Day 2 transfer improves pregnancy outcome in in vitro fertilization cycles with few available embryos

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Objective: Delaying ET to day 3 to optimize embryo selection is well accepted. However, in cases where there are not enough embryos to perform selection, it is not clear whether there is a difference in clinical outcomes with the day of ET.
Design: Cohort study.
Setting: Academic medical center.
Patient(s): Two hundred forty-two fresh IVF/intracytoplasmic sperm injection (ICSI) cycles from 2002–2004, where all generated embryos were transferred irrespective of quality because of an extremely low number of available embryos.
Intervention(s): In time period 1, ET was on day 3. In time period 2, ET was on day 2.
Main Outcome Measure(s): Patient response to stimulation was analyzed along with pregnancy outcome and implantation rate.
Result(s): Miscarriage rates were decreased, and ongoing pregnancy rates were increased with a day 2 ET in patients <40 years of age.
Conclusion(s): In women <40 years of age, the day of transfer is a significant predictor of clinical outcome in cases in which a low number of embryos are available for transfer. The evidence suggests that limiting embryo culture to only 2 days reduces the incidence of miscarriage and increases ongoing pregnancy rates. (Fertil Steril® 2006;86:44–50. ©2006 by American Society for Reproductive Medicine.)

Key Words: Embryo transfer, pregnancy rates, implantation rates, spontaneous abortion

Over the years, pregnancy and implantation rates with IVF have increased substantially. This increase is thought to be due to improvements in embryo culture, laboratory conditions, and the increased ability for embryo selection. Early embryonic development is controlled predominantly by factors stored in the oocyte before the initiation of embryonic gene expression during the early cleavage stages (1–3). Therefore, the selection process often involves delaying ET from day 2 to day 3 to observe this initial transition to embryonic gene expression control. In addition, embryo viability can be further assessed to optimize selection by extending culture to the blastocyst stage since only 40%–50% of human embryos develop into blastocysts (4–7).

Over the past decade, new generations of culture media have been designed to support the embryo in culture for longer periods of time. However, optimization of human embryo culture media has been plagued by a number of problems, particularly the lack of developmentally synchronous embryos for experimentation and the lack of randomized trials. Currently, measuring the performance of culture media is limited to an assessment of blastocyst formation rates. Whether development of cultured embryos in vitro using the standard culture media is equivalent to development in the tubal and uterine in vivo environment is extremely difficult to determine. Furthermore, simply an assessment of blastocyst formation rates may reveal nothing of the epigenetic phenomenon occurring at these stages of development that may possibly affect later embryonic development. A recent meta-analysis comparing day 2 versus day 3 ET demonstrated that there was not sufficient evidence to suggest an improvement in live birth rate when the ET was delayed (8). In addition, Racowsky et al. showed that if no 8-cell embryos are formed by day 3, extending the culture to day 5 resulted in no ongoing pregnancies compared with a 33% clinical pregnancy rate if ET occurred on day 3, suggesting that the in vivo environment can rescue less healthy embryos (9). However, many studies have indicated that implantation rates are higher with blastocysts (4–7, 10, 11); these studies had specific inclusion criteria such as a defined

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number of 7- to 8-cell embryos before engaging in ongoing culture and blastocyst production. Extended culture is likely most suitable for patients who produce an ample number of good-quality cleavage-stage embryos, and it allows enhanced selection.

Currently, there are few data regarding the optimization of pregnancy outcome for patients who do not have a sufficient number of embryos available for selection. In those cases, all embryos are usually transferred, irrespective of their quality. We asked whether it was of any benefit to culture embryos for 1 day less in this subgroup of IVF patients. Current evidence indicates that the outcome of day 2 transfers is at least comparable to those performed on day 3 (8, 12–17). Furthermore, it has even been suggested that limiting culture to day 1 (2 pronuclei stage) results in comparable pregnancy and implantation rates (18–21). Only one study to date suggested that pregnancy outcomes in cycles, in which the number of embryos created was less than the numbers of embryos consented for ET, were improved with a day 2 transfer (22). We therefore started a trial to transfer embryos on day 2 in a similar population and compared day 2 and day 3 ET results to determine whether decreasing the time in culture will increase pregnancy rates and reduce the risk of miscarriage.

MATERIALS AND METHODS

The study was approved by the Committee on Human Research at the University of California, San Francisco.

Study Population

IVF/intracytoplasmic sperm injection (ICSI) cycles from November 2002 to September 2004 where couples created no more than the maximum numbers of embryos to transfer based on American Society for Reproductive Medicine guidelines (2001) were included in the study. The decision to transfer all embryos was based on patient prognosis (age, FSH, and prior failed attempts) and the number of embryos (Table 1). There were no exclusion criteria with regard to infertility diagnosis. All patients underwent ovarian stimulation with standard protocols (down-regulated, flare, antagonist) based on patient characteristics and physician preference. All day 3 ETs occurred from November 2002 to September 2003 (study period 1). All day 2 ETs occurred from October 2003 to September 2004 (study period 2). Patients who participated in both periods were not included in the study.

TABLE 1

Criteria for decision of transferring all embryos in patients who only produce a very low number of embryos.

Patient age	Transfer all embryos
≤34	≤2 embryos if first or second attempt; 3 embryos if failed multiple cycles
35–37	≤3 embryos if first or second attempt; 4 embryos if failed multiple cycles
38–40	≤4 embryos if first or second cycle; 5 embryos if failed multiple cycles
≥41	≤5 embryos if first or second cycle; 6 embryos if failed multiple cycles

Shen. Day 2 versus day 3 ET. Fertil Steril 2006.

Clinical and Laboratory Conditions

The clinical and laboratory conditions from November 2002 through September 2004 were consistent. There were no significant differences in the patient population and volume during the 2-year period. There were no changes in the ovarian stimulation protocols or the physicians. The laboratory is located on the top floor of an eight-story building and is newly renovated with a four-stage air filtration system: stage 1 includes standard pleated prefilters; stage 2, mixed bed gas phase filters from Purafil (Doraville, GA); stage 3, charcoal filter modules; and stage 4, high-efficiency particulate air filters (MERV 14 per ASHRAE 52.2-1999) (HEPA Corp., Anaheim, CA). The stage 2 gas phase filters are "broad spectrum" filters, containing a 50:50 mixture by volume of potassium permanganate and activated charcoal, which are designed to remove volatile organic compounds (VOCs), ozone, nitrogen dioxide, and sulfur dioxide (primarily from vehicle emissions), hydrogen sulfide, ammonia, and formaldehyde. Positive pressure to adjacent rooms was set in the embryology lab, and the ventilation rate was 20 air changes per hour. The temperature range was 22 ± 1°C, and humidity was kept to within 40%–60%. UV-filtered fluorescent lighting was used in the lab. Non-VOC paint was used for the entire floor (ICI Dulux, Lifemaster 2000, Cleveland, OH).

There were no differences in embryo culture conditions or techniques throughout the study period. All embryos were cultured in incubators (Hereus, Langensebold, Germany) with 5%–6% CO_2 in air. CO_2 concentration was calibrated according to the medium pH range 7.25–7.35. The culture dishes consisted of 25 μL medium drops covered by 8 mL oil (Sigma, St. Louis, MO). Vitrolife G media (Englewood, CO) was used for oocyte retrieval (G_1), sperm preparation (G-sperm), insemination (G-fert), micromanipulation (G-MOPS), embryo culture (G_1), and ET (G-MOPS). All media were supplemented with 5% human serum albumin (Sage-Cooper Surgical, Trumball, CT) before use.

To assess whether the conditions between the two different study periods were similar, we determined whether the study period was a predictor of the clinical outcome in good-prognosis patients. This analysis included patients <40 years of age with sufficient embryos available for selection and all anonymous ovum donors.