the speed of blastulation, which determines the day of TE biopsy, influences the IR of euploid embryos.

MATERIALS AND METHODS

Cycle Selection

The Weill Cornell Medicine Institutional Review Board approved this study. All patients undergoing FET of a single euploid blastocyst at the Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine from January 2013 to December 2016 were included.

Clinical Protocols

Controlled ovarian hyperstimulation, trigger of final oocyte maturation with hCG and/or leuprolide acetate, oocyte retrieval, embryo culture, and embryo transfer were performed according to our standard protocols (20). Gonadotropin doses were determined based on age, antral follicular count, weight, serum antimüllerian hormone levels, and previous response to stimulation. Ovarian stimulation was carried out with the use of exogenous gonadotropins (Gonal-F, EMD-Serono; Follistim, Merck; and Menopur, Ferring Pharmaceuticals). Pituitary suppression was attained with the use of GnRH antagonist (ganirelix acetate, Merck; or Cetrotide, EMD-Serono) or GnRH agonist (leuprolide acetate; Abbott Laboratories). Patients who required luteal pretreatment for follicular synchronization received either E_2 patches (Climara; Bayer Healthcare Pharmaceuticals) or oral contraceptive pills (Ortho-Novum; Janssen Pharmaceuticals).

Gonadotropin dosage was adjusted according to each patient's response to stimulation, which was monitored with the use of transvaginal ultrasounds and serial E_2 levels. Final oocyte maturation was triggered with the use of hCG (Novarel; Ferring Pharmaceuticals) and/or GnRH agonist (leuprolide acetate; Abbott Laboratories) when the mean diameter of two or more follicles reached $\geq$17 mm. The dose of hCG was tailored based on serum E_2 levels on the day of trigger according to the following sliding scale: 10,000 IU hCG for E_2 $<$1,500 pg/mL; 5,000 IU hCG for E_2 1,500–2,500 pg/mL; 4,000 IU hCG for E_2 2,501–3,000 pg/mL; and 3,300 IU hCG, 4 mg GnRH agonist, or dual trigger (1,500 IU hCG and 4 mg GnRH agonist) for E_2 $>$3,000 pg/mL. Ultrasound-guided oocyte retrieval was performed under conscious sedation 35–37 hours after trigger.

Embryos that were determined by PGT-A to be euploid were transferred in FET cycles. In general, patients with regular menstrual cycles and normal midluteal serum P levels underwent "natural" FET cycles. E_2 and LH levels were monitored during the follicular phase to identify the LH surge. Embryo transfer was performed 5 days later. Some patients, based on their physicians' discretion, received vaginal P supplementation (Endometrin; Ferring Pharmaceuticals), which was initiated 1 day after transfer. Patients who were not ideal candidates for natural cycles underwent "programmed" cycles, in which E_2 patches were serially increased up to 0.4 mg. After adequate endometrial thickness ($\geq$7 mm) was achieved, and following $\sim$14 days of E_2 supplementation, daily intramuscular P was initiated and the E_2 dose was lowered to 0.2 mg/d. Hormonal levels were monitored to ensure efficiency of supplementation. After 5 days of exposure to P, embryos were transferred with the use of Wallace catheters (Smiths Medical).

Laboratory Protocols

Embryos were cultured in sequential culture media with the use of the Embryoscope (Vitrolife) time-lapse system. Embryologists graded the blastocysts on the mornings of day 5 and day 6 based on the degree of expansion and the morphology of ICM and TE (7). One very experienced embryologist confirmed the scores of all day 5 and day 6 embryos. The degree of expansion included the following six grades: 1: a nonexpanded embryo with the blastocele filling $<$50%; 2: the blastocele fills $>$50% of the embryo; 3: the blastocele fills the entire blastocyst; 4: an expanded blastocyst with a thin zona pellucida; 5: a hatching blastocyst; and 6: a hatched blastocyst. The ICM was graded as follows: A: tightly packed