Blastocyst transfer is not associated with increased rates of monozygotic twins when controlling for embryo cohort quality

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Objective: To compare monozygotic twinning (MZT) rates in patients undergoing blastocyst or cleavage-stage ET.
Design: Retrospective cohort.
Setting: Academic research center.
Patient(s): Autologous, fresh IVF cycles resulting in a clinical pregnancy from 1999 to 2014.
Intervention(s): None.
Main Outcome Measure(s): Monozygotic twin pregnancy in blastocyst-stage transfer vs. cleavage-stage transfer when controlling for patient prognosis and embryo cohort quality factors.
Result(s): There were a total of 9,969 fresh transfer cycles resulting in a pregnancy during the study period. Of these pregnancies, 234 monozygotic twin pregnancies were identified (2.4%). Of all transfers, 5,191 were cleavage-stage and 4,778 were blastocyst-stage transfers. There were a total of 99 MZT identified in the cleavage-stage group (1.9%) and 135 MZT in the blastocyst ET group (2.4%), which was significant. Multivariable logistic regression revealed that increasing age was associated with a significant reduction in MZT, regardless of transfer order. Embryo cohort quality factors, including the number and proportion of six- to eight-cell embryos and availability of supernumerary embryos, were also significant. When controlling for patient age, time period during which the cycle took place, the number and proportion of six- to eight-cell embryos, and availability of supernumerary embryos, there was no longer a difference in MZT rate between blastocyst and cleavage transfer.
Conclusion(s): Patient prognosis and embryo cohort quality seem to be major factors in MZT rate in women undergoing blastocyst transfer. Although technology-based effects cannot be excluded, patient and embryo characteristics play an important role. (Fertil Steril® 2015;103: 95–100. ©2015 by American Society for Reproductive Medicine.)
Key Words: IVF, monozygotic pregnancy, blastocyst, twinning

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In recent years, assisted reproductive technology (ART) has seen significant changes in embryo culture conditions and micromanipulation techniques, leading to improved pregnancy rates with IVF. Nevertheless, some of these techniques, namely extended culture and blastocyst ET, have been implicated in the increased monozygotic twinning (MZT) rates associated with ART (1, 2). Although the natural MZT rate is approximately 0.4% in all births, it has been quoted to range anywhere from 1.57% to 5.6% with blastocyst ET over the last 10 years (1–5).

Although many studies have cited that MZT rates are higher with blastocyst transfer than with cleavage-stage transfer, a recent study with single embryo transfer (SET) showed no significant difference between the two (4). This raises the possibility that transfer order of higher-quality embryos, such as those from cohorts capable of growing blastocysts, is a greater risk

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factor than simply the stage of transfer as previously described. In addition to blastocyst transfer, zona manipulation procedures were thought to contribute to MZT in early studies; however, recent large-scale studies in major centers have shown no association between intracytoplasmic sperm injection (ICSI) or assisted hatching and MZT (2, 5, 6). In 2013 the American Society for Reproductive Medicine Practice Committee implied that these conflicting data "may be related to experience with blastocyst culture and transfer and differing culture systems among programs" but advised that patients need to be counseled about the increased risk of MZT with blastocyst transfer until this issue is clarified (7).

Although laboratory techniques have been an area of active research as risk factors for MZT, some studies have also cited oocyte age as a significant risk factor. Most recently, Knopman et al. (2) found increased MZT rates when maternal age of oocytes is <35 years. Although they also noted elevated MZT rates with blastocyst transfer, these results were confounded by the fact that younger women were more likely to qualify for blastocyst transfer in their IVF program. This points to the possibility that twinning may be related to the inherent quality of the oocyte and thus the embryo cohort rather than the laboratory techniques used for fertilization or culture alone.

The impact of embryo cohort quality on MZT as a result of cleavage-stage vs. blastocyst transfer remains unclear. This study sought to determine the association, if any, between MZT and measures of patient prognosis and embryo quality in a cohort undergoing blastocyst transfer vs. a cohort undergoing a cleavage-stage ET. In addition to assessing MZT rates based on transfer day and type of insemination as previous studies have done, patient and embryonic factors that impact and predict cohort quality were explored, including, patient age, number of oocytes retrieved, day-3 embryo morphology, and number of supernumerary embryos available for cryopreservation. Furthermore, the impact of transfer order on MZT rates among cleavage-stage and blastocyst-stage transfers was analyzed.

MATERIALS AND METHODS

This retrospective cohort study was performed to investigate whether the risk of MZT is related to embryo quality and/or impacted by differential management in the laboratory. The following comparisons were made: [1] MZT rate was compared in cleavage-stage vs. blastocyst-stage transfers; [2] MZT rate was compared in cases of ICSI vs. conventional insemination; [3] markers of embryo quality were analyzed and MZT rate compared; and [4] markers of embryo quality combined with patient characteristics were analyzed and the MZT rate compared. Finally, logistic regression was used to examine the relationship between stage of ET and rate of MZT when controlling for potential confounding variables.

Patient Population

All clinical pregnancies from autologous fresh IVF cycles performed at a single institution from October 1999 through February 2014 were analyzed. Frozen embryo transfer cycles and donor oocyte cycles were excluded from analysis, to standardize the embryo cohort quality analysis. Standard regimens for controlled ovarian hyperstimulation were employed, using purified urinary FSH or recombinant FSH and LH activity in the form of low-dose hCG or hMG along with GnRH agonist (long down-regulation or microdose flare) or GnRH antagonist to prevent a premature LH surge. Monitoring of IVF cycles was per practice routine. Oocyte maturation was induced with recombinant hCG (typically 500 μg) or purified urinary hCG (typically 10,000 IU) or with GnRH agonist (leuprolide acetate 2 mg in two doses 12 hours apart) $\pm$ 1,500 IU of hCG when two or three follicles reached or exceeded 17–18 mm or when the follicular cohort was deemed to be mature by the patient's primary physician.

Transvaginal oocyte aspiration was performed approximately 36 hours later. Cumulus stripping occurred after retrieval. Insemination of mature oocytes was performed by ICSI or by conventional insemination. Embryos were then cultured with sequential media with Quinns Advantage (CooperSurgical) followed by BlastAssist (Origio).

Regardless of transfer day, all embryos were examined on day 3, and the size and quality of the cohort were assessed. Per routine in this program, embryos underwent laser-assisted hatching of the zona pellucida on day 3 of development. During the years 1999 to 2010 embryos were transferred at either the cleavage or blastocyst stage. However, per practice routine, beginning in January 2011 all embryos were placed in extended culture for planned blastocyst transfer on day 5 or 6. Transfer order was then determined by patients and their physician, in consultation with the embryology team.

All pregnant patients underwent transvaginal ultrasound evaluation in the mid-first trimester. Monozygotic twinning cases were identified when the number of fetal heart beats exceeded the number of embryos transferred, and all records that indicated the presence of monozygotic gestations were reviewed.

Data Analysis

Patient demographic variables, laboratory procedures, and embryo cohort variables were analyzed for differences between the cleavage-stage transfer and blastocyst-stage transfer groups. This was done to evaluate our dataset in comparison with the previously published literature on MZT and stage of transfer, which provides context for the remainder of the comparisons. These comparisons were performed using the χ^2 test, Student's t test, or Mann-Whitney U where applicable.

Next the impact of patient prognosis on MZT rates in cleavage- and blastocyst-stage ETs was compared. Given that patient age is both an important indicator of IVF success and a predictor of embryo cohort quality, the overall MZT rate was examined across Society for Assisted Reproductive Technologies age groups and with age as a continuous variable. More direct indicators of embryo cohort quality were also examined. These factors included mean cell number at day 3, absolute number of embryos in the cohort with six to eight cells on day 3, percentage of total embryos in the cohort containing six to eight cells on day 3, and presence of