ORIGINAL ARTICLE: ASSISTED REPRODUCTION

The ratio between inner cell mass diameter and blastocyst diameter is correlated with successful pregnancy outcomes of single blastocyst transfers

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Objective: To evaluate the ability to predict pregnancy outcomes of single-blastocyst transfers by measuring the ratio of inner cell mass (ICM) diameter to blastocyst diameter using time-lapse images.
Design: Retrospective cohort study.
Setting: University-affiliated medical center.
Patient(s): One hundred twenty-seven women undergoing a total of 129 blastocyst transfers with intracytoplasmic sperm injection.
Intervention(s): Embryo monitoring by time-lapse microscopy.
Main Outcome Measure(s): The ratio of ICM diameter to blastocyst diameter in single-blastocyst transfers and clinical pregnancy rates.
Result(s): In phase I of the study, 63 women underwent 65 single blastocyst transfers that resulted in 25 pregnancies (40% of the women). The successfully implanted blastocysts had an average ICM/blastocyst diameter ratio of 0.487 ± 0.086, whereas the average ICM/blastocyst ratio of nonimplanted blastocysts was significantly lower (0.337 ± 0.086). The live-birth rate was 29% (18/63). In phase II, 64 single-blastocyst transfers were performed in 64 women. The ICM/blastocyst diameter ratio was measured, and blastocysts with the highest ratios were chosen for transfer. Forty-three women (67%) with an average ICM/blastocyst diameter ratio of 0.46 achieved pregnancy, and 36 of the 43 pregnancies (84%) resulted in the delivery of a healthy baby. In the 21 women (33%) who failed to achieve pregnancy, the average ICM/blastocyst ratio was 0.45. The resultant positive predictive value was 74%, and the negative predictive value was 70%.
Conclusion(s): The ICM-to-blastocyst diameter ratio is a predictor of implantation and live birth in single-blastocyst transfers, offering a simple, noninterfering method to select blastocysts with high developmental capacity. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Blastocyst grading, inner cell mass, pregnancy, time-lapse

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In assisted reproduction treatments, the optimal outcome is the birth of a single healthy baby. To achieve this objective, accurate prediction of the developmental potential of single embryos is necessary.

The transfer of a single blastocyst-stage embryo has been considered a better option than the transfer of a cleavage-stage embryo because better embryo-endometrium synchrony may exist and the odds of transferring a viable embryo are increased (1).

Gardner and Schoolcraft (2) devised a qualitative scoring assessment for blastocysts, which was based on three major variables: the expansion stage of the blastocyst, the consistency of the inner cell mass (ICM), and the cohesiveness of the trophectoderm (TE). Gardner et al. reported that when two top-scoring blastocysts were transferred, implantation and clinical pregnancy rates of 69% and >80% were achieved. When only one of the transferred blastocysts was top quality, the implantation and pregnancy rates were 50% and 70% (3).

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Further quantitative measurements of blastocyst morphology as related to implantation potential were first reported by Richter et al. (4). Their results demonstrated a strong relationship between the size and shape of the ICM and blastocyst viability.

When time-lapse imaging incubators were introduced, IVF embryo evaluation was extended to continuous assessment, instead of distinct time-point observations. In the literature reviewed by Kirkegaard et al. (5) and Desai et al. (6), numerous time-lapse variables were described as positive or negative predictors of embryo development and implantation potential.

Based on the blastocyst classification system by Gardner and Schoolcraft and colleagues (2, 3) and the morphokinetic variables provided by time-lapse incubators, we attempted to provide a semiquantitative measure of ICM that could be easily applied with the use of time-lapse microscopy images and may significantly contribute to the prediction of implantation potential of single blastocysts.

MATERIALS AND METHODS

Study Design

Phase I.—We performed a retrospective cohort study of all women who underwent assisted reproduction treatments in our unit at Kaplan Medical Center, Rehovot, Israel, from September 2012 (the introduction of time-lapse incubators in our laboratory) until September 2014.

Inclusion criteria were as follows: women who had their autologous fertilized oocytes cultured continuously in the EmbryoScope (Unisense Fertilitech) and who underwent the transfer of a single fresh blastocyst on day 5, irrespective of the women's age, cycle number, and reasons for treatment.

Women receiving ovum donations were excluded from the study. Women with day 5 embryos that had not progressed past the morula stage were also excluded.

Phase II.—Starting in October 2014, based on the results from phase I, a modified evaluation was initiated using the ICM to blastocyst diameter ratio to predict the implantation potential of the blastocysts.

Women whose autologous embryos were cultured in the EmbryoScope until transfer on day 5 were included in the study. Similar to the retrospective study, women whose embryos did not reach the blastocyst stage were excluded. Only women with a measurable ICM diameter were included in the population studied.

Each blastocyst was measured, and the ratio between the ICM diameter and the inner diameter of the embryo was calculated. The blastocyst with the highest ratio was chosen for transfer.

Serum levels of the β-subunit of hCG (β-hCG) hormone were measured 14 days after oocyte retrieval. Clinical pregnancy was confirmed when an intrauterine gestational sac with a fetal heartbeat was observed by ultrasound 3 weeks after a positive β-hCG result.

Study approval was obtained from the Kaplan Medical Center Institutional Review Board for both phases of the study.

Ovarian Stimulation, Ovum Pick-up, Intracytoplasmic Sperm Injection (ICSI), and Culture Conditions

Pituitary down-regulation and controlled ovarian stimulation were carried out with either a GnRH agonist or antagonist (Decapeptyl, Ferring, or Cetrotide, Merck Serono, respectively) followed by daily injections of gonadotropins. The daily doses were adjusted after 4–5 days according to follicle size and serum E_2 levels. Transvaginal oocyte retrieval was performed approximately 32–34 hours after either the administration of hCG (Ovitrelle, Merck Serono Laboratories) or GnRH agonist or both.

Oocytes were cultured in continuous single culture media (CSC; Irvine Scientific) with 10% serum substitute supplement (Irvine Scientific) or in Global Total for fertilization (LifeGlobal) at 5.5% CO_2 and 37°C before denudation with hyaluronidase (Sage CooperSurgical). ICSI was performed in a medium containing HEPES buffer, and the injected oocytes were transferred into preequilibrated EmbryoSlide culture dishes (Unisense Fertilitech) with 12 microwells. The oocytes were cultured individually in 25 μL of CSC or Global Total under a layer of oil in a time-lapse incubator (EmbryoScope, Unisense Fertilitech) until day 5, without media change or removal of the slides from the EmbryoScope.

Blastocyst Grading, Transfer, and Implantation

Fertilization and embryo morphology were assessed based on the images acquired from the time-lapse system. Annotation of morphokinetics was performed on all embryos on culture days 1, 2, 3, and 5. Timings were expressed as hours after ICSI, which was considered time zero. Blastocysts were scored on day 5 according to the criteria of Gardner and Schoolcraft (2) as follows: the size of the blastocoelic cavity (early, less than half the volume of the blastocyst; blastocyst, more than half the volume; full blastocyst, blastocoelic cavity fills the embryo), the ICM grade (A, many tightly packed cells; B, several loosely packed cells; C, very few cells), the number and cohesiveness of the TE cells (A, cohesive layer of many cells; B, several cells forming a loose epithelium; C, very few large cells). Examples of blastocyst grading and pregnancy outcome are shown in Figure 1. Q1

The EmbryoViewer is equipped with ellipse and distance tools that can be easily used for estimating area and diameter of blastomeres and embryos. However, we used external imaging processing software (7) to measure the diameter of the ICM and the inner diameter of the blastocyst because it seemed to us more accurate and sensitive. ImageJ open-source software can read most of the formats used in the field of biomedical imaging. This program supports all common image manipulations, including reading and writing image files, and is able to run on different platforms.

The measurements were taken on images of blastocysts before expansion occurred, between 114 and 116 hours after zero time. The ICM diameter was defined as the perpendicular distance between the apex of the ICM and its base on the inner border of the blastocyst. The blastocyst diameter was