Monozygotic Twins and Triplets in Association with Blastocyst Transfer

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Purpose: To compare the incidence of monozygotic twins following blastocyst versus day-3 embryo transfer (ET).

Methods: A retrospective analysis of the outcome of assisted reproductive technology (ART) cycles utilizing blastocyst ET during 1999–2000 was compared to a similar group of patients undergoing day-3 ET during 1997–1998.

Results: Blastocyst ET was used in 75 cycles with 2.0 ± 2 embryos transferred. The comparison group consisted of 90 cycles with day-3 ET and 3.0 ± 2 embryos transferred.

Conclusions: High pregnancy rates are maintained with blastocyst ET even though fewer embryos are transferred. The rate of monozygotic twins is higher with blastocyst ET than with day-3 ET. This increase may partially negate the benefit of reduced high-order multiple gestations attributed to blastocyst ET.

KEY WORDS: Blastocyst; in vitro fertilization; monozygotic twins; multiple pregnancies.

INTRODUCTION

The high incidence of multiple gestations is the main complication associated with the clinical practice of assisted reproductive technology (ART). According to the 1999 Annual Report of the Society for ART, of The American Society of Reproductive Medicine, 37.1% of all deliveries following in vitro fertilization (IVF) were multiple deliveries with 4.9% being triplets or greater (1).

Recent advances in sequential culture media have led to reliable growth of embryos to the blastocyst stage. These advanced stage embryos have been reported, based on retrospective comparative studies, to be associated with higher per embryo implantation

rates when compared to day-3 embryos (2). As a result, it has been proposed that pregnancy rates can be maintained with fewer embryos transferred per cycle (3). The advantage of this strategy is thought to be the potential to lower the rate of multiple gestations, especially higher-order pregnancies.

Several recent reports have suggested that monozygotic twinning may be increased in association with blastocyst transfer. Peramo *et al.* reported two cases of monozygotic twins following blastocyst transfer (4). Rijnders *et al.* and Behr *et al.* have reported series of blastocyst transfers, both with increased rates of monozygotic twins as compared to rates observed after day-3 embryo transfer (ET) (5,6).

The purpose of this study was to evaluate the incidence and characteristics of multiple gestations associated with blastocyst transfer.

MATERIALS AND METHODS

We analyzed the outcome of all blastocyst transfers taking place at our institution during calendar

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years 1999 and 2000. All egg donation cycles and IVF cycles in women less than 35 years of age who had three or more 8-cell embryos on postaspiration day 3 were eligible for blastocyst transfer. A total of 75 transfer cycles were thus achieved between January 1, 1999 and December 31, 2000, with 55 involving egg donation and 20 with autologous IVF. The comparison group consisted of egg donation cycles ($n = 67$) and patients under the age of 35 undergoing stimulated IVF ($n = 23$) whose cycles took place during calendar years 1997 and 1998. During both time periods, similar stimulation using the long protocol with leuprolide acetate, culture for the first 3 days using P1 media (Irvine Scientific, Santa Ana, CA, USA) and ET using the Soft-Pass catheter (Cook Ob/Gyn, Spencer, IN, USA) were used. However, all transfers were performed 72 h after aspiration during 1997 and 1998. In order to exclude the potentially confounding effects of zona manipulation, cycles in which ICSI were performed were not included in the study. None of the cycles involved assisted hatching. This retrospective study was approved by the Institutional Review Board of the Los Angeles County + University of Southern California Medical Center.

All subjects underwent standard controlled ovarian hyperstimulation with leuprolide (Lupron, TAP Pharmaceuticals, Deerfield, IL, USA) downregulation followed by human menopausal gonadotropin or follicle-stimulating hormone stimulation. Follicular growth was monitored with serial sonography and serum estradiol (E_2) determinations. Human chorionic gonadotropin 10,000 IU was administered when follicle maturity criteria were achieved. Patients underwent ultrasound-guided transvaginal follicle aspiration 36 h later.

Oocytes were fertilized conventionally with 100,000 sperm per 50 μ L drop approximately 4 h after follicle aspiration. Embryos were cultured in 50 μ L of media under oil and were moved to fresh media daily. Embryos were cultured in P-1 media (Irvine Scientific, Santa Ana, CA, USA) with 10% Synthetic Serum Supplement (SSS) (Irvine Scientific, Santa Ana, CA, USA) for the first 72 h. Patients were eligible for blastocyst transfer if there were at least 3 embryos with 8 or more blastomeres on day 3. Embryos were transferred to Blastocyst Media (Irvine Scientific, Santa Ana, CA, USA) with 10% SSS through days 5–6. Embryo transfer was performed using a Soft-Pass Catheter on day 5 or delayed until day 6 if the blastocysts were not full or expanded and had many cells in the inner cell mass or trophectoderm (less than 3AA) (7).

Progesterone supplementation in IVF cycles was achieved with twice daily vaginal administration of 200 mg of micronized progesterone beginning on the day of ET regardless of the day of transfer (day 3, 5, or 6). Recipients of egg donation received 2–6 mg per day of oral E_2 for 14 days. Vaginal progesterone, 200 mg three times daily, was added on the day of follicle aspiration and continued until the end of the first trimester of pregnancy. Pregnancy was defined as fetal cardiac motion at 8 weeks of gestation and implantation rate as the number of chorionic sacs divided by the number of embryos transferred. Chi-square and Fisher's exact test were used to compare rates between the two groups. Identical twinning was ascertained by the observation of two fetal structures, each with a heart-beat, contained in one chorionic sac (8) with one or two amnions. These criteria detect all but dichorionic, diamniotic, monozygotic twins.

RESULTS

Seventy-five of 83 eligible patients underwent blastocyst transfer. Reasons for not performing a blastocyst transfer when at least three 8-cell embryos were available on day 3 include scheduling preferences, low embryo number (≤ 4) and one embryonic arrest. Of the 75 cycles in the blastocyst-ET group, 1 blastocyst was transferred in 4 cases, 2 in 69 cases, 3 in 1 case, and 4 in 1 case. The mean number of blastocysts transferred per case was 2.0 ± 2 with an implantation rate of 45.3% per blastocyst. Thirty-eight of the 75 cycles (50.7%) resulted in a clinical pregnancy with 22 (57.9%) multiple gestations. Of the 22 multiple gestations, 18 were twins, 3 were triplets, and 1 was a quadruplet pregnancy for a higher-order pregnancy rate of 4/38 (10.5%). Five (13.2%) of the pregnancies were monozygotic gestations. Each of these pregnancies was monochorionic, multiamniotic, and were clearly visible by ultrasound between 7 and 8 weeks gestational age. Four of the five monozygotic gestations occurred in women who were the recipients of donor oocytes following day 5 transfers.

One of the higher-order gestations in the blastocyst ET consisted of monozygotic triplets. This patient underwent transfer of two blastocysts on day 5 of extended culture after oocytes were retrieved from a donor. An initial ultrasound performed at 7 weeks gestation revealed 2 chorionic sacs, the first containing 3 discrete embryos, each with cardiac motion, and the second with only a yolk sac (Figs. 1 and 2). Cardiac motion ceased in one of the triplets by the 8th gestational week. An embryo never developed within the second

Fig. 1. Transvaginal sonograph at 7 weeks gestation demonstrating two chorionic sacs, the first with three discrete embryos and the second with only a yolk sac.

chorionic sac. The patient delivered identical female infants at 32 weeks gestational age by caesarean section without complications.

Of the 96 cycles in the day-3 ET group, a mean of 3.0 ± 2 embryos were transferred per cycle with an implantation rate of 32.2%, which is significantly different than the implantation rate of 45.3% observed in the blastocyst transfer group ($P < 0.05$). The clinical pregnancy rate was 47/90 (53.4%) and the multiple gestation rate 23/47 (48.9%). Of these 23 multi-

ple gestations, 16 were twins and 7 were triplets for a higher-order pregnancy rate of 7/47 (14.9%). Only 1/47 (2.1%) of the pregnancies was a monozygotic twin (visualized at 7½ weeks gestational age as mono-chorionic, diamniotic), a statistically significant difference when compared to the blastocyst-ET group ($P < 0.05$).

Of the blastocyst pregnancies complicated by a monozygotic gestation, 4/5 (80%) were also associated with multiple implantation. In three of the cases,

Fig. 2. Transvaginal sonograph at 7½ weeks gestation demonstrating each of three embryos within a single chorionic sac.

including the case of the monozygotic triplets, two blastocysts were transferred resulting in two separate chorionic sacs. In two of these cases, one of the chorionic sacs was noted to have two amnions, each with a heartbeat. In the other case, one of the chorionic sacs had 3 amnions and 3 heartbeats. The fourth case was unusual in that it involved a known balanced translocation. Preconceptual genetic counseling suggested that one of the chromosome arrangements would not implant, whereas another would produce an affected fetus, while others would produce either a carrier state or a normal chromosomal complement. After extensive discussion with the couple, the decision was made to transfer four blastocysts. Three of these implanted (1 affected and 2 normal) and one implantation spontaneously divided into monochorionic diamniotic twins, thus resulting in a quadruplet gestation. The pregnancy was subsequently reduced and the patient delivered a singleton at term. The 5th case was the result of a single blastocyst transfer in a patient who wished to avoid twins. The single implantation yielded a single chorionic sac with 2 amnions and 2 heartbeats.

Of the six pregnancies complicated by monozygotic twins (1 in the day-3 ET group and 5 in the blastocyst transfer group), three underwent selective reduction and delivered a singleton pregnancy at term. One of these pregnancies was complicated by mild pregnancy-induced hypertension, and postdates and was delivered by cesarean section. The other three pregnancies, all twin pregnancies, were delivered by cesarean section between 31½–35½ weeks. In each case, the twins were admitted to the neonatal intensive care unit and discharged home once appropriate weight was attained without significant sequelae.

DISCUSSION

The exact incidence of monozygotic twins associated with blastocyst transfer has not been established. Rijnders *et al.* were the first to report an increased rate of monozygotic twins associated with blastocyst ET (5). They observed a 0.68% rate of monozygotic twins among 735 pregnancies that were the result of the day-3 ET and a 2.7% rate of monozygotic twins among 111 pregnancies that were the result of day 5 transfer, a statistically significant difference. Subsequently, Behr *et al.* reported a 5% rate of monozygotic twinning among 199 pregnancies that were the result of blastocyst transfer (6).

We observed a rate of 13.2% in a relatively small sample of patients. Whereas this does represent the

highest reported incidence of monozygotic twins associated with blastocyst transfer, it is possible that the relatively small denominator played a role in this apparently high rate. It is interesting to note that of the first 12 patients to receive blastocyst transfer at our center, 7 conceived and 3/7 (43%) had a monozygotic twin. This same phenomenon was described by Sheiner *et al.* who reported a 2/9 (22%) rate of monozygotic twins following his initial experience with blastocyst transfer (9). Although a decrease in the rate of monozygotic twinning has been noted with time, the overall rate remains at 13.2%. The higher-order pregnancy rate of 10.5% observed in the blastocyst transfer group is approximately twice the 4.5% rate reported for donor embryos by SART (1). Monozygotic pregnancies were associated with 3 of the 4 higher-order pregnancies and thus clearly contributed to the high rate of higher-order pregnancies seen in this cohort. Patients should therefore be counseled on the possible increased chance of higher-order multiples and consider all options including selective reduction prior to choosing blastocyst transfer.

Several authors have reported an association between manipulation of the zona pellucida and monozygotic twins (10,11). Schieve *et al.* analyzed 11,247 pregnancies from the Society for Assisted Reproductive Technologies database and found that monozygotic twinning was more than twice as likely to occur among IVF–ET cycles in which embryos were treated with assisted-hatching methods (11). Da Costa *et al.* studied the rate of monozygotic twins following blastocyst transfer after ICSI and found a monozygotic twinning rate of 3.9% vs. 0.7% following ICSI and transfer of 4–8 cell embryos (12). These data are different from the study of Sills *et al.* who failed to show a difference between zona manipulated and zona intact embryos in 1911 pregnancies (13). In this study, we excluded patients that had undergone zona manipulation to specifically determine the rate of monozygotic twins following blastocyst transfer as compared to day-3 ET. This study suggests an increased rate of monozygotic gestations as a result of blastocyst transfer alone.

In their report, Behr *et al.* proposed that a possible explanation for monozygotic twinning in association with blastocyst transfer might be hardening of the zona pellucida as a result of extended exposure to culture media (6). As a result, the inner cell mass may be “pinched” during subsequent blastocyst hatching and a disruption of cell-to-cell adhesions might lead to a splitting of the inner cell mass and the consequent

development of two fetal plates rather than just one. This proposed mechanism is consistent with prior observations that monozygotic twinning may be associated with artificial reproductive technologies in which the zona pellucida is breached as outlined above.

We observed that four of the five cases of monozygotic twins were associated with concurrent multiple implantations. If this association proves true by larger series, then another mechanism may be proposed. Two adjacently implanted trophoblasts may come in close apposition to one another and, by an as yet unclear interaction, one of the implantations may be stimulated to form a second fetal plate. In this paradigm, the identical twinning associated with blastocyst transfer could be accounted for by the close apposition of the two implantations. Whereas embryos transferred on day 3 postaspiration remain in the uterine cavity several days prior to implantation and thus have adequate opportunity to move apart prior to implanting, blastocysts are transferred in a small amount of fluid (10 μ L in our program) at a stage of development in which implantation is imminent. Thus, multiple embryo implantations following transfer at the blastocyst stage is more likely to take place in close apposition and thus are associated with an increased incidence of monozygotic twins.

Although all of the monozygotic twin pregnancies resulted in the delivery of at least one healthy baby, 3 (50%) of the patients underwent selective reduction and 4 (66%) ultimately required cesarean section. Although the cesarean section rate is not dissimilar from other ART cohorts (14), the 50% rate of selective reduction is much higher than the 3–5% rate annually observed at our center. The incidence of obstetrical complications associated with monozygotic twins including pregnancy-induced hypertension and postdates (both 1/6 (16.6%) in this cohort) may not be meaningful when compared to singleton and dizygotic twin pregnancies due to the small sample. The experience of larger, pooled samples should be evaluated and compared to singletons and dizygotic twins when they become available.

CONCLUSIONS

We conclude that blastocyst ET allows high pregnancy rates to be maintained even though fewer embryos are transferred. This will no doubt lead

to a decrease in the incidence of fraternal triplets and higher-order multiple gestations. However, the observed increase in monozygotic twins may partially offset some of this benefit.

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