Successful pregnancy rates achieved with day 4 embryo transfers

Josh C. Skorupski, M.D., Daniel E. Stein, M.D., Uchenna Acholonu, M.D., Heather Field, B.A., and Martin Keltz, M.D.

Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, St. Luke's-Roosevelt Hospital Center, Columbia University College of Physicians and Surgeons, New York, New York

Objective: To assess the success of day 4 embryo transfers (ETs) following IVF at one institution.
Design: Retrospective analysis.
Setting: A university hospital IVF program.
Patient(s): Two hundred nondonor, fresh IVF cycles.
Intervention(s): None.
Main Outcome Measure: Outcomes of IVF. Outcome assessments after day 4 ETs included rates of implantation, clinical pregnancy, and singleton and multiple live births.
Result(s): The overall live-birth rate was 54.4%. Implantation rates were highest in younger age groups, and similar in patients 35–40 years of age. Pregnancy and live-birth rates were similar across all age groups up to age 40 years. Multiple gestations were highest in women ≤40 years of age.
Conclusion: Acceptable pregnancy rates can be achieved with day 4 ETs. (Fertil Steril® 2007;87:788–91. ©2007 by American Society for Reproductive Medicine.)

Key Words: Embryo transfer, blastocyst, morula

Although the embryo transfer (ET) resulting in the first pregnancy achieved through IVF was performed at the blastocyst stage (1), the majority of ETs to date have been performed 3 days after insemination. Initial limitations in culture media prevented the growth of prezygotic-stage embryos and thus later ETs (2). With the advent of sequential media, extended human embryo culture to the morula and blastocyst stages is now feasible (3). Extending embryo culture prior to ET may have several advantages over earlier-stage transfers. It was shown that the human embryonic genome is not activated before the 4–8-cell stage (4). Additional hours of in vitro culture allow for improved visualization of proliferating embryos and improved embryo selection (5–7). With the ability to select the most developmentally capable embryos, the number of embryos transferred per cycle could potentially be reduced, thereby limiting high-order multiple pregnancies while maintaining an acceptable pregnancy rate (PR). Day 5 ETs at the blastocyst stage were extensively studied; they maintain certain advantages and risks when compared to transfers on day 3 (8, 9). Advantages include a better selection of embryos and acceptable success rates, with the transfer of fewer embryos. Possible risks of day 5 blastocyst transfers might include in vitro failure of blastulation (10), increased monozygosity (11–13), reduced embryo quality, and an increase in transfer cancellations (14). In our own center during the past several years, we have primarily transferred morulae on day 4. Our rationale is based on the observation that in nature, embryos enter the uterus as morulae (15).

Reports on the use of day 4 ETs are limited. Tao et al. retrospectively compared the use of day 3 versus day 4 ETs in a nonrandomized population, and showed that morula- and compaction-stage embryos allowed better embryo selection than earlier-stage embryos, and significantly reduced the number of embryos needed for transfer (16). Selection of day 3 or day 4 ETs by Tao et al. (16) was based on an arbitrary change of protocol in the participating IVF centers from routine day 3 to routine day 4 transfers. Grifo et al. (17) and Gianaroli et al. (18) reported successful pregnancy outcomes with day 4 ETs after preimplantation genetic diagnosis. Day 4 transfers might allow for improved selection over day 3 embryos by allowing for an additional day of observation and assessment, without some of the potential risks experienced with blastocyst transfers.

Here, our experience with day 4 transfers is presented. The possible advantages of day 4 transfers, including decreasing the risk of multiple pregnancies, facilitating assisted hatching, and preimplantation genetic diagnosis (PGD) analysis prior to implantation, are also discussed.

MATERIALS AND METHODS

We performed a retrospective analysis of all 200 nondonor, fresh IVF cycles at St. Luke's Roosevelt Hospital Center, New York, New York, between January 1, 2003–December 31, 2003. Data collected included age, basal FSH and E_2 levels, infertility diagnoses, number of retrieved oocytes and

Received January 1, 2006; revised and accepted August 11, 2006.
Reprint requests: Josh Skorupski, M.D., Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, St. Luke's-Roosevelt Hospital Center, 425 West 59th Street, Suite 5A, New York, New York 10019 (FAX: 212-523-8348; E-mail: josh_skorupski@hotmail.com).

0015-0282/07/$32.00
doi:10.1016/j.fertnstert.2006.08.093

embryos, embryo grades, medications (and dosages) used, and peak E_2 levels. Our study group consisted of all women undergoing IVF in 2003 regardless of their infertility diagnosis, age, number of follicles, or number of fertilized oocytes. Patients with a basal FSH level >15 IU/L were generally excluded from nondonor IVF. We also collected outcome data including implantation, pregnancy, and live-birth rates. Data were collected in compliance with our institutional review board. There was no conflict of interest between any commercial entity and the contributing authors. Controlled ovarian stimulation was performed using leuprolide acetate, recombinant FSH, or a combination of recombinant FSH and urinary hMG in a long or short (flare) protocol for all subjects (19, 20). Ovarian follicular development was monitored by transvaginal ultrasonography and serial E_2 levels. After ≥3 oocytes ≥18 mm in maximum diameter were detected by sonography, 10,000 IU of hCG were administered. Thirty-five hours after administration of hCG, oocytes were retrieved transvaginally under ultrasound guidance. All retrievals and transfers were performed by two physicians in a single private IVF center under ultrasound guidance. Retrieved oocytes were incubated with spermatozoa, or in cases of severe male factor (21), intracytoplasmic sperm injection was performed. The presence of two pronuclei 24 after insemination confirmed fertilization (day 1). Assisted hatching is performed in our center in approximately 80% of IVF cycles. Fragment removal was performed on embryos with >10% fragmentation. Assisted hatching and fragment removal were performed on day 3 embryos at the 6–8-cell stage. Micromanipulation was performed in prewarmed petri dishes (Falcon 1006; Beckton, Dickson and Company, Bedford, MA) in 10-μL droplets consisting of modified human tubal fluid (HEPES-buffered; Sage Biopharma, Trumbull, CT) supplemented with 15% synthetic serum (Irvine Scientific, Irvine, CA). All micromanipulation procedures were performed on the heated stage of an inverted microscope (Olympus America Inc., Melville, NY) with the use of hydraulic manipulators (Narishige International, USA, East Meadow, NY). The assisted hatching procedure was performed as initially described by Cohen et al. (22).

Extracellular fragments were removed, with an effort to avoid damage to proximal blastomeres. Micromanipulated embryos were washed with Tyrode's solution, and cultured for an additional 24 hours prior to transfer. Transfers were performed on day 4 after retrieval, with the use of a soft catheter (Wallace; Sims Portex Ltd., Kent, UK). Serum beta hCG levels were drawn on days 12 and 14 after transfer. The presence of a gestational sac was verified by transvaginal sonography 19 or 20 days after transfer. Biochemical, nonclinical pregnancies were included in the statistical analyses as nonpregnancies.

RESULTS

The data for all patients who underwent ET on day 4 after insemination are shown in Table 1. In our population, the average patient was 34.8 years of age. A mean of 8.37 oocytes was retrieved, of which 6.51 were fertilized and 3.05 were transferred.

TABLE 1

In vitro fertilization data for day 4 ETs.

Mean age at ET (y)	34.8 ± 4.2
Mean basal FSH (mIU/mL)	6.28 ± 2.6
Mean no. of gonadotropins used/cycle	50.72 ± 25.6
Mean peak ampules per day	5.68 ± 1.94
Mean peak E_2 (pg/mL)	2,223.4 ± 1,037.9
Mean peak endometrial thickness	10.53 ± 2.2
Mean no. of oocytes retrieved	8.37 ± 4.6
Mean no. of M2	6.96 ± 4.4
Mean no. of oocytes fertilized	6.51 ± 4.4
Mean no. of ETs	3.05 ± 1.1

Note: M2 = metaphase II oocyte.

Skorupski. Success with day 4 embryo transfers. Fertil Steril 2007.

The outcomes of day 4 ETs stratified by age are given in Table 2. The implantation rate was highest in the youngest age groups, and similar in patients 35–40 years of age. Pregnancy and live-birth rates were similar across all age groups up to age 40 years. The rate of multiple gestations was highest in women ≤40 years of age.

DISCUSSION

Advances in embryo culture media have allowed a longer period of embryo culture prior to transfer. Extension of culture beyond the point of embryonic genome activation permits discrimination of embryos with a slower or arrested cleavage rate from those undergoing regular cell division, potentially enhancing the capacity for implantation (23–25). While the implantation rates, PRs, and live-birth rates reported in this study appear to be higher across all age groups in comparison to the national average from the Society of Assisted Reproductive Technology (SART) database, comparisons to the national cumulative SART rates may not be relevant, because the SART database does not delineate implantation rates, PRs, and live-birth rates according to day of transfer. In current literature, controversy exists concerning the applicability of extended embryo culture to improve PRs across all patient subgroups. Studies addressing the use of blastocyst transfer in IVF found that extended embryo culture may be more beneficial in a selected patient population (26–29). Our results are in agreement with other studies performed in nonselected couples undergoing IVF (30, 31).

Improvements in embryo selection might reduce the number of embryos transferred while maintaining an acceptable