Incidence of monozygotic twins in blastocyst and cleavage stage assisted reproductive technology cycles

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Objective: To study the incidence of monozygotic twins (MZT) in blastocyst and cleavage stage ET.
Design: Retrospective review.
Patient(s): Four hundred ninety-six women undergoing IVF/intracytoplasmic sperm injection cycles at a private assisted reproductive technology (ART) center.
Intervention(s): Patients undergoing ART were divided according to the stage of ET into blastocyst transfer (BT) and cleavage stage (CS) ET.
Main Outcome Measure(s): Incidence of MZT as noted on vaginal ultrasound at 6 to 8 weeks.
Result(s): There were 374 (75.4%) BT cycles, and 122 (24.6%) CS cycles. Women in the BT group were significantly younger, had more oocytes retrieved, and had less embryos transferred compared with the CS group. The clinical pregnancy rate was significantly higher in the BT group at 67.9% (254 of 374), compared with 37.7% (46 of 122) among the CS group. There was a significantly higher incidence of multiple gestation in the BT group compared with CS group (37.4% compared with 19.6%). The overall incidence of MZT was 1.3%, but differed with the stage of ET: the incidence of MZT was 1.57% (4 of 254) in the BT group, and 0 (0 of 46) in the CS group.
Conclusion(s): Contrary to the older published literature on MZT in BT cycles, the incidence of MZT is low. Women undergoing ART therefore should not be discouraged to undergo BT for fear of MZT, especially in light of the higher pregnancy rate and lower number of transferred embryos noted in those cycles compared with cleavage stage transfers. (Fertil Steril® 2009;■:■–■. ©2009 by American Society for Reproductive Medicine.)

Key Words: Monozygotic twins, blastocyst, cleavage stage, ART, pregnancy rate, ET

The incidence of monozygotic twins (MZT) as related to infertility treatment and specifically with assisted reproductive technology (ART) and the stage of ET has been the source of confusion in the literature. The early papers addressing this topic showed a very high incidence of MZT in blastocyst transfer (BT) compared with cleavage stage (CS) ET cycles, with some questioning whether BT cycles are preferable to CS ET cycles despite higher pregnancy rates (PRs) noted in several trials (1–9). However, most of these studies were based on a small number of cycles with inconsistent results. In 2006, the ASRM Practice Committee noted that, based on the available literature, BT "results in an increased incidence of MZT varying between 2.7% and 13.2%," and therefore, MZT "remains a major drawback to routine blastocyst transfer for all ART patients" (10). The ASRM statement was based on few papers, and none of those were published after 2002. Only two studies have been published evaluating data after 2002 (11, 12), and a recent meta-analysis evaluating MZT in ART showed that the incidence of MZT after 2002 was not increased with BT compared with CS ET, in contrast to the studies published earlier (13). A reassuring recent study from the Danish National Cohort project found no increase in MZT between women conceiving naturally (0.3%, whether they conceive in <12 or >12 months of trying to conceive [TTC]), and those infertile couples conceiving after fertility treatment (0.3%, adjusted odds ratio [OR] 1.5, 95% confidence interval [CI] 0.7–3.2) (14).

Monozygotic twins are derived from splitting of a single embryo, and depending on when the embryo splits, different MZT configurations arise. If the embryo splits between day 0 and day 4 after fertilization, dichorionic diamniotic twins are formed (thus indistinguishable from dizygotic twins arising from two separate embryos). If the split occurs between day 4 and day 8, the result is a monochorionic diamniotic twin. If the split occurs after day 8, the result is a monochorionic monoamniotic twin. A split after day 12 results in conjoined twins (15).

Monozygotic twins are a rare event outside the context of ART. They occur in 0.4% to 0.45% of all births (16). Monozygotic twins are associated with significant obstetric and perinatal morbidity. These include increased fetal loss, intrauterine growth restriction (IUGR), preterm deliveries, and perinatal loss. Cord accidents are increased in those cases where a separating amniotic membrane is not noted. Twin-to-twin transfusion complicates 30% of monochorionic diamniotic twins and account for 15% of perinatal mortality,

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and those surviving have an increased risk of neurologic damage (16, 17). Maternal morbidity includes gestational hypertension and preeclampsia along with gestational diabetes (16, 17). It is therefore critical to avoid MZT as much as possible while maximizing patients' chances at a livebirth.

Multiple mechanisms for the increased incidence of MZT with ART have been proposed. These include extended culture, zona manipulation such as intracytoplasmic sperm injection (ICSI), and assisted hatching, ovarian stimulation, increased maternal age, and temperature effects (1–9, 16–19).

MATERIALS AND METHODS

In the present study, a retrospective review of all IVF/ICSI cycles performed at our institution between August 2003 and August 2008 was undertaken. The former was the date when we started blastocyst transfers in our program. All cycles with an embryo transfer were included in this review. Exclusion criteria included donor oocyte cycles, preimplantation genetic diagnosis, and frozen embryo transfer cycles. All women underwent ovarian stimulation using one of three stimulation protocols: long luteal GnRHa, GnRH antagonist, or microdose flare GnRHa. All cycles used a mixed protocol using urinary or recombinant FSH (Bravelle; Ferring, Suffern, NY; Gonal-F; Serono, Rockland, MA; and Follistim; Organon, Roseland, NJ) and hMG (Repronex and Menopur, Ferring). When at least three follicles reached 16 to 18 mm in diameter, 5,000 to 10,000 U of urinary hCG (Novarel, Ferring) was administered subcutaneously, and oocyte retrieval was performed 35 hours later. All patients received intramuscular P (50 mg) along with vaginal estradiol (2 mg two times a day) starting the day after oocyte retrieval. All women underwent ICSI to ensure optimal fertilization.

Patients proceeded with a BT or CS ET based on the following criteria: those with a total mean antral follicle count of <6, mean ovarian volumes <3 cm^3, those with baseline FSH >10 IU/L, or those with elevated FSH on day 3 or day 10 using a Clomid challenge test, those with less than four oocytes in a prior cycle, and those who had poor blastocyst development in a prior cycle underwent a CS ET (it is important to note that age was not a criterion for selecting CS over BT). All others underwent a BT. All women in the BT group underwent an embryo transfer, that is, none were canceled for lack of a blastocyst available for transfer on day 5.

Oocytes were rinsed and denuded using a hyalurinadase solution combined with mechanical stripping, and ICSI was performed 4 to 6 hours later. Embryos were cultured in groups under mineral oil in droplets of culture media (P1/cleavage stage, Irvine Scientific, Irvine, CA; in 2003–2004; and Global Medium, Life Global, Life Global, CT; from 2004–present) with 10% serum substitute supplement. For those progressing to blastocysts, the embryos were grown in sequential medium (Blastocyst media, Irvine Scientific, from 2003–2004, and Global Medium, Life Global; from 2004–present). All embryos were cultured under 37°C in a 5% O_2, 5% CO_2 environment for 3 or 5 days. For those undergoing a CS transfer, assisted hatching was routinely performed using acidified Tyrode's solution before ET. All BT were performed on day 5. All ET were performed under ultrasound guidance using a Wallace Sure-view catheter (Marlow, Irvine Scientific) or Cook echo-tip (Cook, Chicago, IL). A pregnancy test was performed 10 to 12 days after ET. Biochemical pregnancies were recorded as negative. Pregnant patients underwent a vaginal ultrasound at 6 to 7 weeks, and the number of gestational sacs was recorded. Monozygotic twins were diagnosed when more than one fetus with cardiac activity was seen in the same gestational sac. In addition, the presence or absence of a separating amniotic membrane was noted. The ultrasound examination was repeated 1 to 2 weeks later to confirm the prior findings.

Statistical analysis was performed using χ^2. A value of $P<.05$ was considered significant. Our center's institutional review board approved this retrospective review.

RESULTS

A total of 496 cycles were evaluated: 254 (75.4%) were BT cycles and 122 (24.6%) were CS transfers. All women who had a CS transfer underwent assisted hatching, and none of the women who had a BT did. The women who underwent BT were significantly younger than those who underwent CS transfer (Table 1). They also had a significantly higher number of retrieved oocytes (and MII oocytes) than the CS group, and had significantly fewer embryos replaced, but no difference in fertilization rates. The overall clinical PR was 60.5% (300 of 496), but differed significantly with the stage of ET: the clinical PR was significantly higher at 67.9% (254 of 374) in the BT group, compared with 37.7% (46 of 122) in the CS group (Table 1). As expected, there was a significantly higher rate of dizygotic twins in the BT compared with the CS group (37.4% compared with 19.6%), despite the significantly lower number of embryos transferred.

The overall incidence of MZT was 1.3% (4 of 300), but differed by stage of ET: the incidence of MZT was 1.57% (4 of 254) in the BT, compared with 0 (0 of 46) in the CS group. It is interesting to note that two of the MZT cases occurred in women who had elective single-embryo transfer (eSET) in an attempt to avoid a twin gestation (one delivered MZT at 30 weeks, and the other delivered MZT at 36 weeks). The other two women had two blastocysts transferred each and conceived with a MZT and another twin. Both women elected to have selective reduction of the MZT at 11 weeks after extensive counseling with maternal–fetal medicine specialists, and both delivered healthy singletons (one at 36 weeks and one at 28 weeks). There were no cases of monochorionic monoamniotic pregnancies.

DISCUSSION

Our present study shows that the incidence of MZT is rather low (1.57%) in a relatively large number of BT cycles, and although this is about a three times increase over the

TABLE 1

Incidence of MZT in BT and CS ET among 496 ART cycles.

	Blastocyst transfer	Day 3 transfer
Number of patients	374	122
Age (years) (mean ± SD)*	34.3 ± 5.1	37.7 ± 4.3
Eggs retrieved (mean ± SD)*	13.5 ± 6.5	6.9 ± 4.3
MII Oocytes (mean ± SD)*	10.2 ± 5.4	4.8 ± 2.9
Fertilization rate %	84.7%	84.5%
ET (mean ± SD)*	2.2 ± 0.7	2.7 ± 0.4
Clinical pregnancy rate/ET*	254/374 (67.9%)	46/122 (37.7%)
Multiple gestation rate*	95/254 (37.4%)	9/46 (19.6%)
Monozygotic twins (%)	4/254 (1.57%)	0/46 (0%)

Note: MZT = monozygotic twins; BT = blastocyst transfer; CS = cleavage state; ART = assisted reproductive technology; ET = embryo transfer.

* $P < .05$ for BT vs. cleavage stage ET.

Sharara. MZT in blastocyst and cleavage stage cycles. Fertil Steril 2009.

incidence of MZT found in the general population (16), this incidence is significantly less than the earlier results obtained with BT. Because BT cycles are associated with higher PR and lower number of ET in our study, these results should encourage other programs to proceed with BT to maximize their patients chances while lowering the number of transferred embryos, and thus the higher order multiple gestation rate. Our results also show that using the criteria noted above prospectively as far as who will benefit the most from a BT, good prognosis patients undergoing BT can achieve an excellent clinical outcome.

Multiple mechanisms for the increased incidence of MZT with ART have been proposed. These include extended culture, zona manipulation such as ICSI and assisted hatching, ovarian stimulation, increased maternal age, and temperature effects (1–9, 16–19). Which one seems to be critical is hard to point out. Unfortunately no animal model is available that can provide answers to this dilemma.

The benefits of BT cycles have been questioned as the number of reports of high MZT incidence increased (1–9). The first reported association between BT and MZT was in 1998 (1). However, most of these studies were based on a small number of cycles, especially in the BT group. The largest study evaluated the Society of Assisted Reproductive Technologies 1999 and 2000 data with an overall incidence of MZT of 0.6% (226 MZT in 39,198 pregnancies) (20). Of those, 7,921 pregnancies resulted from a BT and 29,144 from day 3 ET (20). There was a four fold increase in MZT with BT compared with CS ET after controlling for multiple treatment and patient factors (20). More recent studies are much scarcer. Surprisingly, only 2 studies have been published that have evaluated data obtained after 2002 (11, 12). The first study evaluated 175 BT cycles and 176 cleavage stage cycles undergoing eSET, resulting in 58 clinical pregnancies in the BT group compared with 41 in the CS group (11), whereas the second larger study evaluated 385 BT and 547 cleavage stage pregnancies (12). In the study by Papanikolaou et al. (11), there were no cases of MZT in 73 BT pregnancies, compared with 3.4% in the cleavage stage group. In the larger study by Moayeri et al. (12), the incidence of MZT before March 2002 and between March 2002 and December 2005 were compared; the incidence of MZT decreased over time from 5.6% to 2.3%, not significantly different from the 1.8% incidence noted in cleavage stage group, raising the point that the decrease in MZT incidence could be because of an increase in the embryologists' experience with extended culture (12). Barritt et al. (21) presented similar results in an abstract form and also found that as the experience of the embryologists in their program increased, the incidence of MZT decreased from 4.4% (18 of 412) in BT cycles in their first 29 months of laboratory operation (2002–2004), to 2.0% (10 of 510) from 2005 to 2007 (21). In our present study, we only had four cases of MZT in 256 BT pregnancies, and they occurred throughout the study period (one in 2004, one in 2006, one in 2007, and one in 2008), and therefore, we did not have a "learning" curve. In our program, all ovarian stimulation and ET were performed by a single physician, and all the embryo manipulations were handled by the same embryologist, resulting in a relatively low incidence of MZT over those past 5 years. Unlike the absence of MZT noted in the BT eSET study noted above (11), two of our MZT cases occurred in the context of eSET, and both were recent (one in 2007 and one in 2008). Therefore, eSET will not eliminate the occurrence of MZT.

A recent study evaluating 2,501 pregnancies after ART between 1998 and 2004 reported 41 monochorionic pairs (which for all practical purposes relating to ART are labeled as MZT), and 2,460 pregnancies without a monochorionic pair (1,456 singletons and 1,004 multiples) (22). Using a multivariate analysis, the investigators found an increased incidence of monochorionic twins with assisted hatching (OR 2.23, 95% CI 1.06–4.67), ICSI (OR 2.42, 95% CI 1.22–4.83), day 5 ET (OR 2.48, 95% CI 1.62–3.80). The worst predictor was in cycles with ICSI and day 5 ET (OR 24.42, 95% CI 7.03–84.42) (22). Of note, only 273 pregnancies in that large study were after a BT (no note on during which time frame were these cycles done, and whether the investigators plan to change their policy regarding BT in their program in response to their results). In our study, we did not specifically evaluate monochorionic twins (as this will require a careful examination of the placentae at birth or DNA fingerprinting on all same sex twin births) (23), but all our patients with dizygotic twins had separate sacs with clearly separate placentae (otherwise they were labeled as MZT for our study). We did not find

a negative association, as noted by Skiadis and colleagues (22), especially as we perform ICSI routinely on all our patients and 75% of whom underwent a BT. The above investigators (22) also did not evaluate whether there was a decrease in their monochorionic twins over time during their 7-year study (during which one would expect different laboratory conditions including different embryologists were present), as noted below by Moayeri et al. (12).

In a recent meta-analysis, Chang et al. (13) reviewed the published literature and found that the incidence of MZT is indeed increased in BT compared with cleavage stage cycles. However, they found substantial heterogeneity among the included studies and found that with studies published after 2002, there was no increase in the incidence of MZT compared with cleavage stage cycles (OR 1.00, 95% CI 0.43–2.32). This observation is unfortunately based on only two studies, one of which evaluated a small number of cycles; hence, the only study of significance is the one by Moayeri et al. (12). The overall incidence of MZT in the above meta-analysis was 1.64% after BT, and 0.41% after cleavage stage ET. Although we had no cases of MZT in our cleavage stage ET group (most likely because of the low number of cases in that group), the figure of 1.64% noted in the meta-analysis of Chang et al. (14) in the BT group is very close to the 1.57% found in our study. A recent and reassuring population-based study from the Danish National Cohort project found no increase in MZT between women conceiving naturally (0.3%, whether they conceive in <12 or >12 months of TTC), and those infertile couples conceiving after fertility treatment (0.3%, adjusted OR 1.5, 95% CI 0.7–3.2), despite the significantly increased incidence of dizygotic twin gestation noted with fertility treatment. The low number of MZT in the Danish National Cohort study in women who received fertility treatment (8 of 2798 pregnancies) precluded any meaningful examination of the association between different treatment modalities (hormonal treatment, intrauterine insemination, IVF, ICSI) and MZT incidence. We therefore believe that separate patient-specific underlying factors (familial MZT clusters have been reported) (24), rather than the ART technique used (ovarian stimulation, ICSI, assisted hatching [AH], BT), could predispose a particular patient to MZT.

Larger studies from large centers are therefore clearly needed. We believe more studies such as our current one will continue to show a low MZT incidence, and that more programs will move toward more BT cycles, especially in the good prognosis patients where eSET will become the norm. This will hopefully result in a new revised ASRM Practice Committee publication that encourages BT for most patients.

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