SHORT COMMUNICATION

DENVER, COLORADO

Pronuclear Uterine Transfer Lowers In Vitro Fertilization Success¹

Submitted: April 30, 1998

Accepted: May 7, 1998

INTRODUCTION

The ideal time to perform embryo transfer (ET) has not been established. A prospective, randomized study showed no difference in pregnancy outcomes when 2-, 3-, and 4-day transfers were compared (1). Furthermore, no difference in success rates were seen between transfer on days 3 or 5 in another study (2). Currently, most in vitro fertilization (IVF) programs transfer embryos on day 2 or 3 and perform assisted hatching when this approach is believed to be beneficial. With extended culture times, there is a risk of developmental delay of healthy embryos. Assisted hatching may help overcome artificial zona hardening in culture, but this procedure adds to the complexity and expense of IVF.

Embryo development is almost always enhanced in the presence of coculture, and endometrial cells appear to provide a beneficial effect (3). Unfortunately, coculture also adds to the complexity and expense of IVF. Since embryo development is enhanced with endometrial coculture, we hypothesized that early embryo uterine transfer may enhance implantation and pregnancy rates with IVF. To test the benefit of early ET, we initiated a study to determine whether pronuclear uterine transfer 1 day after oocyte retrieval was more effective than transferring cleaving embryos on day 2.

MATERIALS AND METHODS

We conducted a prospective, randomized study of pronuclear uterine transfer on the morning after oocyte retrieval vs. uterine transfer of cleaving embryos 2 days after retrieval at a university-assisted reproduction program. The initial study called for a total of 50 patients. Institutional Review Board approval and informed consent were obtained for all study participants. Patients were randomized by computer prior to starting stimulation. Study eligibility included an indication for IVF, an age younger than 38 years, a basal follicle-stimulating hormone (FSH) level of 10 mIU/ml or less (conversion to SI units, 1.0), normal semen parameters (>20 million/ml, >40% motility, and >10 normal morphology by strict criteria), regular menses, and a normal uterine cavity.

All patients were treated with a minimally monitored, fixed stimulation protocol (4). All received mid-luteal leuprolide acetate and were treated with a fixed schedule for FSH, 225 IU per day until follicles were mature. A single ultrasound was performed after 8 days for women younger than the age of 33, and 9 days for those between 33 and 37 years of age. Human chorionic gonadotropin (hCG) was given when two or more follicles measured at least 18 mm. Retrieval was performed 35 hr after the administration of hCG. Oocytes were inseminated in microdroplets under oil. Embryo transfer was done under abdominal ultrasound guidance.

Day 1 pronuclear uterine transfers were performed between 8:00 A.M. and 9:00 A.M., approximately 18–24 hr after retrieval, in order to minimize the in vitro incubation time. Pronuclear embryos were selected with a preference for those with a regular shape, homogeneous cytoplasm, pronuclear symmetry, and smooth plasma membrane.

Day-2 cleaving embryo uterine transfers were performed 48–54 hrs after retrieval. Embryos were selected with a preference for those with the most blastomeres and minimal cytoplasmic fragmentation. Assisted hatching was performed for day-2 embryos

¹ Presented at the 53rd Annual Meeting of the American Society for Reproductive Medicine, Cincinnati, Ohio, October 18–22, 1997.

when the zona was thickened, when moderate or severe fragmentation was noted, or when development was delayed.

Groups were compared by one-way analysis of variance, or one-way analysis of variance on ranks, or Dunn's test when the normality test failed. Pregnancy rates were compared using the Fischer exact test. Implantation rates were compared by chi-square analysis. The initial study of 50 patients had a power of 0.8 to determine a 10% difference in implantation rates at the level of 0.05. A *P* value of <0.05 was considered statistically significant.

RESULTS

An interim review of the data after enrollment of our first 25 subjects demonstrated a 6.8% implantation rate with day-1 pronuclear uterine transfer, compared with a 22.8% implantation rate with the transfer of cleaving embryos on the second day after oocyte retrieval (Table I). This difference was significant (*P* = 0.02). The clinical pregnancy rate was only 15% with day-1 transfers, significantly lower than the 67% rate with transfer on day 2. A similar trend was seen with the delivery rate, which was only 8% with pronuclear transfer, vs. 42% with transfer on day 2. Otherwise, there did not appear to be a difference in the demographics between the two groups, nor was there a difference in the number

of oocytes obtained, fertilization rate, or number of embryos transferred.

DISCUSSION

Because of the unexpectedly dramatic difference in the implantation and pregnancy rates between the two groups, the study was closed after the first 25 cycles. Even with the limited number of patients in this study, the power to detect a difference in pregnancy rates at the 0.05 level was 0.77, and was 0.56 for the difference seen in implantation rates. It is possible that pronuclear uterine transfer eventually may have proved to be no different than transfer of cleaving embryos on day 2. However, it is highly improbable that a day-1 transfer would have been superior if the study had been carried to completion.

After the study was closed, we continued to monitor subsequent patients who met study criteria to determine if implantation and pregnancy rates seen with day-2 transfers were maintained. The implantation rate was 27% in the 39 who met study criteria (Table I). A 56% clinical pregnancy and a 53% delivery rate were been achieved in these patients with traditional IVF and day-2 ET. Although there seems to be a national trend toward more prolonged incubation and culture intervals, ET 2 days after oocyte retrieval allows for high implantation and pregnancy rates in a successful IVF program.

Day-2 ET with contemporary laboratory conditions may allow better embryo selection than pronuclear transfer. Furthermore, the uterine environment may be detrimental to the developing pronuclear embryo. Whatever the explanation, pronuclear ET resulted in unacceptably low implantation and pregnancy rates in this study.

In conclusion, we experienced compromised results with pronuclear uterine transfer, and excellent results with day-2 ET. Despite the lower complexity of IVF with pronuclear transfer, this approach cannot be recommended.

REFERENCES

1. Huisman GJ, Alberda AT, Leerentveld RA, Verhoeff A, Zeilmaker GH: A comparison of in vitro fertilization results after embryo transfer after 2, 3, and 4 days of embryo culture. *Fertil Steril* 1994;61:970-971
2. Scholtes MC, Zeilmaker GH: A prospective, randomized study of embryo transfer results after 3 or 5 days of embryo culture in in vitro fertilization. *Fertil Steril* 1996;65:1245-1248

Table I. Results of Day 1 vs. Day 2 Prospective Transfer Study

	Transfer day		
	Day 1	Day 2	Day 2 poststudy
No. cycles	13	12	39
Age ^a	31.3 ± 5.1	31.8 ± 3.9	31.9 ± 4.2
Prior pregnancy	6	5	16
Diagnosis			
Tubal	9	6	24
Endometriosis	2	5	11
Unexplained	1	1	4
Oocytes ^a	13.2 ± 6.6	13.9 ± 4.9	16.6 ± 9.4
Embryos ^a	9.0 ± 6.1	10.1 ± 3.9	10.8 ± 6.6
Embryos transferred	59	58	161
Implantation rate ^b	6.8%	22.8%	27.0%
Clinical pregnancy rate ^b	15%	67%	56%
Delivery rate ^c	8%	42%	53%

^a ± Standard deviation.

^b *P* < 0.05 day 1 vs. day 2, and day 1 vs. day 2 poststudy.

^c *P* = 0.004 day 1 vs. day 2 poststudy.

3. Jayot S, Parneix I, Verdaguer S, Discamps G, Audebert A, Emperaire JC: Coculture of embryos on homologous endometrial cells in patients with repeated failures of implantation. *Fertil Steril* 1995;63:109-114
4. Hurst BS, Tucker KE, Awoniyi CA, Schlaff WD: Preprogrammed, unmonitored ovarian stimulation reduces expense without compromising the outcome of assisted reproduction. *Fertil Steril* 1997;68:282-286

Bradley S. Hurst²
Kathleen E. Tucker
Caleb A. Awoniyi
William D. Schlaff

Division of Reproductive Endocrinology
University of Colorado Health Sciences Center
Denver, Colorado

² To whom correspondence should be addressed at Department of Obstetrics and Gynecology, University of Kentucky, Room C359, 800 Rose Street, Lexington, Kentucky 40536-0084.