Blastocyst-ET and Monozygotic Twinning¹

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Purpose: To examine the rate of monozygotic twinning associated with blastocyst transfer using commercially available, cell-free culture systems with unmanipulated blastocysts.

Methods: A retrospective analysis was conducted in multiple private and academic infertility centers throughout the United States, of 199 pregnant patients following in vitro fertilization (IVF) blastocyst embryo transfer (ET). Human embryos obtained through standard IVF stimulation protocols were cultured in commercially available, cell-free media systems and transferred as blastocysts. The main outcome measure was the rate of monozygotic twinning.

Results: A total of 199 blastocyst-ET pregnancies were achieved during the study period at the fertility centers examined. Monozygotic twinning was noted in 10/199 (5%) of these pregnancies. All were monochorionic diamnionic.

Conclusions: Monozygotic twinning previously has been reported following IVF, especially in relation to assisted hatching. While blastocyst transfer has been available for many years using coculture, there have been no published multicenter reports of monozygotic twinning associated with unmanipulated blastocysts. In a multicenter analysis, a definite increase in monozygotic twinning was seen following blastocyst-ET. We believe this phenomenon is real and that this information should be considered when counseling patients for treatment.

KEY WORDS: Blastocyst transfer; in vitro fertilization; monozygotic twinning; pregnancy.

INTRODUCTION

Increased implantation and pregnancy rates following blastocyst transfer (BET) using chemically defined media now have been reported by several investigators (1). We also previously have reported an increased incidence of multiple pregnancy following BET (2). Dizygotic twinning is an established risk associated with assisted reproductive technology (ART), and monozygotic twinning has also been reported especially in relation to micromanipulation or assisted hatching (3). In nature, the frequency of monozygotic twinning has been estimated to be approximately one set per 250 births, although ovulation induction has been reported to double that risk (4). While blastocyst transfer has been available for many years using coculture, there have been no published reports of monozygotic twinning associated with unmanipulated blastocysts using this method of culture. Since BET is a relatively novel treatment modality for most centers in the United States, it would be difficult for any one center to have sufficient volume to draw adequate conclusions from their experience. In this article, we present data from multiple centers examining the rate of monozygotic twinning associated with BET using commercially available, cell-free culture systems with unmanipulated blastocysts.

MATERIALS AND METHODS

In vitro fertilization (IVF) patients at all centers studied were stimulated using standard gonadotropin-releasing hormone-agonist (GnRH-a)/follicle-stimulating hormone (FSH) protocols after signing approved consent forms. Ovulation was triggered with human chorionic gonadotropin (hCG). Oocytes were retrieved transvaginally under ultrasound guidance and were inseminated conventionally or with intracytoplasmic sperm injection (ICSI). Embryos were either cultured

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in P1 (Irvine Scientific, Santa Ana, CA) supplemented with 10% synthetic serum substitute (SSS) from day 0–3 and then were transferred into blastocyst medium (Irvine Scientific) supplemented with 10% SSS for an additional 48 hr, or were cultured in IVF50 (Scandinavian IVF Science, Gothenburg, Sweden) from day 0–3 and then were transferred into S2 (Scandinavian) for an additional 48 hr.

Blastocysts exhibiting a well-defined trophoctoderm, a distinct inner cell mass, and an expanded blastocoele cavity were transferred to the uterus on day 5 after harvest using either a Wallace (Marlow Surgical Technology, Willoughby, OH) or Tefcat (Cook Ob/Gyn, Spencer, IN) catheter. Assisted hatching was not used. All patients were less than 40 years of age. A maximum of three blastocysts were transferred. A monozygotic twin pregnancy was defined as two fetuses identified within the same gestational sac on ultrasound examination and confirmed at birth.

RESULTS

A total of 199 pregnancies were achieved following blastocyst transfer at the fertility centers studied. A monozygotic twin gestation occurred in ten of these pregnancies (5%). Of the ten monozygotic pregnancies, two occurred from ICSI. Characteristics of these pregnancies are presented in Table I. All were monochorionic, diamnionic, as evidenced by a dividing membrane seen on ultrasound and confirmed at birth. Two of the ten monozygotic twins spontaneously converted to a singleton gestation. There was no apparent effect from the type of transfer catheter or the brand of culture medium used on the potential for developing a monozygotic twin gestation. Thickness of the zona pellucida on the day of embryo transfer also did not

seem to correlate with the chance of developing a monozygotic twin pregnancy.

DISCUSSION

Sequential, cell-free media systems are relatively new. While the success with these systems has been substantial, until now, only the positive experiences from blastocyst transfer have been reported using these new media. In vitro culture has been reported to cause increased hardening of the zona pellucida, which could potentially lead to pinching of the inner cell mass upon spontaneous or assisted hatching (3). It is interesting to note that we found no correlation between zona pellucida thickness on the day of embryo transfer and the chance of developing a monozygotic twin gestation, although thickness of the zona pellucida may not correlate with hardness.

In addition, despite significant experience with blastocyst transfer using coculture, there have been no reports of monozygotic twinning using that system. It is possible that current generation-extended culture media could cause perturbations of cell-to-cell adhesion, facilitating splitting of the inner cell mass. Finally, a potentiating effect from the embryo transfer itself cannot be excluded. However, the fact that monozygotic twinning occurred despite the use of varied media and catheter types makes a significant impact from these factors less likely. It is also of interest that the incidence of monozygotic pregnancies appears to be unrelated to ICSI since the a higher incidence of monozygotics came from conventional insemination. What ever the mechanism, we believe the increased incidence of monozygotic twinning seen following blastocyst transfer to be a real phenomenon that should be considered when counseling patients and making treatment decisions. Further investigation is warranted. For

Table I. Characteristics of Monozygotic Pregnancies Resulting from Blastocyst Transfer

Monozygotic twin pregnancy	Culture medium	Transfer cath	Spontaneous resolution
1	P1/Blastocyst medium	Tefcat	No
2	P1/Blastocyst medium	Tefcat	No
3	P1/Blastocyst medium	Tefcat	Yes
4	P1/Blastocyst medium	Tefcat	Yes
5	IVF50/S2	Wallace	No
6	IVF50/S2	Wallace	No
7	IVF50/S2	Wallace	No
8	P1/Blastocyst medium	Wallace	No
9	P1/Blastocyst medium	Wallace	No
10	P1/Blastocyst medium	Wallace	No

those interested, there is an international blastocyst registry website at www.eshre.com. Programs may enter their blastocyst data so that information such as the data presented in this article may be disseminated more rapidly.

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