Number of supernumerary vitrified blastocysts is positively correlated with implantation and live birth in single-blastocyst embryo transfers

Micah J. Hill, M.D.,[a,b] Kevin S. Richter, Ph.D.,[b] Ryan J. Heitmann, M.D.,[a,b] Terrance D. Lewis, M.D., Ph.D.,[a] Alan H. DeCherney, M.D.,[a] James R. Graham, M.S.,[b] Eric Widra, M.D.,[b] and Michael J. Levy, M.D.[b]

[a] Program in Reproductive and Adult Endocrinology, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda; and [b] Shady Grove Fertility Reproductive Science Center, Rockville, Maryland

Objective: To estimate whether live birth in single-blastocyst transfers is correlated with the number of sibling supernumerary vitrified blastocysts (embryos not transferred) generated from that same cycle.
Design: Retrospective cohort study.
Setting: A large academic assisted reproduction clinic.
Patient(s): All single-blastocyst transfers in 2010 graded as "good" embryos by Society for Assisted Reproductive Technologies (SART) criteria.
Intervention(s): None.
Main Outcome Measure(s): Implantation and live birth.
Result(s): Of the 655 single-blastocyst transfers that met inclusion criteria, implantation occurred in 65% and live birth in 54% of cycles. In chi-square analysis, patients with supernumerary vitrified blastocysts had a statistically higher implantation rate (65% versus 50%) and live-birth rate (56% versus 41%) when compared with patients without supernumerary blastocysts. Univariate logistic regression demonstrated an increase in implantation (OR 1.09; 95% CI, 1.03–1.15) and live birth (OR 1.06; 95% CI, 1.02–1.09) with increasing number of supernumerary blastocysts. Multivariate logistic regression analysis demonstrated that patient age and the number of supernumerary blastocysts were statistically significantly associated with implantation and live birth.
Conclusion(s): The number of supernumerary vitrified blastocysts correlated positively with the odds of implantation and live birth in good quality single-blastocyst transfers. Patients with supernumerary blastocysts are good candidates for single-embryo transfer. (Fertil Steril® 2013;99:1631–6. ©2013 by American Society for Reproductive Medicine.)
Key Words: Blastocyst, implantation, live birth, supernumerary embryos, vitrified

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/hillmj-supernumerary-embryos-vitrified-blastocysts-implantation-live-birth/

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.

Received December 3, 2012; revised January 16, 2013; accepted January 21, 2013; published online February 20, 2013.
M.J.H. has nothing to disclose. K.S.R. has nothing to disclose. R.J.H. has nothing to disclose. T.D.L. has nothing to disclose. A.H.D. has nothing to disclose. J.R.G. has nothing to disclose. E.W. has nothing to disclose. M.J.L. has nothing to disclose.
Supported in part by the Intramural Research Program of the Program in Reproductive and Adult Endocrinology, National Institute of Child Health and Human Development, National Institutes of Health.
The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the U.S. Government.
Reprint requests: Michael J. Levy, M.D., Program in Reproductive and Adult Endocrinology, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, 10 Center Drive, Building 10, CRC, Room E1–3140, Bethesda, Maryland 20892 (E-mail: michael.levy@integremed.com).

Fertility and Sterility® Vol. 99, No. 6, May 2013 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.01.130

The ability to predict embryo implantation is clinically useful for counseling patients and in determining the ideal number of embryos to transfer. Cycles with the highest implantation potential are ideal cycles for single-embryo transfer to minimize the risks of multifetal gestation while maximizing the likelihood of pregnancy (1). It is well established that embryo quality correlates with implantation potential (1–5). Supernumerary embryos

are excess embryos obtained during assisted reproductive technologies (ART) that are not transferred in the fresh cycle in which they are generated. The presence of supernumerary embryos for cryopreservation has been used as a marker of embryo quality and is an independent predictor of implantation (2, 6–8). In cleavage-stage embryo transfers, supernumerary embryo development to the blastocyst stage was more likely to occur in cycles where the transferred embryos implanted (9). Cleavage-stage embryo transfers with additional cryopreserved embryos were more likely to implant than similar grade embryo transfers without supernumerary embryos available for cryopreservation (2).

There are gaps in the current knowledge about supernumerary embryos. The current literature comes primarily from cleavage-stage transfers. It is possible that the importance of supernumerary embryos in predicting outcomes may not apply to blastocysts, which benefit from a higher implantation rate as a result of improved embryo selection. The statistical analyses in the current studies rely primarily on chi-square statistics. While this has demonstrated that the presence of supernumerary embryos is superior to the absence, this statistical test does not allow a prediction of whether the number of supernumerary embryos correlates with ART outcomes or a control for important potential confounders in the analysis. Finally, the current studies have been performed in multiple-embryo transfer cycles. A single-embryo transfer study design would control for the actual number of embryos transferred.

Although the absence or presence of supernumerary embryos is correlated with implantation, we are not aware of any data examining the correlation of the *number* of supernumerary embryos on implantation in single-blastocyst transfers. We estimated whether live birth in single-blastocyst transfers was correlated with the number of sibling supernumerary vitrified blastocysts generated from that same cycle. We hypothesized that the number of supernumerary blastocysts available for vitrification would positively correlate with implantation and live birth in single-blastocyst transfers.

MATERIALS AND METHODS

Study Design

This was a retrospective cohort analysis of 720 fresh, autologous single-blastocyst embryo transfer cycles during 2010. Because the quality of the actual embryo being transferred would be a significant confounder in the implantation and live birth of each cycle, we limited the analysis to only embryos transferred that were scored by Society for Assisted Reproductive Technologies (SART) criteria as "good" grade to control for embryo quality. These are embryos with an inner cell mass grade A and a trophectoderm grade A or B. Of 720 single-blastocyst transfers, 655 were transfers of a SART grade good embryo. Supernumerary blastocysts were defined as additional blastocysts generated in that same fresh ART cycle that were not transferred and were of a quality to be cryopreserved. Vitrification was performed for inner cell mass/trophectoderm grade BB or better embryos. The study was performed at the Shady Grove Fertility Reproductive Science Center in Rockville, Maryland, and approval was obtained from the institutional review board.

Patients

All patients who underwent a fresh autologous embryo transfer of a SART grade good embryo during 2010 were included in the analysis. Additional subgroup analyses were performed on embryos that were expanded or hatched blastocysts with a grade A trophectoderm and grade A inner cell mass, representing the highest quality embryos for transfer. The study included elective single-blastocyst transfers as well as patients with only a single blastocyst available for transfer. Exclusion criteria included cycles with multiple embryos transferred, donor-recipient cycles, frozen-thaw transfers, cleavage-stage transfers, morula-stage transfers, and transfers of SART grade fair or poor blastocysts.

Stimulation Protocol

Ovarian stimulation was accomplished with mixed follicle-stimulating hormone/luteinizing hormone (FSH/LH) protocols under gonadotropin-releasing hormone (GnRH) antagonist or GnRH agonist pituitary suppression, as previously described elsewhere (10). For most patients, oral contraceptive treatment was initiated 19 days before stimulation. For GnRH antagonist cycles, the antagonist (Ganirelix) was initiated when the lead follicle was 14 mm in size. During the last 3 days of oral contraceptives, 20 units of leuprolide acetate (Lupron) was initiated in the GnRH agonist cycles. The leuprolide acetate dose was decreased to 5 units when ovarian suppression was confirmed. Ovarian stimulation was typically achieved with a mixed protocol employing recombinant FSH and human menopausal gonadotropin (hMG). When the lead follicle was $\geq$18 mm, final oocyte maturation was triggered with 10,000 units of hCG or with 40 units of GnRH agonist in some of the GnRH antagonist cycles. Oocyte retrieval occurred 36 hours later, and insemination was achieved with conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), as indicated. Ultrasound-guided embryo transfer was performed on day 5 or day 6. Serum hCG levels were assessed 2 weeks after the trigger injection, and ultrasonographic confirmation of pregnancy was obtained in all pregnant patients.

Embryo Grading

All blastocysts were evaluated by an embryologist using a modification of the grading system of Gardner and Schoolcraft (11, 12). The trophectoderm was assigned one of the following grades: A = many cells organized in epithelium, B = several cells organized in loose epithelium, or C = few large cells. The inner cell mass was assigned one of the following grades: A = numerous, tightly packed cells, B = several and loosely packed cells, or C = very few cells. Blastocyst expansion was assigned one of the following descriptors: early = blastocele less than half the blastocyst; expanded = blastocele fills the blastocyst with thin zona; hatched = blastocyst has hatched out of the zona. Morula