ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Morphologic grading of euploid blastocysts influences implantation and ongoing pregnancy rates

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Objective: To determine whether blastocyst grading can predict pregnancy outcomes in the frozen-thawed embryo transfer (FET) of euploid blastocysts.
Design: Retrospective cohort study.
Setting: Academic medical center.
Patient(s): Women who underwent FET of euploid embryo(s) between January 2013 and December 2015, with blastocysts were divided into four groups based on their morphologic grading before cryopreservation: excellent (≥3AA), good (3–6AB, 3–6BA, 1–2AA), average (3–6BB, 3–6AC, 3–6CA, 1–2AB, 1–2BA), and poor (1–6BC, 1–6CB, 1–6CC, 1–2BB).
Intervention(s): FET.
Main Outcomes Measure(s): Ongoing pregnancy rate (OPR).
Result(s): A total of 417 FET cycles (477 embryos) were included. Excellent-quality embryos (n = 38) yielded a statistically significantly higher OPR than poor-quality embryos (n = 106) (84.2% vs. 35.8%; adjusted odds ratio 11.0; 95% confidence interval, 3.8–32.1) and average-quality embryos (n = 197) (84.2% vs. 55.8%; adjusted odds ratio 4.8; 95% confidence interval, 1.7–13.3). Good-quality embryos (n = 76) were associated with a statistically significantly higher OPR than poor-quality embryos (61.8% vs. 35.8%). These odds ratios were adjusted for patient's age, body mass index, number of transferred embryos, type of frozen cycle, peak endometrial thickness, day of trophectoderm biopsy (5 or 6), and total number of euploid embryos for each patient. An inner cell mass grade of A yielded a statistically significantly higher OPR than ICM grade C (76.2% vs. 13.5%) or grade B (76.2% vs. 53.6%) after controlling for all confounders.
Conclusion(s): Contrary to prior published studies, the current data suggest that blastocyst morphologic grading and particularly inner cell mass grade is a useful predictor of OPR per euploid embryo. Morphologic grading should be used to help in the selection among euploid blastocysts. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Blastocyst morphologic grading, euploid embryo, inner cell mass, IVF outcome, PGS, preimplantation genetic screening

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Advances in clinical and laboratory techniques over the last few decades have substantially improved pregnancy rates after in vitro fertilization (IVF) but have also increased multifetal pregnancy rates. These outcomes underscore the importance of having the ability to select and transfer the single best embryo that has the highest potential of achieving a live birth. Preimplantation genetic screening (PGS) and blastocyst morphologic grading have been used to select the best embryo(s) in a given cohort (1–6).

Embryo aneuploidy is one of the main factors influencing IVF success rates (7, 8). Screening strategies to select euploid embryos have been a focus of the field for the last two decades. Over the last several years, blastocyst biopsy with analysis of all chromosomes has supplanted day 3 analysis via fluorescence in situ hybridization. Three randomized controlled trials have shown a beneficial role of PGS using comprehensive chromosome screening technology on trophectoderm (TE) cells (2–4). In general, PGS allows for a decrease in embryo transfer number, higher implantation rates per transfer, and a lower rate of miscarriage once implantation occurs for older patients undergoing IVF.

Conventional blastocyst grading systems include the following three

Received September 17, 2016; revised November 2, 2016; accepted November 14, 2016.
M.I. has nothing to disclose. D.R. has nothing to disclose. A.R. has nothing to disclose. A.M. has nothing to disclose. O.D. has nothing to disclose. N.Z. has nothing to disclose. K.X. has nothing to disclose. Z.R. has nothing to disclose.
M.I. and D.R. should be considered similar in author order.
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Fertility and Sterility® Vol. ■, No. ■, ■ 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.11.012

morphologic parameters: degree of blastocoel expansion, inner cell mass (ICM), and TE cells. These parameters are good predictors of live-birth rate (LBR) after fresh and frozen-thawed embryo transfer (FET) cycles (1, 9–11). Moreover, morphologic grading has been used to predict the ploidy status of blastocysts (12). However, this association is not perfect as 48% of top-quality blastocysts were aneuploid and 37% of poor-quality blastocysts were euploid in a representative study (12).

Capalbo et al. (13) recently reported that blastocyst grading does not predict FET outcome of euploid embryos (using the same four-tier rubric presented here). However, they only included 13 poor-quality embryos. On the contrary, our anecdotal experience suggested that blastocyst grading provides additional valuable information that can be used as an adjunct to PGS to select the best embryo(s) for transfer. Thus, the current retrospective cohort study was developed to evaluate the hypothesis that blastocyst grading allows for further optimization of pregnancy rates in transfers of known euploid embryos.

MATERIALS AND METHODS

Cycle Selection

The Weill Cornell Medicine institutional review board approved this study. All FET cycles in which only PGS euploid embryos were transferred at the Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine from January 2013 through December 2015 were reviewed for inclusion. Only the first FET cycle for each patient was included to avoid repeated measures bias. Cycles in which patients had transfer of two embryos of different grading or patients with a history of uterine factor infertility were excluded from the study.

Clinical Protocols

Controlled ovarian hyperstimulation, human chorionic gonadotropin (hCG) and/or leuprolide acetate trigger, oocyte retrieval, embryo culture, and embryo transfer were conducted per our standard protocols (14). The majority of cycles used gonadotropin-releasing hormone (GnRH) antagonist protocols; treatment with gonadotropins (Follistim, Merck; Gonal-F, EMD-Serono; and/or Menopur, Ferring) was followed by pituitary suppression using GnRH antagonists (Ganirelix acetate, 0.25 mg, Organon; Cetrotide, 0.25 mg, EMD-Serono) when either estradiol (E_2) level surpassed 300 pg/mL or the lead follicle reached 13 mm. Alternatively, patients were down-regulated with a GnRH agonist (Lupron, Abbott Pharmaceuticals). Women with diminished ovarian reserve were pretreated with E_2 patches or oral contraceptive pills for follicular synchronization. Gonadotropin doses were formulated according to patient's age, weight, antral follicular count, antimüllerian hormone, and previous response to stimulation.

Serial E_2 levels and transvaginal ultrasounds were performed to monitor response to stimulation; the gonadotropin dose was adjusted accordingly. Final oocyte maturation was triggered with hCG (Pregnyl, Schering-Plough; Novarel, Ferring Pharmaceuticals; Profasi, EMD-Serono) and/or GnRH-agonist (Lupron, Abbott Pharmaceuticals) when the mean diameter of ≥ 2 follicles attained ≥ 17 mm. The following slide scale was used to adjust the dose of hCG according to serum E_2 levels on the day of trigger: $E_2 < 1{,}500$ pg/mL: hCG 10,000 IU; E_2 1,501–2,500 pg/mL: hCG 5,000 IU; E_2 2,501–3,000 pg/mL: hCG 4,000 IU; $E_2 > 3{,}000$ pg/mL: hCG 3,300 IU, dual trigger (GnRH-agonist 2 mg, and hCG 1,500 IU) or GnRH agonist 4 mg. Patients underwent ultrasound-guided oocyte retrieval under conscious sedation 35 to 37 hours after the trigger.

Confirmed euploid embryos were transferred in subsequent FET cycles. The embryos selected for transfer were the best morphologically graded euploid embryo available for any individual patient. Patients who underwent transfer of excellent, good, or average morphologically graded blastocysts had also poorer graded embryos for selection. Those who underwent transfer of poor morphologically graded blastocysts did not have better graded euploid embryos for selection. Patients with regular menstrual cycles typically underwent "natural FET," in which they were monitored with serial serum E_2 and luteinizing hormone (LH) measurements. Embryo transfer was performed 5 days after detection of the LH surge.

Based on physician preference, some patients started vaginal progesterone supplementation (Endometrin, Ferring Pharmaceuticals) 1 day after embryo transfer. Alternatively, "programmed FET" cycles were performed in which E_2 patches were serially escalated typically up to a dose of 0.4 mg and attainment of an endometrial thickness ≥ 7 mm. Then daily intramuscular progesterone supplementation was started, and the E_2 dose was decreased to 0.2 mg. Progesterone and estrogen levels were measured after transfer to confirm the adequacy of the supplementation. Embryos transfers were performed 5 days after starting progesterone using Wallace catheters (Marlow/Cooper Surgical).

Laboratory Procedures and Blastocyst Grading

Embryos were cultured using the EmbryoScope (Vitrolife) time-lapse system. Blastocysts were graded immediately before TE biopsy according to the degree of expansion and ICM and TE morphology (1). The degree of expansion and hatching status were as follows: [1] the blastocoel filling $<50\%$ of the nonexpanded embryo; [2] the blastocoel filling $>50\%$ of the embryo; [3] the blastocoel filling $\sim$ 100 % of the full blastocyst; [4] an expanded blastocyst with a thin zona pellucida; [5] a hatching blastocyst; [6] a blasctocyst that has completely hatched out of the zona pellucida. The ICM grading was as follows: [A] tightly packed cells; [B] loosely grouped cells; [C] cells that are not identifiable. Similarly TE grading was [A] many cells creating cohesive epithelial layer; [B] uneven size cells; [C] few large cells squeezed to the side (1). The embryologists were blinded to results of PGS, as the embryos were graded before TE biopsy.

For the purpose of this study, and to compare our results to the findings of the published study by Capalbo et al. (13), blastocysts were divided into four groups based on their morphologic grading assessed immediately before TE biopsy: excellent ($\geq$3AA), good (3AB, 4AB, 5AB, 6AB, 3BA, 4BA, 5BA, 6BA, 1AA, and 2AA), average (3BB, 4BB, 5BB, 6BB, 3AC, 4AC, 5AC, 6AC, 3CA, 4CA, 5CA, 6CA, 1AB, 2AB, 1BA,