Blastocoele expansion degree predicts live birth after single blastocyst transfer for fresh and vitrified/warmed single blastocyst transfer cycles

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Objective: To evaluate the independent effects of the degree of blastocoele expansion and re-expansion and the inner cell mass (ICM) and trophectoderm (TE) grades on predicting live birth after fresh and vitrified/warmed single blastocyst transfer.
Design: Retrospective study.
Setting: Reproductive medical center.
Patient(s): Women undergoing 844 fresh and 370 vitrified/warmed single blastocyst transfer cycles.
Intervention(s): None.
Main Outcome Measure(s): Live-birth rate correlated with blastocyst morphology parameters by logistic regression analysis and Spearman correlations analysis.
Result(s): The degree of blastocoele expansion and re-expansion was the only blastocyst morphology parameter that exhibited a significant ability to predict live birth in both fresh and vitrified/warmed single blastocyst transfer cycles respectively by multivariate logistic regression and Spearman correlations analysis. Although the ICM grade was significantly related to live birth in fresh cycles according to the univariate model, its effect was not maintained in the multivariate logistic analysis. In vitrified/warmed cycles, neither ICM nor TE grade was correlated with live birth by logistic regression analysis.
Conclusion(s): This study is the first to confirm that the degree of blastocoele expansion and re-expansion is a better predictor of live birth after both fresh and vitrified/warmed single blastocyst transfer cycles than ICM or TE grade. (Fertil Steril® 2016;105:910–9. ©2016 by American Society for Reproductive Medicine.)
Key Words: Blastocyst morphology, blastocoele expansion degree, live birth, single blastocyst transfer, ART

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The goals of assisted reproductive technology are to attain a high live-birth rate of healthy children and to minimize the risk of multiple pregnancies (1–4). New generations of culture media (5, 6) have increased the rate of two pronuclei reaching the blastocyst stage (7, 8). Vitrification is a successful method of blastocyst cryopreservation (9–11). Artificial shrinkage (12) of the blastocoele before vitrification improves the survival rates of vitrified/warmed blastocysts (13, 14). With the application of vitrification and artificial shrinkage, vitrified/warmed blastocyst transfer results in a live-birth rate that is similar to that of fresh blastocyst transfer (11, 15). Studies demonstrate that single blastocyst transfer results in clinical pregnancy and live birth at rates that are similar to those with double ET and higher than those with single cleavage-stage ET; furthermore, for fresh and vitrified/warmed blastocysts, single

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blastocyst transfer generates a reduced incidence of multiple pregnancies than double ET (4,15–17).

Several classification and grading systems have been proposed to evaluate blastocysts. The blastocyst grading system by Gardner and Schoolcraft (18) is based on morphological parameters and remains largely unchallenged. However, studies continue to debate which parameter is the most important predictor of live birth. Several studies have reported that the inner cell mass (ICM) grade is positively correlated with the clinical pregnancy rate (19, 20). However, other recent studies have demonstrated that the trophectoderm (TE) grade correlates with clinical pregnancy and live-birth rates in both fresh and vitrified/warmed blastocyst cycles (21–24). In addition, evidence suggests that the degree of blastocoele expansion is a predictor of clinical outcomes after single blastocyst transfer (23, 25, 26).

Therefore, this study aimed to estimate the independent effects of ICM, TE, and degree of blastocoele expansion and re-expansion on the live-birth rate after fresh and vitrified/warmed single blastocyst transfer cycles.

MATERIALS AND METHODS

Study Design

This project was a retrospective study of single blastocyst transfer, including fresh cycles and vitrified/warmed cycles, performed from August 2009 to September 2014 at the Reproductive Medical Center of the First Affiliated Hospital of Zhengzhou University in China. The inclusion criteria included patients undergoing elective or nonelective single blastocyst transfer on day 5 in fresh or vitrified/warmed cycles. The main exclusion criteria included the oocyte donation cycle, fresh cycles undergoing assisted hatching, and cycles undergoing preimplantation genetic diagnosis. The First Affiliated Hospital of Zhengzhou University Ethics Committee approved this retrospective study.

Ovarian Stimulation Protocol

Patients underwent pituitary suppression with SC administration of triptorelin acetate (0.1 mg Decapeptyl, Ferring; or 3.75 mg Diphereline, Ipsen). When the patient achieved the criteria for pituitary suppression, ovarian stimulation was initiated with gonadotropin (Gonal-F, Merck Serono; Puregon, Organon; or Fostimon, ISBA). When the lead follicle was $\geq$20 mm and more than half the follicles were $\geq$16 mm, hCG (Ovitrelle, Merck Serono) was injected to trigger oocyte maturation. Follicle aspiration guided by transvaginal ultrasound was conducted 36–38 hours after hCG administration.

Blastocyst Culture and Grading

Oocytes were identified and isolated from the follicular aspirate and rinsed in culture medium (G-MOPS, Vitrolife). Insemination was achieved by IVF for 4 hours of incubation (27) or via the intracytoplasmic sperm injection (ICSI) technique in fresh preequilibrated culture medium (G-IVF, Vitrolife). The inseminated oocytes were then placed in cleavage medium (G1, Vitrolife), and fertilization was confirmed based on the formation of two pronuclei after 16–18 hours. On the third day, cleavage-stage embryos were graded according to the following criteria (12): number and regularity of blastomeres, percentage of fragmentation, and presence of granulation. On day 3, the embryos were regrouped, and selected embryos were cultured for 48 hours in a specific medium to form blastocysts (G2, Vitrolife).

On day 5, the blastocysts were evaluated by at least two local embryologists according to Gardner and Schoolcraft's grading system (18) (Fig. 1 A). Briefly, blastocysts were mainly evaluated based on three morphological parameters: the degree of blastocoele expansion and the grades of the ICM and TE. Expansion was categorized based on the following degrees: 1, an early blastocyst with its blastocoele less than half its size; 2, an early blastocyst with a blastocoele over half its size; 3, a full blastocyst with a blastocoele filling the space; 4, an expanded blastocyst with a blastocoele larger than its size; 5, a hatching blastocyst escaping from the zona pellucida; and 6, a hatched blastocyst that has completely escaped from the zona pellucida. ICM included the following grades: A, numerous tightly packed cells; B, a few loosely grouped cells; and C, very few cells. The TE was evaluated based on the following categories: A, many cells forming a cohesive epithelium; B, several cells forming a loose epithelium; and C, very few cells. High-quality blastocysts were transplanted with guidance from ultrasound within 2 hours of grading, and the remaining blastocysts were vitrified on day 5 or 6 according to the procedure described below.

Vitrification and Warming

The blastocoele was artificially shrunken using a laser pulse (OCTAX laser shot, MTG) before vitrification. Vitrification and warming protocols were conducted following traditional methods (28) according to the instructions of the Vit Kit (Kitazato Biopharma). Each blastocyst was transferred to equilibration solution (7.5% [v/v] DMSO + 7.5% [v/v] ethylene glycol) for 8–10 minutes. The blastocyst was then transferred into vitrification solution (15% [v/v] DMSO + 15% [v/v] ethylene glycol + 0.5 M sucrose) for 30 seconds. Subsequently, the blastocyst was placed on the Cryotop, and excess vitrification medium was removed by aspiration. The blastocyst was stored under liquid nitrogen.

During warming, the Cryotop was removed under liquid nitrogen, and the blastocyst was immersed in thawing solution (1.0 M sucrose) for 1 minute. The blastocyst was then transferred to diluent solution (0.5 M sucrose) at room temperature for 3 minutes. The blastocyst was washed twice in washing solution at room temperature for 10 minutes and cultured in blastocyst medium for 3 hours. A blastocyst was regarded as surviving if more than half the cells were intact and the blastocoele expanded again. Vitrified/warmed blastocysts were graded as described above according to Gardner and Schoolcraft's grading system (18) 2 hours after warming of blastocysts (28) (Fig. 1B).

Laser-assisted hatching was subsequently conducted as described elsewhere (29, 30). Two-thirds of the zona