Embryo afterloading: a refinement in embryo transfer technique that may increase clinical pregnancy

Adrienne B. Neithardt, M.D.,[a,b,c] *James H. Segars, M.D.,*[a,b,c] *Sasha Hennessy, M.S.,*[c] *Aidita N. James, Ph.D.,*[c] *and Jeffrey L. McKeeby, M.D.*[d]

[a] Pediatric and Reproductive Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland; [b] Uniformed Services University of Health Sciences, Department of Obstetrics and Gynecology, Bethesda, Maryland; [c] Walter Reed Army Medical Center, Department of Obstetrics and Gynecology, and The ART Institute of Washington, Washington, D.C.; and [d] Shady Grove Fertility Reproductive Science Center, Rockville, Maryland

Objective: Given the importance of ET technique during assisted reproductive technology cycles, we evaluated the effect of embryo afterloading subsequent to placement of the ET catheter on pregnancy rates vs. a standard direct ET.
Design: Retrospective cohort analysis.
Setting: University-based assisted reproductive technology program.
Patient(s): Patients undergoing a fresh nondonor day 3 ET by a single provider over a 1-year period.
Intervention(s): None.
Main Outcome Measure(s): Clinical pregnancy.
Result(s): One hundred twenty-seven patients met inclusion criteria, and the overall pregnancy rate was 46.5%. There was no difference between the two groups with respect to age, basal FSH, or number of embryos transferred. The ET method used was at the discretion of the provider. There was no difference between the two groups in the presence of blood on the transfer catheter. However, there were significantly more transfer catheters with mucus contamination in the direct transfer group (25.58% vs. 5.95%). The clinical pregnancy rate in the group with ET using the afterloading technique was higher than in the direct ET group (52.4% vs. 34.9%).
Conclusion(s): There was a trend toward an increase in pregnancy rate when an embryo afterloading technique was used. A prospective randomized trial is needed to examine this issue. (Fertil Steril® 2005;83:710–4. ©2005 by American Society for Reproductive Medicine.)

Key Words: Embryo transfer, technique, IVF-ET, pregnancy rate

Over the past 10–15 years there have been increasing success rates with assisted reproductive technologies (ART) in all age groups. The Society for Assisted Reproductive Technology reported an increase in live birth rates from 28% in 1996 to 32% in 2002 (1). This increase has been attributed to multiple factors including improved stimulation protocols (2–4), advances in embryology laboratory techniques (5), and improvement in ET techniques (6, 7).

Embryo transfer is universally accepted as a crucial last step in any ART cycle. The importance of this step has been emphasized by the fact that different providers at the same institution may have disparate pregnancy rates after ET (8, 9). Other variables affecting pregnancy include the ease of ET (7, 10, 11), presence or absence of blood on the transfer catheter (12), type of catheter used (13), technique used to perform the transfer (14–16), and experience of the physician (17).

Received April 27, 2004; revised and accepted August 5, 2004.
Presented at the Annual Meeting of the American Society for Reproductive Medicine, San Antonio, Texas, October 2003.
Reprint requests: Adrienne Neithardt, M.D., PREB, NICHD, National Institutes of Health, Bldg. 10, Room 9D-42, 9000 Rockville Pike, Bethesda, Maryland 20892 (FAX: 301-402-0884; E-mail: neithara@mail.nih.gov).

In the early 1990s, studies were first published on the use of a mock or "dummy" ET before the start of an IVF cycle (11, 18). A mock ET allows the physician to choose the appropriate transfer catheter, measure the depth of the endometrial cavity, and anticipate potential problems at ET. However, a mock transfer remote from the actual ET is done under different circumstances and may not be reflective of actual conditions encountered on the day of ET. Sharif et al. (19) proposed to circumvent this problem by performing a mock ET immediately before the actual ET.

To avoid additional trauma by the passage of two separate catheters, we began transferring embryos by an afterload technique, in which an empty catheter is placed at, or just past, the internal cervical os. The inner sheath is withdrawn, and a second inner sheath containing the embryos is passed. This gives the provider the benefit of an immediate mock transfer while minimizing manipulation of embryos and possibly reducing trauma to the endometrium.

We performed a retrospective analysis of 127 ETs done during a 1-year period of time to determine whether there were differences in pregnancy rates based on the transfer method used.

0015-0282/05/$30.00
doi:10.1016/j.fertnstert.2004.08.022

MATERIALS AND METHODS

Under an approved protocol reviewed by the Department of Clinical Investigation, we performed a retrospective analysis of patients undergoing a day 3 ET at the Walter Reed Army Medical Center ART program from July 2001 to July 2002 by a single provider. Information regarding patient age, day 3 FSH level, number of embryos transferred, method of ET, and clinical pregnancy rates were collected. The ET method used was at the discretion of the provider performing the procedure, and the number of women receiving the afterload technique was proportional throughout the study. Transfers of blastocysts, cryopreserved embryos, and donor oocytes were not included in the analysis. Patients were excluded from analysis if they were greater than 43 years of age or had an FSH level >14 mIU/L on cycle day 3 (or on cycle day 3 or 10 after a clomiphene citrate challenge test). A total of 127 patients met criteria for study.

All patients had undergone controlled ovarian stimulation by using a combination of long-term gonadotropin-releasing hormone (GnRH) agonist (Lupron, 1.0 mg/day; TAP Pharmaceuticals, Deerfield, IL) or microdose flare GnRH agonist (40 μg twice daily and gonadotropins Gonal-F (Serono, Rockland, MA) or a combination of Gonal-F and Repronex (Ferring Pharmaceuticals, Suffern, NY) as described elsewhere (20). The dose of gonadotropins was individualized based on the patient's age, history, and response to medication. Cycles were monitored using serial transvaginal ultrasounds to chronicle follicular growth and the measurement of serum E_2 levels. Administration of hCG occurred when follicular size and E_2 levels were appropriate. Transvaginal oocyte retrieval was performed approximately 36 hours later (20). Patients received IM progesterone in oil (50 mg/d) beginning the night of the retrieval until 8 weeks' gestation.

Embryo Transfer Techniques

A mock ET and a saline sonohysterogram were performed in all patients before enrollment in the ART cycle. All ETs were performed with a full bladder under ultrasound guidance (Ultramark 4 with a 5-MHz transducer; ATL, Bothell, WA) using the Edward-Wallace catheter (Simcare, West Sussex, UK). The difficulty of the ET was determined by the performing physician and scored as easy, moderate, or difficult.

Direct Embryo Transfer The patient was placed in the lithotomy position. A sterile bivalve speculum was placed in the patient's vagina, and the cervix was exposed. Excess mucus and debris were cleared from the ectocervix using sterile cotton swabs dampened with phosphate-buffered saline. The embryos were loaded into the transfer catheter by the embryologist as described elsewhere, and the catheter was passed to the transfer physician (4). The embryos were then deposited approximately 1.5 cm from the uterine fundus under ultrasound guidance. After approximately 5 seconds the catheter was gently rotated 180° and removed, with care being taken to keep the plunger of the catheter depressed until it had been completely removed from the cervix, usually 5–10 seconds. The embryologist immediately flushed the catheter with media to check for retained embryos, blood, or mucus. Patients remained supine for 20 minutes after the procedure.

Afterloaded Embryo Transfer The patients were placed in the lithotomy position, and the cervix was exposed and prepped in the same manner as the direct technique. An empty Wallace catheter was passed to the level of the lower uterine segment under ultrasound guidance to a point where the inner catheter entered the endometrial cavity, typically about 5 cm. The inner sheath was slowly removed, leaving the outer sheath just beyond the internal os. A second inner sheath was loaded by an embryologist who then assisted the transfer physician in threading the inner sheath into the catheter. The inner catheter was slowly advanced by the physician, and the embryos were deposited 1.5 cm from the fundus. After approximately 5–10 seconds, the catheter was gently rotated and removed, with care being taken to keep the plunger of the catheter depressed until it had been completely removed from the cervix over 15 seconds. The embryologist flushed the catheter with media to check for retained embryos. Patients remained supine for 20 minutes after the transfer.

All patients from both groups who had appropriately rising quantitative hCG values underwent transvaginal ultrasound at 6–8 weeks' gestation to look for gestational sacs.

Statistical Analysis

Since these data were retrospectively analyzed, sample size was determined by the study time interval and not a power analysis. The method of transfer was recorded in our ART database along with other cycle parameters/outcomes kept for the purpose of Society for Assisted Reproductive Technology reporting. The primary outcome analyzed was clinical pregnancy rate per transfer as defined by the presence of a gestational sac on ultrasound at 6–8 weeks of gestation. Nonsustained rises in hCG levels and ectopic pregnancies were not scored as clinical pregnancies. Implantation rates were defined as number of sacs on ultrasound per number of embryos transferred. Statistical differences were determined using Student's *t*-test, Fisher's exact, and χ^2 analysis where appropriate. A Mann-Whitney *U*-test was used to determine the statistical difference between nonparametric variables. An alpha error of $<.05$ was considered significant.

RESULTS

One hundred twenty-seven day 3 ETs were performed by a single provider over a 1-year period of time. The average age of all the patients was 33.6 $\pm$ 4.38 years, with a range of 20–42 years. The overall pregnancy rate was 46.5%. Eighty-four ETs were performed by the afterload technique, and 43 as direct ETs.

As depicted in Table 1, there was no difference between the two groups with respect to age (33.4 vs. 33.9), basal FSH