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Does assisted hatching pose a risk for monozygotic twinning in pregnancies conceived through in vitro fertilization?

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Objective: To examine the association between assisted hatching and monozygotic (MZ) twinning.

Design: Case-control.

Setting: Population-based sample of IVF-ET cycles initiated in U.S. clinics, 1996.

Patient(s): The IVF-ET (n = 35,503) cycles and 11,247 resultant pregnancies.

Intervention(s): Use of an assisted hatching procedure on embryos transferred.

Main Outcome Measure(s): Cases were pregnancies for which number of fetal hearts observed on ultrasound exceeded number of embryos transferred. These pregnancies were considered to contain at least one MZ set of twins. Cases were compared with two control groups: other multiple-gestation pregnancies (≥2 fetal hearts but number of fetal hearts ≤ number of embryos transferred); and singleton pregnancies (1 fetal heart).

Result(s): Women with a case pregnancy were more likely to have received embryos treated with assisted hatching procedures than were women in either control group. After adjustment for patient age, number of embryos transferred, prior cycles, infertility diagnosis, intracytoplasmic sperm injection, and whether embryos from the current cycle were cryopreserved for later use, odds ratios and 95% CIs for use of assisted hatching were 3.2 (1.2–8.0), compared with other multiple-gestation pregnancies, and 3.8 (1.8–9.8), compared with singleton pregnancies.

Conclusion(s): Assisted hatching may pose a risk for MZ twinning. (Fertil Steril® 2000;74:288–94. ©2000 by American Society for Reproductive Medicine.)

Key Words: Assisted hatching, monozygotic, multiple birth

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Monozygotic (MZ) multiple gestation is of concern because of the associated increase in infant mortality and morbidity due to multiple gestation in general (1–5) and MZ multiple gestation in particular. Perinatal mortality risk among MZ twins has been reported to be two to three times higher than the risk among dizygotic (DZ) twins (6, 7).

Throughout the world and over recorded time periods, the prevalence of MZ twinning has remained relatively constant (8–12). However, in the late 1980s it was suggested for the first time that an extrinsic factor, use of ovulation induction medication, might impact this rate. Derom and colleagues (13) reported a twofold increase in the MZ twin birth rate among women who had conceived after therapy with an ovulation inducing medication, compared with women who conceived naturally from their population-based study in East Flanders, Belgium. Recently, it was suggested that another infertility treatment factor, assisted hatching procedures used in conjunction with IVF-ET, might also be associated with MZ twinning.

Assisted hatching techniques involve making a small opening in the zona pellucida of a fertilized embryo through chemical, laser, or mechanical manipulation. These techniques have been developed over the past 10 years to overcome low embryo implantation rates, which are thought to be related to abnormal hardening and/or thickening of the zona pellucida of some embryos fertilized in vitro (14–17).

Studies examining the effectiveness of assisted hatching methods in increasing pregnancy rates suggest that these procedures may present some benefit to certain subgroups of difficult to treat patients, such as older women or women with multiple failed cycles (16, 18–24). However, the safety of assisted hatching has been brought into question by case reports and case series of MZ twins borne by women undergoing IVF-ET with assisted hatching (25–28) and by animal studies showing that MZ twinning can be induced through mechanical or chemical manipulation of the zona pellucida (29–32).

We performed a case-control analysis of the association between assisted hatching and MZ twinning with use of a large population-based dataset of IVF-ET cycles initiated in U.S. clinics in 1996. Although we did not have specific data on the zygosity of multiple births, we were able to surmise that a proportion of pregnancies contained at least one MZ multiple gestation; these were pregnancies in which the number of fetuses exceeded the number of embryos that had been transferred. We compared these MZ pregnancies with two control groups: other multiple-gestation pregnancies and singleton gestation pregnancies.

MATERIALS AND METHODS

Study Population

The Fertility Clinic Success Rate and Certification Act of 1992 (FCSRA) requires that each medical center in the United States that performs IVF-ET or related assisted reproductive technologies (ART) report data for every ART cycle initiated to the Centers for Disease Control and Prevention (CDC) annually for the purpose of reporting clinic-specific pregnancy success rates (33). The Society for Assisted Reproductive Technology (SART) annually creates a database of ART cycles performed in U.S. clinics and shares these data with CDC under contract. All U.S. clinics that perform ART (both clinics that are members of SART and clinics that are not) are asked to submit data to SART for inclusion in the database. All clinics that submit their data to this CDC-supported SART system are in compliance with FCRSA.

An ART cycle begins when a woman begins taking fertility drugs or starts ovarian monitoring with the intent of having embryos transferred. The ART clinics submit data obtained from clinic records for each cycle initiated for a given reporting year, January 1 through December 31, in a standardized format. The data file is organized with one record per cycle. Multiple cycles from a single patient are not linked. Data collected include patient demographics, medical history and infertility diagnoses, clinical information pertaining to the ART procedure, and information on resultant pregnancies and births. In 1996, 300 U.S. clinics reported >60,000 ART cycles to SART and CDC. Some clinics did not report their data, despite the federal mandate; however, it is estimated that >95% of all cycles were reported.

We limited the present analysis to the most common ART approach, fresh, nondonor IVF-ET (n = 44,723 cycles). This refers to cycles in which eggs were removed from a woman's ovaries, combined with sperm, and if fertilized, the resulting embryo(s) were replaced into the same woman's uterus. Thus, we excluded cycles in which donor eggs or embryos were used, cycles in which cryopreserved embryos were used, cycles in which embryos or oocytes were transferred into a woman's fallopian tubes (gamete intrafallopian transfer [GIFT], zygote intrafallopian transfer [ZIFT], and tubal embryo transfer [TET]), and cycles with a gestational carrier or surrogate. Because these various cycle types may vary in implantation and pregnancy rates, they were not combined. Separate analysis for each cycle type was precluded by small sample sizes.

Among fresh, nondonor IVF-ET cycles initiated in 1996, we excluded cycles that did not progress to embryo transfer (n = 8890), cycles for which patient age was either missing (n = 79), <20 (n = 6), or >44 (n = 194), and cycles missing data for assisted hatching (n = 51). This left 35,503 cycles (transfer procedures) available for study. A total of 12,095 of these transfer procedures resulted in a clinical pregnancy. Data pertaining to number of fetuses were available for 11,247 of these pregnancies.

Case Definition

We defined cases as pregnancies for which the number of fetal hearts observed on an early ultrasound exceeded the number of embryos that had been transferred. We considered these pregnancies to contain at least one MZ set of twin fetuses. We acknowledge that such pregnancies might not all be solely MZ multiple gestations; our definition allows for the inclusion of pregnancies with a mix of MZ and DZ fetuses. Twenty-two cases were thus identified (Table 1). For 20 cases the number of fetuses exceeded the number of embryos transferred by one and for two cases the number of fetuses exceeded embryos transferred by two.

Because of fetal loss, the number of infants born was often reduced compared with the number of fetal hearts reported early in pregnancy. Nonetheless, even though we defined cases based on early gestation rather than birth data, our case definition is likely to have missed some cases of MZ pregnancy. We could not identify pregnancies with an MZ multiple gestation among cycles in which the number of fetuses did not exceed number of embryos transferred. Although we assume that multiple-gestation pregnancies arising from IVF-ET treatment are primarily DZ stemming from the transfer of multiple embryos, a small proportion of MZ fetuses are also likely contained among IVF-ET multiples gestations that were not considered cases, according to our case definition.

A second way our case definition may have undercounted MZ multiple gestations relates to ultrasound timing; some cases of both MZ and DZ multiple gestation may have been

TABLE 1

Cases of monozygotic (MZ) multiple pregnancy.

Case no.	No. of embryos transferred	No. of fetal hearts on ultrasound	No. born
1	1	2	2
2	1	2	2
3	1	2	2
4	1	2	0
5	3	4	4
6	3	4	3
7	3	4	2
8	3	4	2
9	3	4	2
10	3	4	2
11	3	4	2
12	3	4	2
13	3	4	0
14	3	5	0
15	4	5	5
16	4	5	5
17	4	5	2
18	4	5	2
19	4	5	2
20	4	5	2
21	4	6	3
22	6	7	2

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missed if multiple fetal hearts were not discernable at the time of ultrasound. We do not consider this to have been a major source of error because in the general IVF-ET population >99% of multiple births had been documented as having two or more fetal hearts.

Selection of Controls

We constructed two control groups: [1] other IVF-ET multiple gestation pregnancies (≥2 fetal hearts on ultrasound, but number of fetal hearts ≤ number of embryos transferred); and [2] IVF-ET singleton pregnancies (1 fetal heart). These control groups included 4,623 and 6,602 pregnancies, respectively.

Having two control groups was desirable because each had a possible limitation. First, comparison of our cases of MZ pregnancies with the control group of other multiple gestation pregnancies could be biased toward the null hypothesis of no association between assisted hatching and MZ twinning. As previously mentioned, the “other multiples” control group could contain some MZ and DZ multiple pregnancies, a misclassification that would be independent of use of assisted hatching, i.e., a nondifferential misclassification. Nondifferential misclassification has been shown to attenuate associations between two variables (34).

In contrast, comparison of MZ multiples with a control group of singleton pregnancies might produce a spurious association. Previous studies suggest assisted hatching may increase the implantation rate (16, 18–24); if true, such a comparison might overstate an association with assisted hatching if the MZ case pregnancies were actually a mix of MZ and DZ fetuses. This in fact is likely the case with our data (see Table 1; in 18 of 22 MZ cases the number of embryos transferred was >1, and the number of fetal hearts was >2). An observed association between assisted hatching and MZ twinning, then, might actually reflect a higher implantation rate (hence, an increase in DZ multiples) among cycles using assisted hatching rather than an effect on embryo splitting. Thus, each control group has a possibility for bias, albeit in opposite directions.

Exposure of Interest

Assisted hatching was defined as the use of an assisted hatching technique on either some or all embryos transferred. Data were not available to classify the specific type of assisted hatching procedure used.

Potential Confounding Factors

We considered several indicators of potential confounding including patient age (<35, ≥35 years), number of embryos transferred (<4, ≥4), number of prior ART cycles (0, ≥1), infertility diagnosis (tubal, not tubal), use of intracytoplasmic sperm injection (ICSI, a micromanipulation technique in which a sperm cell is directly injected into the ooplasm of an egg), and whether ≥1 embryos retrieved and fertilized during the current IVF-ET cycle were cryopreserved for use in a later cycle (a general indicator of both ovarian response and embryo quality; i.e., if some of the embryos were set aside for cryopreservation, it is thought that higher quality embryos may have been available for transfer).

Data Analysis

We first examined the percentage of pregnancies that resulted from transfer procedures that [1] did not use assisted hatching procedures for any embryos; [2] used assisted hatching with some embryos transferred; [3] used assisted hatching with all embryos transferred. In addition, we calculated the percentage of pregnancies that were multiple gestations and the percentage of pregnancies with at least one MZ multiple gestation for these three groups.

Because of small sample sizes, we collapsed the definition of assisted hatching into a dichotomous variable (none vs. some or all) for the remainder of the analyses. We used this variable in calculating ORs and 95% CIs. We calculated the odds that a cycle resulting in an MZ pregnancy had used embryos treated with assisted hatching techniques relative to the odds that [1] cycles resulting in other multiple-gestation pregnancies had used assisted hatching and [2] cycles resulting in singleton pregnancies had used assisted hatching. We evaluated the possibility of confounding and effect modification by stratifying our analysis by each of the potential confounding factors listed above. In addition, we constructed

TABLE 2

Relationship between assisted hatching and pregnancy, multiple gestation, and monozygotic (MZ) multiple gestation—United States, 1996.

Assisted hatching	No. of cycles	Pregnancy (%)[a] (pregnancies/transfer procedure)	Multiple gestation (%) (multiple gestations/ pregnancy[b])	At least one MZ multiple gestation (%) (MZ multiple gestations/pregnancy[b])
No assisted hatching	21,490	33.3	41.5	0.13[c]
Assisted hatching with some transferred embryos	3,310	40.1	46.2	0.16
Assisted hatching with all transferred embryos	10,703	33.9	39.1	0.33
Total	35,503	34.1	41.3	0.20

[a] Based on 12,095 pregnancies.
[b] Percent multiple gestation and percent at least one MZ multiple gestation based on 11,247 pregnancies (848 subjects were missing necessary data).
[c] $P<.05$ for χ^2 test for trend comparing rates across assisted hatching categories.

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logistic regression models that included assisted hatching and all the potential confounders as independent variables.

This research was approved by the Institutional Review Board at CDC.

RESULTS

Among the 35,503 transfer procedures considered in this analysis, 21,490 (61%) did not use assisted hatching procedures, 3,310 (9%) used assisted hatching for some transferred embryos, and 10,703 (30%) used assisted hatching for all transferred embryos. The overall pregnancy rate among transfer procedures was 34%; although this rate was slightly increased among cycles in which some embryos had been treated with an assisted hatching procedure, there was no appreciable difference in pregnancy rates between cycles in which no embryos were treated and cycles in which all embryos were treated (Table 2). Results were similar after stratification by both age and number of embryos transferred (data not shown).

The percentage of pregnancies with multiple fetuses was 41% overall; again, assisted hatching did not materially affect the multiple-gestation rate (Table 2). The percentage of pregnancies that contained at least one set of MZ multiples was 0.20%; however, the rate varied, depending on assisted hatching status. In comparison with cycles in which no embryos had been treated (0.13%), the percentage of MZ multiple pregnancies we were able to identify was increased more than twofold among cycles in which all embryos had been treated with use of assisted hatching (0.33%). In addition, when all three categories (none, some, and all) were considered together, a statistically significant test for trend was noted.

Women with an MZ multiple gestation pregnancy tended to be younger and had fewer embryos transferred on average than women with pregnancies included in either of the two control groups (Table 3). In addition, in comparison with women with pregnancies in either control group, women with a case pregnancy were slightly less likely to be undergoing their first ART cycle, more likely to have been diagnosed with tubal factor infertility, less likely to have used ICSI, and more likely to have had extra embryos cryopreserved for later use. We were not able to determine whether the differences noted for embryos cryopreserved was because extra embryos were more likely to be available for cases or whether some other factor related to patient choice or clinical evaluation was different between cases and controls.

Women with a case pregnancy were more likely to have

TABLE 3

Distribution of potential confounding factors among cases and controls.

	Cases (n = 22)	Control group 1 other multiple gestations (n = 4,623)	Control group 2 singleton gestations (n = 6,602)
Mean age (y)	32.2 (3.8)[a,b]	33.1 (3.9)	34.1 (4.1)
Mean number embryos transferred	3.1 (1.2)[a]	4.3 (1.3)	4.0 (1.4)
First ART cycle (%)	59.1[a]	63.6	60.4
Infertility due to tubal factor (%)	50.0	35.4	35.3
Using ICSI (%)	23.8[c]	36.0	38.2
One or more embryos cryopreserved (%)	50.0[a]	46.0	35.2

Note: ART = assisted reproductive technology; ICSI = intracytoplasmic sperm injection.
[a] $P<.01$ for comparison of distributions between case and control groups; means were compared with use of analysis of variance, percentages were compared with χ^2 tests.
[b] SD in parentheses.
[c] $P<.05$.

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TABLE 4

Association between assisted hatching and monozygotic multiple gestations before and after adjustment for potential confounding factors.

	Control group 1 other multiple gestations				Control group 2 singleton gestations			
	No. of cases	No. of controls	OR	95% CI	No. of cases	No. of controls	OR	95% CI
Crude association assisted hatching								
Yes	13	1,860	2.1	0.9–5.0	13	2,692	2.1	0.9–4.9
No	9	2,763			9	3,910		
Adjusted associations (single factor)								
Age			2.5	1.04–5.9			2.6	1.1–6.2
No. of embryos transferred			2.5	1.1–5.8			2.3	0.96–5.3
Prior ART cycles			2.1	0.9–4.9			2.1	0.9–4.9
Tubal infertility			2.3	0.96–5.3			2.2	0.96–5.3
ICSI			2.7	1.1–6.5			2.6	1.1–6.4
One or more embryos cryopreserved			2.2	0.9–5.3			2.3	0.98–5.5
Adjustment for all factors			3.2	1.2–8.0			3.8	1.5–9.8

Note: OR = odds ratio; CI = confidence interval.

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received embryos treated with assisted hatching procedures than women included in either control group (Table 4). The unadjusted ORs were 2.1 for analyses with both control groups; however, the CIs included 1.0 in both instances. The associations suggested in the crude analyses persisted after adjustment for each of the potential confounding factors; in fact, both associations increased and became statistically significant when all factors were included in a multivariable model. Stratified analyses did not suggest any effect modifications between assisted hatching and any of the potential confounding factors included in Table 4 (data not shown).

Although our final analyses examined assisted hatching as a dichotomous variable, we also evaluated the effects of an ordinal assisted hatching variable (none, some, or all). Because of the small number of cases in which some embryos were treated with assisted hatching, the ORs for this group were unstable with CIs that included 1.0; nonetheless, they were in the direction expected. When we compared case pregnancies with the other multiple-gestations control group, we observed adjusted ORs of 1.7 and 3.7 for associations with some and all embryos treated with assisted hatching, respectively; when we compared case pregnancies to the singleton gestation control group, we observed adjusted ORs of 2.5 and 4.2 for associations with some and all embryos treated with assisted hatching, respectively.

DISCUSSION

These findings support prior case reports and case series that have suggested a link between use of assisted hatching and MZ twinning (25–28). We found that MZ twinning was more than twice as likely to occur among IVF-ET cycles in which embryos were treated with assisted hatching methods. This association was not explained by age, number of embryos transferred, previous ART cycles, infertility diagnosis, use of ICSI, or availability of extra embryos for cryopreservation.

Although case ascertainment was limited by lack of specific information on zygosity, we do not believe this biased the relative comparisons presented here. We compared cases of MZ pregnancy to two different control groups: other multiple-gestation pregnancies and singleton pregnancies. Potential biases related to case ascertainment were expected to be in opposite directions for these control groups: toward the null for comparisons with other multiple-gestation pregnancies and away from the null for comparisons with singleton pregnancies. However, both unadjusted and adjusted ORs were similar across control groups.

To further address potential misclassification or misreporting of the number of fetal hearts reported from ultrasound, we repeated our analyses after defining MZ pregnancies solely based on whether number of infants *born* exceeded the number of embryos transferred. As can be seen in Table 1, this reduced our number of cases to six; nonetheless, the association with assisted hatching persisted (data not shown). We also considered the possibility that MZ embryos might have been created through natural conception; we repeated our analysis after limiting the sample to women with tubal factor infertility and again found no change in our risk estimates.

We lacked data to distinguish specific methods of assisted hatching used; however, if certain hatching procedures pose a higher risk for MZ twinning than others, combining all procedures as was done in this analysis would tend to dilute the effect. Thus, the true association for certain types of assisted hatching may be even stronger than our results indicate. Likewise, the association for some assisted hatching procedures may be smaller than the overall association presented here.

Although the prevalence of DZ twinning has been influenced by factors such as maternal age, race, and infertility treatments, until recently the prevalence of MZ multiple births has been remarkably similar across countries and time periods; estimates range from three to five MZ multiple births per 1000 total births (8–12). Derom et al. (13) estimated an increase in this rate to 12 per 1,000 among births conceived with use of artificial ovulation induction medications. Because our method of MZ case ascertainment is subject to undercounting, we are unable to provide an accurate estimate of MZ multiple gestation or birth rates associated with assisted hatching procedures. However, in our study, all but one (4.6%) case and eight (0.1%) singleton controls were given one or more medications to stimulate ovulation as part of their treatment protocol. Thus, assisted hatching posed an increased risk for MZ twinning above and beyond the risk that may have already been present from treatment with ovulation medications.

A mechanism by which assisted hatching might lead to MZ twin formation has been proposed. Animal studies have shown that embryos with holes in the zona layer may be trapped during hatching (35, 36). In humans, if hatching occurs through the artificial gap created by assisted hatching treatments rather than by natural autolysis of the zona pellucida, a portion of the inner cell mass may become trapped, possibly forcing a split into two separate embryos. This hypothesized mechanism suggests that embryo division would occur near the time of implantation. Thus, not only would assisted hatching increase the risk of MZ twin formation, but the timing of the division might be related to higher risk subsets of MZ twins, monochorionic, and possibly even monoamniotic MZ twins (37).

This thesis has some support in the literature. A recent report identified four monoamniotic multiple gestations associated with assisted hatching IVF-ET cycles (25). A separate case report described a set of conjoined twins in a triplet pregnancy that developed during an assisted hatching IVF-ET cycle (26).

Several previous studies have reported benefits in terms of higher pregnancy rates when assisted hatching was used among identified groups of poor prognosis patients (16, 18–24). In the present study, we observed little difference in pregnancy rates between cycles that treated embryos with assisted hatching and those that did not. These results persisted after stratification by patient age and number of embryos transferred. However, we did not have data on specific embryo characteristics such as zona thickness; therefore, we cannot exclude the possibility that assisted hatching may provide a benefit to certain subgroups. Cohen et al. (24) found that selective use of assisted hatching among embryos with thick zona provided the optimal benefit. The Society for Assisted Reproductive Technology is currently reviewing the literature on the effectiveness of assisted hatching (personal communication).

The benefits of assisted hatching remain unclear. Moreover, even if we assume these techniques do afford some gains in pregnancy rates, this study suggests that assisted hatching poses a risk for a potentially serious outcome. Multiple-birth infants are at increased risk for preterm delivery, low birth weight, congenital malformations, fetal and infant death, and long-term morbidity and disability among survivors (1–5). The MZ multiples, particularly monoamniotic MZ multiples, are the highest risk subgroups of multiple-birth infants (6, 7). Medical interventions, such as assisted hatching that may impact the MZ twinning rate, must, therefore, be carefully evaluated. Future studies are needed to examine associations between MZ twinning and specific types of assisted hatching procedures. In addition, in light of the potential risks assisted hatching poses, further research on whether assisted hatching provides a benefit to certain subgroups is also warranted.

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