Trophectoderm morphology significantly affects the rates of ongoing pregnancy and miscarriage in frozen-thawed single-blastocyst transfer cycle in vitro fertilization

Hiroyuki Honnma, M.D., Ph.D.,[a] Tsuyoshi Baba, M.D., Ph.D.,[b] Masahiro Sasaki, M.D., Ph.D.,[a] Yoshiki Hashiba, M.D., Ph.D.,[a] Hiroshi Ohno,[a] Takanori Fukunaga, Ph.D.,[a] Toshiaki Endo, M.D., Ph.D.,[b] Tsuyoshi Saito, M.D., Ph.D.,[b] and Yoshimasa Asada, M.D., Ph.D.[a]

[a] Asada Ladies' Clinic, Aichi; and [b] Department of Obstetrics and Gynecology, Sapporo Medical University School of Medicine, Sapporo, Japan

Objective: To determine which parameter of blastocyst morphology is the most important predictor of ongoing pregnancy or miscarriage.
Design: Retrospective analysis.
Setting: One in vitro fertilization (IVF) center.
Patient(s): Women who underwent a total of 1,087 frozen-thawed single-blastocyst transfer cycles.
Intervention(s): First IVF treatment with blastocysts after frozen-thawed cycle.
Main Outcome Measure(s): Ongoing pregnancy and miscarriage rates as related to blastocyst morphology (blastocyst expansion, inner cell mass, and trophectoderm), and interaction tests in unadjusted logistic regression models to assess clinical factors affecting outcomes.
Result(s): After adjustment for trophectoderm, inner cell mass, and age as confounders, trophectoderm was determined to be statistically significantly related to the rate of ongoing pregnancy. Trophectoderm was also statistically significantly related to the miscarriage rate. By contrast, neither inner cell mass nor blastocyst expansion was statistically significantly related.
Conclusion(s): In frozen-thawed embryo transfer cycles, trophectoderm morphology is statistically significantly related to the rates of ongoing pregnancy and miscarriage after adjusting for confounders. Trophectoderm morphology may be the most important parameter when selecting a single blastocyst for transfer. (Fertil Steril® 2012;98:361–7. ©2012 by American Society for Reproductive Medicine.)
Key Words: Blastocyst grading, frozen-thawed embryo transfer, logistic regression analysis, miscarriage rate, pregnancy rate, trophectoderm morphology

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/honnmah-trophectoderm-morphology-pregnancy-and-miscarriage-frozen-thawed-blastocyst-transfer/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

The most important of the reported advantages of blastocyst-stage transfer in vitro fertilization (IVF) is that it yields a higher pregnancy rate than cleavage-stage embryo transfer because embryos are selected through delayed culture. Moreover, the high pregnancy rate obtained with blastocyst transfer enables the use of single-embryo transfer, thereby reducing the incidence of multiple pregnancies (1, 2). We have been using a three-parameter grading system (3)—blastocoel expansion, inner cell mass (ICM), and trophectoderm—to evaluate blastocyst quality, as this method is relatively easy and is noninvasive. Several reports have indicated that trophectoderm morphology is predictive

Received January 24, 2012; revised April 23, 2012; accepted May 7, 2012; published online June 6, 2012.
H.H. has nothing to disclose. T.B. has nothing to disclose. M.S. has nothing to disclose. Y.H. has nothing to disclose. H.O. has nothing to disclose. T.F. has nothing to disclose. T.E. has nothing to disclose. T.S. has nothing to disclose. Y.A. has nothing to disclose.
Reprint requests: Hiroyuki Honnma, M.D., Ph.D., Asada Ladies' Clinic, Renak 5F, Matsushincho 1–4, Kasugai, Aichi 486–0931, Japan (E-mail: hihonma@sapmed.ac.jp).

Fertility and Sterility® Vol. 98, No. 2, August 2012 0015-0282/$36.00

doi:10.1016/j.fertnstert.2012.05.014

of cycle outcome (pregnancy rate) in IVF treatment (4, 5). However, it also has been reported that there is a positive correlation between ICM and pregnancy rate (6–9), but no correlation between trophectoderm morphology and ICM (10). This discrepancy reflects that the mechanisms underlying these blastocyst parameters and their relationship to pregnancy outcome are still not fully understood. In addition, there have been no studies examining the relationship between blastocyst parameters and the miscarriage rate.

It is recognized that embryo chromosomal abnormalities significantly affect embryo development and morphology, and pregnancy outcomes (11). For example, it was recently reported that blastocyst aneuploidy negatively affects blastocyst developmental speed, ICM morphology, and trophectoderm morphology. Embryo gender also affects blastocyst development in several mammalian species (12). Selection of a morphologically robust blastocyst reduces the risk of aneuploidy and increases the likelihood of a good pregnancy outcome. However, although blastocyst morphology and aneuploidy are linked, morphological analysis cannot be relied upon to ensure transfer of chromosomally normal embryos although it could reduce the risk of aneuploidy (12).

With the recent advent of embryo cryopreservation technology, which has led to a surplus of good quality embryos in a considerable proportion of IVF cycles and has diminished the incidence of ovarian hyperstimulation syndrome (OHSS), frozen-thawed embryo transfer (F-TET) cycles have become an integral part of IVF treatment (13, 14). Moreover, although endometrial receptivity reportedly differs significantly between fresh embryo transfer (FET) and F-TET cycles (15), there has been only one report on the relationship between single blastocyst quality and pregnancy rates in F-TET cycles (10). We evaluated the relationship between three blastocyst parameters and the rates of ongoing pregnancy and miscarriage in patients who were undergoing F-TET cycles for their first IVF treatment.

MATERIALS AND METHODS

Patients and Treatment

From October 2007 through December 2010, 2,916 cycles of frozen-thawed single-blastocyst transfers were performed in our clinic. From among these, 1,087 first IVF treatment cycles (excluding mild stimulation protocols) with a blastocyst expansion grade ≥ 3 were enrolled. Inclusion criteria for this study were as follows: [1] the patient was in her first cycle of IVF treatment; [2] her age was <45 years; [3] there was no evidence of an endocrinologic disorder (normal prolactin and thyroid-stimulating hormone levels); [4] her basal serum follicle-stimulating (FSH) levels were <13.0 mIU/mL; and [5] her body mass index (BMI) was <30.0 kg/m^2. In addition to the patient's age, the IVF protocol selection was based on her basal serum FSH level, as previously described elsewhere (16): briefly, gonadotropin-releasing hormone (GnRH) agonist long protocol (long protocol) serum FSH <8.0 mIU/mL; GnRH agonist flare-up protocol (short protocol) serum FSH $=$ 8.0–13.0 mIU/mL; suspected polycystic ovary syndrome (PCOS) and OHSS, GnRH antagonist flexible protocol (antagonist protocol) serum FSH <8.0 mIU/mL.

Controlled Ovarian Hyperstimulation Protocol

The daily gonadotropin administered was either human menopausal gonadotropin (Menopur; Ferring Pharmaceuticals) or recombinant FSH (Gonal-f; Merck Serono). The long protocol began with daily nasal buserelin of 600 μg/day (Buserecure; Fuji Pharmaceutical) from day 21 of the prestimulation cycle. The short protocol began with daily nasal buserelin of 600 μg/day from day 3 of the stimulation cycle. In both the long and short protocols, the GnRH agonist was continued until the day of human chorionic gonadotropin (hCG) administration. In the antagonist protocol, a GnRH antagonist, Ganrelix (Ganirest; MSD) or Cetrorelix (Cetrotide; Nippon Kayaku), was administered when the leading follicle reached a maximum diameter of 14 to 16 mm or a premature luteinizing hormone (LH) surge was suspected based on serum LH monitoring (16). When three or more follicles reached a maximum diameter of 18 mm, urinary hCG (5,000 or 10,000 IU) was administered. Transvaginal oocyte retrieval was performed 34 to 35 hours after hCG injection, as described previously elsewhere (17). Oocytes were fertilized conventionally or via intracytoplasmic sperm injection (ICSI). Whether split (ICSI + conventional) or ICSI was indicated depended on the number of metaphase II (M2) oocytes (<5 M2 oocytes) and oligozoospermia (total number of zoosperm $<10^{*}10^{6}$ determined using the swim-up method).

The cryopreservation criteria without FET were [1] serum estradiol (E_2) $>6,000$ pg/mL, [2] serum progesterone (P4) >2.0 ng/mL on the day of hCG administration, and [3] symptoms of OHSS after oocyte retrieval. Cryopreservation with FET was performed at the blastocyst stage (283 cycles). Cryopreservation without FET was performed at the pronuclear stage (352 cycles based on criteria 1 + 2) or blastocyst stage (452 cycles based on criterion 3).

Embryo Culture and Cryopreservation

The embryos were cultured individually in 15-μL droplets of Sage Cleavage Medium until day 3 or Sage Blastocyst Medium until the blastocyst stage (Sage BioPharma). Embryos were vitrified at the pronuclear (one cell) stage (352 cycles) or the blastocyst stage (735 cycles). Among the embryos vitrified at the blastocyst stage, 448 were vitrified 5 days after fertilization and 287 were vitrified 6 days after fertilization. With the exception of those that were both ICM and trophectoderm grade C, blastocysts that expanded beyond grade 3 were vitrified and transferred. Pronuclear-stage embryos were developed into blastocysts after warming and then transferred. Cooling and embryo warming were performed using the Cryotop methodology for human embryo vitrification described by Kuwayama et al. (18). Equilibration, vitrification, warming, dilution, and washing solutions were provided in the Vitrification Kit (VT-101 and VT-102; Kitazato Biopharma).

Embryo Evaluation

The morphology of prefreeze blastocysts and blastocysts developed from the pronuclear stage after thawing was assessed with respect to the corresponding ongoing pregnancy and