Casting for determinants of blastocyst yield and of rates of implantation and of pregnancy after blastocyst transfers

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Objective: To identify determinants of blastocyst yield, implantation rate, and pregnancy outcome.
Design: Retrospective analysis of outcomes of 1,653 cycles of IVF.
Setting: Private infertility clinic.
Patient(s): Couples presenting to an infertility clinic for IVF.
Intervention(s): None.
Main Outcome Measure(s): Blastocyst yield, implantation rate, and pregnancy.
Result(s): Of a broad array of potential determinants, only the total numbers of oocytes retrieved and properties of day 3 embryos were consistently predictive of blastocyst formation. Relative to numbers of oocytes fertilized by intracytoplasmic sperm injection (ICSI), yields of quality blastocysts were highest in cycles in which <10 oocytes were retrieved. Blastocyst yield was closely linearly correlated with average numbers of blastomeres in embryos on day 3. As oocyte yields rose, average grades and the implantation potential of the blastocysts selected for transfer increased by approximately 0.015 and 0.15%, respectively, for each additional oocyte. Independently, the implantation potential of blastocysts decreased 1.1% for each advancing year in age of the oocyte provider, and, for autologous transfers, uterine receptivity declined an additional 0.6% per year. Higher yields of blastocysts from cycles with high oocyte numbers afforded better selection of blastocysts for transfer, supporting higher overall implantation and pregnancy rates.
Conclusion(s): While the proportion of fertilized oocytes that progressed to quality blastocysts diminished as numbers of recovered oocytes rose, rates of implantation and pregnancy after transfer of the selected best blastocysts increased. The age of the oocyte provider and oocyte yields independently impacted blastocyst implantation potential and uterine receptivity after controlled ovarian hyperstimulation, ICSI, and blastocyst transfer. (Fertil Steril® 2014;102:1055–64. ©2014 by American Society for Reproductive Medicine.)
Key Words: Oocyte yield, blastocyst formation, uterine receptivity, implantation, pregnancy

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After ovulation and fimbrial capture, mature human oocytes fertilize and undergo cleavage to the compacting 8-cell stage within the fallopian tubes. About 3 days after fertilization, compacting morulae migrate into the uterine cavity for continued cleavage, cavitation (blastocoele), hatching from the zona pellucida, and implantation (initiated on about day 5). Blastocysts with the highest implantation potential will have cavitated by the fifth day after oocyte retrieval and will have a distinct compacting inner-cell mass (ICM) (1). Coincident with blastocoele expansion, the zona pellucida thins and is lined with a contiguous layer of trophectoderm cells. The total number of cells in a blastocyst on day 5 should be near 200 (1). These quantifiable properties of blastocysts are broadly incorporated into current grading schemes and into national proficiency testing challenges for grading of human blastocysts (e.g., American Association of Bioanalysts).

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Not all structurally normal morulae and compacting embryos will progress to structurally normal blastocysts, and approximately half of all structurally normal blastocysts do not implant. Consequentially, expression of mRNA transcripts in the embryo is initiated at about the 4- to 8-cell stage (2–4), reflecting activation of the paternal genome (5). When disruptions in spermatogenesis alter the timing and pattern of paternal genome expression, cell adhesion during compaction and the ensuing steps in blastocyst formation are compromised (6). Overall, implantation rates of blastocysts are approximately double those of transferred day 2–3 embryos, and this ascendancy reflects the elimination of those cleaved embryos that fail to progress normally because of any of (or combination of) the multitude of properties of oocytes and/or sperm that have the potential to jeopardize normal blastocyst formation (2, 4–21).

Nationally, the proportion of blastocyst transfers relative to (earlier) transfers of cleaved embryos continues to rise (statistics published annually through the Centers for Disease Control [CDC] and the American Society for Reproductive Medicine, Society for Assisted Reproductive Technology [SART]), with attendant overall improvements in reported pregnancy outcomes. The exploding peer-reviewed literature in clinical assisted reproductive technology (ART) continues to fuel this and other changes in clinical and laboratory practices that parallel improvements in success rates. However, despite uniform adoption of the better established clinical and laboratory procedures, patient outcomes (between and within centers) continue to vary widely (CDC/SART). The present study was therefore undertaken to identify which of the gamut of the popularly controlled and/or measured elements of IVF cycles might relate to the yield of quality blastocysts, their likelihood of implantation, and pregnancy outcomes.

MATERIALS AND METHODS

Institutional Review Board (IRB) Approval/ Exemption

This study was a noninterventional retrospective analysis of outcomes of IVF cycles completed between January 2009 and December 2012 in our center. Patients were referred with varying histories of male and/or female factor infertility, single parenting, or same-sex parenting. Overall, the study population leaned toward subfertility and did not represent a cross section of all couples of reproductive age. This study analyzes existing data that were exported from a database without identifiers that could be linked to the subjects. The results of this study were/will not be submitted to the Food and Drug Administration (FDA) for marketing approval, and the investigation did not involve research on an FDA-regulated product. This research was determined to be exempt from the requirements of 45 CFR 46 by the Western IRB, an independent IRB located in Olympia, Washington.

Study Endpoints

As development of human embryos beyond the blastocyst stage necessitates apposition to, and invasion of, the endometrium, indices of development of cultured human embryos through to the blastocyst stage provide practical endpoints for analysis of any potential clinical or laboratory determinant of pregnancy outcome after IVF and ET. In this study, the index of the potential for blastocyst transformation from fertilized oocytes was the total yield of quality blastocysts (transferrable or freezable), within 6 days of culture, relative to the total number of 2-pronuclear zygotes (2PN; %). Quality blastocysts were defined as those with grades ≥ 2.5 (out of a possible maximum of 5.0), these being expanding blastocysts with thinning zonae, distinct compacting ICMs, and $\geq 70\%$ of the inside surface of the zona pellucida contiguously enveloped with sickle-shaped trophectoderm cells (1). On the basis of survival of blastocysts through vitrification, and thawing and pregnancy outcomes after blastocyst transfers (fresh and frozen thawed), our laboratory has also determined this grade and these elements of blastocyst grading to most consistently differentiate viable from nonviable blastocysts. The clinical endpoints in this study were implantation rates of transferred blastocysts (number of gestational sacs relative to the number of blastocysts transferred; %) and pregnancy outcomes (live birth).

Cycles Qualifying for Inclusion

The study endpoints restricted qualifying cycles to those in which all fertilized oocytes were cultured for a minimum of 4 additional days (no freezing of 2PN or of cleaved embryos or morulae) before ET and/or cryopreservation. Cycles in which previously frozen embryos were added to the fresh embryo cohort were excluded. To limit the effects of products of sperm necrosis (e.g., lipid peroxidases and peroxides (22)) on the quality of fertilized oocytes and their progression, cycles in which fertilization occurred by IVF (representing $<5\%$ of all cycles in this center during the study period) were also excluded. All 2PNs followed in this study were therefore produced by intracytoplasmic sperm injection (ICSI). Cycles in which preimplantation genetic screening (PGS) was performed were excluded because biopsy can reduce implantation potential (23) and results of PGS would have narrowed the candidate population of embryos retained in culture and/or available for transfer or cryopreservation. Applying these restrictions, the number of cycles that qualified for inclusion and proceeded to ET was 1,653 (1,096 autologous ETs in which embryos were returned to the oocyte provider and 557 third-party transfers in which the gestational carrier was not the oocyte provider).

During this time period (January 2009 through December 2012), culture media were procured from the same manufacturer (Vitrolife). The pH ranges for fertilization, growth, and blastocyst development were constant (7.10–7.30, 7.20–7.34, and 7.20–7.34, respectively; Orion 2 Star pH meter, Thermo Scientific), and incubator atmospheres necessary to maintain these conditions were attainable through adjustment within narrow ranges (O_2 held at 5% with CO_2 adjusted between 6.0 and 6.3%; Bacharach).

Potential Determinants

A broad range of potential determinants of blastocyst yield were examined, all of which are routinely recorded for each