The risk of monozygotic twins after assisted reproductive technology: a systematic review and meta-analysis

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TABLE OF CONTENTS
- Introduction
- Materials and Methods
- Results
- Discussion

BACKGROUND: It is estimated that there is at least a 2-fold rise in the incidence of monozygotic twinning after assisted reproductive technology compared with natural conception. This can result in adverse pregnancy outcomes.

METHODS: We searched MEDLINE, EMBASE and SCISEARCH for studies that estimated the risk of monozygotic twinning and its association with any particular assisted reproductive technique. Monozygotic twinning was defined by ultrasound or Weinberg criteria. A meta-analysis of the proportion of monozygotic twins was performed using both fixed and random effects models.

RESULTS: The search revealed 37 publications reporting on the incidence of monozygotic twins after assisted reproductive techniques. Twenty-seven studies met the inclusion criteria and were included in the meta-analysis. The summary incidence of monozygotic twins after assisted conception was 0.9% (0.8–0.9%). The incidence of monozygotic twins in natural conception is 0.4%. Blastocyst transfer and intracytoplasmic sperm injection are associated with 4.25 and 2.25 times higher risk of monozygotic twins.

CONCLUSIONS: The risk of monozygotic twins in assisted conception is 2.25 times higher than the natural conceptions. Larger studies reporting on monozygotic twinning following single-embryo transfer or after postnatal confirmation of zygosity with DNA analysis are warranted before definitive conclusions can be drawn and guidelines produced. In order to provide adequate pre-conceptional counselling, it is important to monitor the incidence of monozygotic twins in both natural and assisted conceptions. We suggest building a national multiple pregnancy database based on accurate diagnosis of zygosity.

Key words: monozygotic and monochorionic twins / in vitro fertilization / intracytoplasmic sperm injection / blastocyst / assisted hatching

Introduction

Although assisted reproductive technology has improved the chances for many subfertile couples to achieve a pregnancy, it has resulted in an increased incidence of multiple births, the majority of which are dizygotic twins due to the replacement of more than one embryo. It is estimated that there is at least a 2-fold rise in the incidence of monozygotic twinning after assisted reproductive techniques, with some studies (Wenstrom et al., 1993; Blickstein et al., 1999; Hulvert et al., 1999; Behr et al., 2000; Saito et al., 2000; Milki et al., 2003; Jain et al., 2004; Frankfurter et al., 2004) reporting an incidence seven to eight times higher than natural conception rate. The reason for the increased incidence of monozygotic twins after assisted reproductive techniques has been a matter of debate for a long time without any definitive explanation. Most of the available hypotheses fail to explain the mechanism of zygotic splitting and which structure is likely to control this phenomenon. Theoretically, the increased monozygotic twinning rate after assisted
reproductive techniques could be due to one or more of the following:

(i) A small proportion of oocytes might have an inborn propensity to undergo splitting upon fertilization leading to the constant prevalence of spontaneous monozygotic conceptions among different populations. Ovarian stimulation may increase the number of available splitting-prone oocytes and consequently increase the risk of monozygotic twins (Blickstein and Keith, 2007).

(ii) Alterations of the zona pellucida. The zona maintains embryonic structure and integrity prior to the formation of intercellular junctions between the blastomers. If the zona is damaged or prematurely breached, then the blastomers of the early cleavage embryo could separate to form two (or more) fetuses. It is possible that human IVF embryos are more prone to zygotic splitting because of reduced embryonic integrity, perhaps, for example, as a result of late or reduced expression of tight junctions (Alikani et al., 1994).

(iii) Hardening of the zona pellucida as a result of extended exposure to culture media before blastocyst transfer may result in pinching of the inner cell mass at the time of hatching. This may result in splitting of the inner cell mass and the consequent development of two fetal plates (Behr et al., 2000).

(iv) Changes in the culture media like absence of growth factors or cytokines and presence of high level free radicals. This can induce apoptosis leading to disruption of the inner cell mass at the time of hatching, thus resulting in zygotic splitting (Ménézo and Sakkas, 2002; Milki et al., 2003).

(v) It has been suggested that in case of double implantation, continuation of pregnancy is more likely, because embryos are selected from a better cohort of embryos (Tummers et al., 2003). Therefore, when two good-quality embryos are transferred, patients have a higher chance of multiple implantations and once there are multiple implantations, they have lower chance of pregnancy loss, thus leading to multiple gestations (Lambers et al., 2007). This phenomenon can increase the random event of monozygotic twinning with multiple implanted embryos (Lambers et al., 2008).

The monozygotic twins are traditionally identified by Weinberg’s differential rule (Weinberg, 1902) from the twin live births. This method of diagnosis of monozygosity, which estimates the rates from the number of unlike-sex twins, was also adopted in identification of monozygotic twins after assisted reproductive techniques. Early first trimester ultrasound scan can identify approximately two-thirds of monozygotic twins. The visualization of uteri containing more than one fetus, either in one sac (monochorionic monamniotic; MC MA) or in two sacs separated by a septum (monochorionic diamniotic; MC DA) is suggestive of monozygotic twins. Monozygotic twinning is also implied when the number of gestational sacs or fetal heart movements exceeds the number of transferred embryos into the uterus following IVF with or without intracytoplasmic sperm injection (ICSI). The true incidence of monozygotic twins is difficult to estimate as a substantial proportion of like-sexed dizygotic twins cannot be differentiated from dichorionic monozygotic twins by either ante-partum ultrasonography or postpartum clinical examination (daCosta et al., 2001). The definitive diagnosis of monozygosity is only possible by DNA analysis of all the twins born (live births and still births).

A major obstacle in evaluating the impact of assisted reproductive techniques on monozygotic twinning is the overall low incidence of monozygotic twins. Monozygotic twinning is a relatively rare finding. Very large studies including more than 10,000 cases are needed to achieve satisfactory statistical power. In an attempt to reach a more realistic estimation of the incidence of monozygotic twinning after assisted reproductive techniques and investigate its relationship with various assisted reproductive techniques modalities, we have conducted this systematic review and meta-analysis.

Materials and Methods

Objective

To estimate the risk of monozygotic twinning after various assisted reproductive techniques.

Types of studies, inclusion and exclusion criteria

We included all types of studies that reported on the incidence of monozygotic twins in relation to the total number of pregnancies (single and multiple) achieved after assisted reproductive techniques. The quality of all studies was assessed with respect to the study design, the inclusion and exclusion criteria and the methods used for diagnosis of zygosity. The primary outcome of interest was the incidence of monozygotic twin pregnancies after various assisted reproductive techniques. The criteria for identification of monozygotic twins were defined before undertaking the literature search, as follows: (i) Visualization of a gestational sac containing two fetuses, either in one sac (MC MA) or in two sacs separated by a septum (MC DA) by ultrasound scan at 6–13 weeks gestation, (ii) The number of gestational sacs or fetal heart movements seen on the ultrasound scans exceeding the total number of transferred embryos, (iii) Twin pregnancies diagnosed following single-embryo transfer (SET) or (iv) Estimate of monozygotic twins at birth by Weinberg’s method.

The following studies were excluded from our analysis: (i) those failing to report monozygotic twins either as clinical pregnancies or live births, (ii) those failing to mention the method of diagnosis or confirmation of zygosity after live births or (iii) those reporting on the incidence of monozygotic twins as a proportion of multiple pregnancies rather than total number of pregnancies.

Literature search

We searched MEDLINE (1966–July 2007), EMBASE (1974–July 2007) and SCISEARCH (1974–July 2007) for all the relevant studies. The search strategy used terms such as monozygotic, monochorionic, twins, multiple pregnancies, assisted reproduction, in vitro fertilization, intracytoplasmic sperm injection, blastocyst transfer, zona manipulation, assisted hatching and ovulation induction. We also searched the Cochran Library, the Intercolligate Study Institute (ISI) Proceedings for conference abstracts, the International Standard Randomized Controlled Trial Number (ISRCTN) Register and Meta-register for RCT (mRCT) for ongoing and archived randomized controlled trials using the same key words. The references of retrieved articles together with the proceedings of relevant conferences were hand-searched in order to identify other potentially eligible studies for inclusion in the analysis missed by the initial search or any unpublished data. Articles frequently cited were
Monozygotic twins after assisted reproductive techniques

used in the Science Citation Index to identify additional citations. No language restrictions were placed in any of the searches.

The literature search, inclusion and exclusion criteria, quality of studies and extraction of data were independently undertaken and verified by two investigators (S.V. and T.G.). The results were then compared and, in case of discrepancies, a consensus was reached with the involvement of a third investigator (L.G.N.).

Data collection

Descriptive tables for population and study characteristics of all eligible studies were generated. For each eligible study we recorded the name of the first author, publication year, sample size, the characteristics of the study, the mean number of embryos replaced, laboratory techniques and methods used for diagnosis of monozygotic twinning. We then generated separate tables for the purpose of the meta-analysis with respect to (i) monozygotic twin pregnancies in natural and assisted conceptions, (ii) monozygotic twin pregnancies resulting from different assisted reproductive techniques including IVF, ICSI, frozen embryo transfer (FET), assisted hatching and blastocyst transfer. In order to simplify the data and avoid exclusion of many trials, assisted hatching was considered as a dominant treatment modality whenever combined with any other modalities. Whether the embryos were fresh or frozen, Day 2–3 or Day 5–6 and whether they were developed after IVF with or without ICSI, as long as assisted hatching was performed, the resulting pregnancy (single or multiple) was added to the assisted hatching group. Similarly, blastocyst transfer was considered a dominant treatment modality whether it resulted from fresh or frozen embryos, IVF with or without ICSI. Studies were not included in the subgroup analysis if different assisted reproductive techniques were combined at random without reporting the exact number of cycles involved in each technique. For instance, if the author stated that ICSI was performed with or without assisted hatching and did not mention the percentage of patients who had ICSI only and those who had both ICSI and assisted hatching, subgroup analysis was not possible. All authors were contacted in an attempt to obtain missing and/or additional data.

Statistical analysis

Using Stats Direct software (version 2.6.5; Tidestone Formula One®, 2005, Tidestone Technologies Inc., UK, http://www.statsdirect.com), both fixed and random effects models were used to calculate the pooled proportions of monozygotic twinning and the 95% confidence intervals (CI). Cochran Q and I² tests were used to assess the heterogeneity between the studies and to assess the heterogeneity beyond the chance.

Results

A total of 1368 citations were initially identified after the electronic search and 1331 citations were excluded after screening the titles and/or abstracts. A total of 37 publications were identified and scrutinized in full text (Fig. 1). Ten studies were excluded from the meta-analysis (Supplementary material, Table S1). Of those, three studies (Derom et al., 1993, 2006; Tong et al., 1997) reported on the ratio of monozygotic to dizygotic twins without actually reporting the monozygotic twin rate. Some studies (Derom et al., 1993; Tong et al., 1997; Abusheika et al., 2000) did not report the total number of pregnancies (single and multiple) after assisted reproductive techniques, and therefore it was not possible to calculate the incidence of monozygotic twinning. Five studies (Meldrum et al., 1998; Gerris et al., 1999; Sheiner et al., 2001; Cassuto et al., 2003; Day et al., 2005) did not report the method of diagnosis of zygosity and it was not clear whether the reported monozygotic twins were live births or clinical pregnancies and, when authors were contacted they did not reply. One study (Slotnick and Ortega, 1996) reported only on MC MA twins rather than monozygotic twin pregnancies.

Table I summarizes the characteristics of the included studies that reported the number of monozygotic twins in natural and assisted conceptions along with the method used for determination of zygosity. Twenty-seven studies were incorporated in the meta-analysis, including 21 retrospective analyses, one prospective observational study, four population-based surveys and one sequential cohort. All articles were published between 1993 and 2007. Two population-based surveys reported on monozygotic live birth twins after both natural and assisted conceptions (Derom et al., 1987; Tandberg et al., 2007). Though the rate of monozygotic twins was nearly constant at 0.4% in natural conception, it varied between studies reporting on assisted conceptions from 0.2 to 12.5%. The average number of embryos transferred varied between studies from 1 to 3.6 embryos per cycle. Only three studies (Blickstein et al., 1999, 2003; Saito et al., 2000) reported on the incidence of monozygotic twins after SET, with a total of 1850 pregnancies. In one of those studies (Blickstein et al., 1999), there were four monozygotic twins out of 82 IVF pregnancies and none out of 94 ICSI pregnancies. In another study (Saito et al., 2000), the monozygotic twins rate was not different between pregnancies that followed conventional IVF and IVF with
ICSI (1.5 and 1.6%, respectively) but was very high (22.7%) after assisted hatching. Data from the Human Fertilization and Embryology Authority (HFEA) involving SET during the period 1991–1998 (Blickstein et al., 2003) did not provide information on the techniques used. However, no difference was found in monozygotic twinning rate between fresh and FETs.

The diagnosis of monozygotic live birth twins was made after assisted conception in five studies. Of those, three (Edwards et al., 1986; Pinborg et al., 2004; Tandberg et al., 2007) estimated the rate of monozygotic live birth twins from the total number of live birth twins by using Weinberg’s differential rule. Wenstrom et al. (1993) reported on clinical pregnancies that resulted in live births and were confirmed by placental histology, while the population-based survey by Derom et al. (1987) confirmed the diagnosis of the monozygotic twins by post-natal DNA analysis, placental histology and determination of the ABO blood groups.

The diagnosis of monozygotic twin clinical pregnancies was based on first trimester ultrasound scan in 19 studies. Of those, some (Hu et al., 1996; Hershlag et al., 1999; Hulvert et al., 1999; Behr et al., 2000; Tarlatzis et al., 2002; Milki et al., 2003; Jain et al., 2004; Moayeri et al., 2007) made their diagnosis by determination of mono-chorionicity while others (Alikani et al., 1994, 2003; Rijnders et al., 1998; Domitrz et al., 1999; Blickstein et al., 2003) used TVS plus PH.

### Table 1 MZT in natural and assisted conceptions

<table>
<thead>
<tr>
<th>First author/year</th>
<th>Country</th>
<th>Type of study</th>
<th>Year</th>
<th>Average number of embryos transferred</th>
<th>Total pregnancies</th>
<th>MZT, n (%)</th>
<th>Method of diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Natural conceptions</strong></td>
<td></td>
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</tr>
<tr>
<td>1. Derom et al. (1987)</td>
<td>Belgium</td>
<td>PBS</td>
<td>1987</td>
<td>NA</td>
<td>100 052</td>
<td>449 (0.44)</td>
<td>Weinberg’s method</td>
</tr>
<tr>
<td>2. Tandberg et al. (2007)</td>
<td>Norway</td>
<td>PBS</td>
<td>2007</td>
<td>NA</td>
<td>2 181 698</td>
<td>9529 (0.43)</td>
<td>Weinberg’s method</td>
</tr>
<tr>
<td><strong>Assisted conception—live births</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1. Edwards et al. (1986)</td>
<td>UK</td>
<td>R</td>
<td>1986</td>
<td>NR</td>
<td>320</td>
<td>0 (0)</td>
<td>Weinberg’s method</td>
</tr>
<tr>
<td>4. Derom et al. (1987)</td>
<td>Belgium</td>
<td>PBS</td>
<td>1987</td>
<td>NR</td>
<td>1624</td>
<td>18 (1.1)</td>
<td>DNA/PH/ABO</td>
</tr>
<tr>
<td>5. Wenstrom et al. (1993)</td>
<td>USA</td>
<td>R</td>
<td>1993</td>
<td>4</td>
<td>218</td>
<td>7 (3.2)</td>
<td>PH</td>
</tr>
<tr>
<td><strong>Assisted conception—clinical pregnancies (diagnosed by early trimester ultrasound)</strong></td>
<td></td>
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</tr>
<tr>
<td>1. Hershlag et al. (1999)</td>
<td>USA</td>
<td>R</td>
<td>1999</td>
<td>NR</td>
<td>391</td>
<td>8 (2.0)</td>
<td>TVS + PH</td>
</tr>
<tr>
<td>2. Frankfurter et al. (2004)</td>
<td>USA</td>
<td>SC</td>
<td>2004</td>
<td>NR</td>
<td>111</td>
<td>8 (7.2)</td>
<td>TVS + PH</td>
</tr>
<tr>
<td>3. Alikani et al. (1994)</td>
<td>USA</td>
<td>R</td>
<td>1994</td>
<td>NR</td>
<td>737</td>
<td>6 (0.8)</td>
<td>TVS</td>
</tr>
<tr>
<td>4. Hu et al. (1996)</td>
<td>USA</td>
<td>R</td>
<td>1996</td>
<td>3.5</td>
<td>109</td>
<td>2 (1.8)</td>
<td>TVS</td>
</tr>
<tr>
<td>5. Rijnders et al. (1998)</td>
<td>Holland</td>
<td>R</td>
<td>1998</td>
<td>NR</td>
<td>1806</td>
<td>13 (0.7)</td>
<td>TVS</td>
</tr>
<tr>
<td>6. Domitrz et al. (1999)</td>
<td>Poland</td>
<td>R</td>
<td>1999</td>
<td>NR</td>
<td>549</td>
<td>6 (1.1)</td>
<td>TVS</td>
</tr>
<tr>
<td>7. Blickstein et al. (1999)</td>
<td>Israel</td>
<td>R</td>
<td>1999</td>
<td>1</td>
<td>176</td>
<td>4 (2.3)</td>
<td>SET</td>
</tr>
<tr>
<td>8. Hulvert et al. (1999)</td>
<td>Poland</td>
<td>R</td>
<td>1999</td>
<td>NR</td>
<td>521</td>
<td>13 (2.5)</td>
<td>TVS</td>
</tr>
<tr>
<td>9. Schieve et al. (2000)</td>
<td>USA</td>
<td>PBS</td>
<td>2000</td>
<td>3.1</td>
<td>11 247</td>
<td>22 (0.2)</td>
<td>TVS</td>
</tr>
<tr>
<td>10. Saito et al. (2000)</td>
<td>Japan</td>
<td>P</td>
<td>2000</td>
<td>1</td>
<td>279</td>
<td>9 (3.2)</td>
<td>SET</td>
</tr>
<tr>
<td>11. Behr et al. (2000)</td>
<td>USA</td>
<td>R</td>
<td>2000</td>
<td>NR</td>
<td>199</td>
<td>10 (5.0)</td>
<td>TVS</td>
</tr>
<tr>
<td>12. Sills et al. (2000)</td>
<td>USA</td>
<td>R</td>
<td>2001</td>
<td>3.6</td>
<td>1911</td>
<td>23 (1.2)</td>
<td>TVS</td>
</tr>
<tr>
<td>13. Schachtet al. (2001)</td>
<td>Israel</td>
<td>R</td>
<td>2001</td>
<td>2.5</td>
<td>731</td>
<td>7 (1.0)</td>
<td>TVS</td>
</tr>
<tr>
<td>14. daCosta et al. (2001)</td>
<td>Brazil</td>
<td>R</td>
<td>2001</td>
<td>3.2</td>
<td>943</td>
<td>11 (1.2)</td>
<td>TVS</td>
</tr>
<tr>
<td>15. Tarlatzis et al. (2002)</td>
<td>Greece</td>
<td>R</td>
<td>2002</td>
<td>2.98</td>
<td>48</td>
<td>6 (12.5)</td>
<td>TVS</td>
</tr>
<tr>
<td>16. Elzur et al. (2004)</td>
<td>Israel</td>
<td>R</td>
<td>2004</td>
<td>NR</td>
<td>1066</td>
<td>10 (0.9)</td>
<td>TVS</td>
</tr>
<tr>
<td>17. Blickstein et al. (2003)</td>
<td>Israel</td>
<td>R</td>
<td>2003</td>
<td>1</td>
<td>1395</td>
<td>25 (1.8)</td>
<td>SET</td>
</tr>
<tr>
<td>18. Milki et al. (2003)</td>
<td>USA</td>
<td>R</td>
<td>2003</td>
<td>NR</td>
<td>554</td>
<td>18 (3.2)</td>
<td>TVS</td>
</tr>
<tr>
<td>19. Alikani et al. (2003)</td>
<td>UK</td>
<td>R</td>
<td>2003</td>
<td>2.78</td>
<td>4305</td>
<td>81 (1.8)</td>
<td>TVS</td>
</tr>
<tr>
<td>20. Jain et al. (2004)</td>
<td>USA</td>
<td>R</td>
<td>2004</td>
<td>2.5</td>
<td>85</td>
<td>6 (7.1)</td>
<td>TVS</td>
</tr>
<tr>
<td>21. Wright et al. (2004)</td>
<td>USA</td>
<td>PBS</td>
<td>2004</td>
<td>2.86</td>
<td>39 198</td>
<td>226 (0.6)</td>
<td>TVS</td>
</tr>
<tr>
<td>22. Moayeri et al. (2007)</td>
<td>USA</td>
<td>R</td>
<td>2007</td>
<td>NR</td>
<td>932</td>
<td>19 (2.0)</td>
<td>TVS</td>
</tr>
</tbody>
</table>

MZT, monozygotic twins; P, prospective observational study; R, retrospective analysis; SC, sequential cohort; PBS, population-based survey; TVS, first trimester transvaginal ultrasound; SET, single-embryo transfer; PH, placental histology; NR, not reported; NA, not applicable; DNA, DNA analysis; ABO, blood groups.
Monozygotic twins after assisted reproductive techniques

Discussion

Data from this meta-analysis demonstrate that the risk of monozygotic twins in women undergoing assisted reproduction is 0.9%. This is a 2-fold increase in comparison with monozygotic twins in natural conceptions (0.4%). Of interest, blastocyst transfer seems to be associated with even higher rate of monozygotic twins (1.7%).

About one-quarter of pregnancies after IVF with or without ICSI are twins, the majority are dizygotic due to the transfer of more than one embryo. Multiple pregnancies are the single most serious risk to the health and welfare of the children born after assisted reproductive techniques. It is well established that the rate of dizygotic twins can be substantially reduced by adopting an elective single-embryo transfer (e-SET) policy. The debate about patient’s selection for e-SET remains open.

Monozygotic twinning is an uncommon phenomenon, the etiology of which is still unclear. It carries additional risks above and beyond those of multiple pregnancies including higher perinatal mortality and morbidity, and increased risk of developmental anomalies, prematurity and discordant growth (Schinzel et al., 1979; Colburn and Pasquale, 1982; Fowler et al., 1991). Several studies in the literature have suggested increased incidence of monozygotic twins after assisted conception when compared with natural conception. As yet, there are insufficient data to support this conclusion. The risk of monozygotic twins can only be reliably identified following SET or by DNA analysis of the twins born from women who had more than one embryo transferred. Traditionally, estimation of the rates of monozygotic and dizygotic live birth twins is by Weinberg’s differential rule (Weinberg, 1902). According to this rule, the rate of dizygotic twin can be calculated by doubling the number of unlike-sex twins.

Using Weinberg’s differential method in natural conception, the incidence of monozygotic twins at birth is estimated to be 0.35–0.4% (Bulmer, 1970; Tandberg et al., 2007). However, Weinberg’s differential rule is based on the assumptions that the number of males equals the number of females at birth (halved sex ratio, where sex ratio equals males/females). The Weinberg’s assumption has been questioned by many investigators (James 1979, 1980, 1992; Machin et al., 1995). Furthermore, Weinberg’s method was also based on live birth data following natural conceptions without including miscarriages or prenatal losses, hence, underestimating the true incidence of monozygotic twins (Bulmer, 1970). More importantly, there is some evidence to suggest that the sex ratio may vary in assisted conceptions, suggesting that Weinberg’s method would not be very reliable in estimating monozygotic twinning in assisted conception. A prospective observational study (Dumoulin et al., 2005) found a higher sex ratio for IVF-derived blastocysts than for ICSI-derived blastocysts (0.55 versus 0.50). A Danish follow-up study (Fedder et al., 2007) reported a lower sex ratio in children born following ICSI using epididymal or testicular sperm than in children conceived after conventional IVF (0.45 versus 0.53). Other retrospective studies reported significantly higher sex ratio after blastocyst compared with early cleavage stage transfer (Ménézo et al., 1999; Luna et al., 2007).

However, recently, Fellman and Eriksson (2006) critically scrutinized Weinberg’s differential method and compared it with a new variance formula for monozygotic twinning rates in Finnish and Swedish birth registers. They concluded that the Weinberg’s differential rule is rather robust and reliable in estimation of monozygotic twins despite its simplicity.

With the exception of three studies (Blickstein et al., 1999, 2003; Saito et al., 2000), all studies included in our review have attempted to calculate the incidence of monozygotic twins in a group of women who had transfer of more than one embryo. This can lead
### Table II  MZT after assisted reproductive technologies

<table>
<thead>
<tr>
<th>Study</th>
<th>Ovulation induction n/N = MZT (%)</th>
<th>IVF n/N = MZT (%)</th>
<th>Frozen-embryo transfer n/N = MZT (%)</th>
<th>ICSI n/N = MZT (%)</th>
<th>Blastocyst transfer n/N = MZT (%)</th>
<th>Assisted hatching n/N = MZT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Derom et al. (1987)</td>
<td>18/1624 (1.1)</td>
<td></td>
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<tr>
<td>2. Wenstrom et al. (1993)</td>
<td>4/72 (5.5)</td>
<td>0/14 (0.0)</td>
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<tr>
<td>3. Hu et al. (1996)</td>
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<tr>
<td>4. Rijnders et al. (1998)</td>
<td>10/1695 (0.6)</td>
<td></td>
<td></td>
<td></td>
<td>3/111 (2.7)</td>
<td>2/109 (1.8)</td>
</tr>
<tr>
<td>5. Blickstein et al. (1999)</td>
<td>4/82 (4.9)</td>
<td></td>
<td></td>
<td></td>
<td>0/94 (0.0)</td>
<td></td>
</tr>
<tr>
<td>6. Hulvert et al. (1999)</td>
<td>3/322 (0.9)</td>
<td>3/76 (3.9)</td>
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<td>5/90 (5.5)</td>
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<td>7. Herslag et al. (1999)</td>
<td>0/141 (0.0)</td>
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<td>8. Schieve et al. (2000)</td>
<td>9/6682 (0.1)</td>
<td></td>
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<td>13/4565 (0.3)</td>
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<td>9. Saito et al. (2000)</td>
<td>3/196 (1.5)</td>
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<td>1/61 (1.6)</td>
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<td>5/22 (22.7)</td>
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<td>10. Behr et al. (2000)</td>
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<td>11. Sills et al. (2000)</td>
<td>3/157 (1.9)</td>
<td>1/73 (1.3)</td>
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<td>1/160 (0.6)</td>
<td></td>
<td>18/1521 (1.18)</td>
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<td>1/139 (0.7)</td>
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<td>13. doCosta et al. (2001)</td>
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<td>15. Elizur et al. (2004)</td>
<td>4/389 (1.0)</td>
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<td>16. Milk et al. (2003)</td>
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<td>11/197 (5.6)</td>
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<td>3/155 (2.0)</td>
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<td>19. Wright et al. (2004)</td>
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<td>20. Moayen et al. (2007)</td>
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n, number of monozygotic twins (MZT); N, total number of pregnancies or live births; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.
significant underestimation of the incidence of monozygotic twins after assisted reproductive techniques. While it is true that monozygotic twins are identical and dichorionic unlike-sexed twins are dizygotic, dichorionic like-sexed twins may be monozygotic or dizygotic. Using blood group typing and physical characteristics or DNA studies after birth, several investigators reported that 22–46% of monozygotic twins are dichorionic (Potter, 1963; Bulmer, 1970; Fujiwara and Froehlich, 1971; Corey et al., 1979; Machin et al., 1995). The diagnosis of monozygotic twinning, when the number of gestational sacs or fetal hearts seen on ultrasound exceeds the total number of transferred embryos, underestimates the incidence of zygotic splitting. When one of the two transferred embryos is lost and the other splits into dichorionic like-sexed twins, the number of fetuses will not exceed the number of transferred embryos and the diagnosis of monozygotic twins will be missed. This can explain why the majority of the studies that diagnosed monozygotic twins by other methods than ultrasound reported a risk of monozygotic twins higher than 0.9%.

There is a wide variation in the reported incidence of monozygotic twins after assisted reproductive techniques. This could be due to

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**Figure 2** (A) MZT-CP diagnosis by ultrasound. Pooled proportion (fixed effects) = 0.007 (95% CI = 0.006–0.008), pooled proportion (random effects) = 0.017 (95% CI = 0.012–0.022), Cochran Q = 268.54; P < 0.0001; I² (inconsistency) = 92.9% (95% CI = 90.9–94.3). (B) MZT-CP confirmed by PH. Pooled proportion (fixed effects) = 0.031 (95% CI = 0.018–0.048), pooled proportion (random effects) = 0.042 (95% CI = 0.006–0.11), Cochran Q = 6.00; P = 0.0143; I² (inconsistency) = % (95% CI = ±%). (C) MZT-LB (Weinberg’s method). Pooled proportion (fixed effects) = 0.013 (95% CI = 0.011–0.015), pooled proportion (random effects) = 0.011 (95% CI = 0.006–0.017), Cochran Q = 17.70; P = 0.0001; I² (inconsistency) = 88.7% (95% CI = 57.7–94.5%). (D) MZT-LB confirmed by DNA ± PH ± ABO. Pooled proportion (fixed effects) = 0.013 (95% CI = 0.009–0.019), Cochran Q = 4.83; P = 0.0279; I² (inconsistency) = % (95% CI = ±%), pooled proportion (random effects) = 0.020 (95% CI = 0.004–0.047).
Figure 3 (A) Ovulation induction. Pooled proportion (fixed effects) = 0.012 (95% CI = 0.007–0.017), pooled proportion (random effects) = 0.012 (95% CI = 0.007–0.017), Cochran Q = 0.478832; P = 0.489; I² (inconsistency) = % (95% CI = %–%). (B) IVF. Pooled proportion (fixed effects) = 0.0035 (95% CI = 0.0029–0.0041), pooled proportion (random effects) = 0.008895 (95% CI = 0.005–0.013), Cochran Q = 59.07; P < 0.0001; I² (inconsistency) = 79.7% (95% CI = 64%–86.7%). (C) ICSI. Pooled proportion (fixed effects) = 0.0097 (95% CI = 0.005–0.016), pooled proportion (random effects) = 0.011579 (95% CI = 0.004–0.022), Cochran Q = 6.871; P = 0.2304; I² (inconsistency) = 27.2% (95% CI = 0–72.9%). (D) FET. Pooled proportion (fixed effects) = 0.03 (95% CI = 0.0096–0.0612), pooled proportion (random effects) = 0.03 (95% CI = 0.0096–0.0612), Cochran Q = 0.939; P < 0.0001; I² (inconsistency) = 0% (95% CI = 0–72.9%). (E) Blastocyst transfer. Pooled proportion (fixed effects) = 0.0167 (95% CI = 0.014–0.02), pooled proportion (random effects) = 0.051 (95% CI = 0.029–0.081), Cochran Q = 57.67; P < 0.0001; I² (inconsistency) = 86.1% (95% CI = 74.9–91.1%). (F) Assisted hatching. Pooled proportion (fixed effects) = 0.007 (95% CI = 0.005–0.09), pooled proportion (random effects) = 0.029 (95% CI = 0.013–0.05), Cochran Q = 69.29; P < 0.0001; I² (inconsistency) = 89.9% (95% CI = 82.7–93.3%).
variation in the sample size with a tendency for underpowered studies (Tarlatzis et al., 2002; Frankfurter et al., 2004; Jain et al., 2004) to report higher incidence than the larger population-based trials (Schieve et al., 2000; Wright et al., 2004). The disparity between studies with respect to number of embryos transferred and the technique involved could also contribute to the wide variation in the reported incidence of monozygotic twins.

Using a proportion meta-analysis, we found a higher incidence of monozygotic twins after assisted conception as compared with natural conception. The incidence of monozygotic twins varied according to the method used for the diagnosis of zygosity in assisted conception, from 0.7 to 3.1%. This variation is more likely to be related to the sample size for different methods used for diagnosis of monozygotic twins rather than the accuracy of the method itself. Unfortunately, only few studies (Blickstein et al., 1999, 2003; Saito et al., 2000) reported on the monozygotic twinning rate after SET with small number of pregnancies, the majority being reported from HFEA (Blickstein et al., 2003), with no information on the technique used or the embryonic stage at the time of transfer. Therefore, it is not known whether the high incidence of monozygotic twins after SET was related to any specific assisted reproductive technique.

Similarly, the higher incidence of monozygotic twin clinical pregnancies, which were later confirmed by placental histology, is not a reliable estimation as it was based only on two small studies (Hershlag et al., 1999; Frankfurter et al., 2004) and the placental histology was only performed in pregnancies that resulted in live births.

We found a higher monozygotic twinning rate in couples who underwent blastocyst transfer or ICSI than those who underwent conventional IVF or assisted hatching. Although the number and size of studies reporting on monozygotic twinning after blastocyst transfer was larger compared with that of ICSI, there was significant heterogeneity between the studies reporting on blastocyst transfer compared with those reporting on ICSI. While the monozygotic twinning rate after FET was 3.0%, the total number of reported pregnancies was very small to make this estimation reliable and conclusive.

The connection between monozygotic twinning and assisted hatching is highly speculative. Theoretically, artificial breach of the zona during the process of assisted hatching can lead to herniation and subsequent splitting of the inner cell mass resulting in monozygotic twinning. The rate of monozygotic twinning probably depends on the size of the hole in the zona. The expertise and the method of assisted hatching employed can therefore affect the incidence of monozygotic twinning. While both assisted hatching and ICSI involve an artificial breach of the zona pellucida, they differ markedly in the size of the associated defect. Zona opening formed by assisted hatching is ~25–30 μm in diameter, while the puncture site following ICSI is much smaller (7–8 μm in diameter) (Sills et al., 2000). The size and the number of zona openings have been shown to influence hatching and trophoblast outgrowth in mouse embryos (Cohen and Feldberg, 1991). Interestingly, this meta-analysis found a higher incidence of monozygotic twinning after ICSI than after assisted hatching.

Transfer of the embryos at the blastocyst stage evolved as a method to identify developmentally competent embryos to help reducing the number of transferred embryos, thus reducing the risk of fraternal multiple pregnancies without compromising pregnancy success rates (Gardner et al., 1998). Several mechanisms have been suggested to explain the observed increased monozygotic twinning rate with blastocyst transfer including extended culture time (daCosta et al., 2001; Sheiner et al., 2001) and culture media composition (Ménézo and Sakkas, 2002; Cassuto et al., 2003). Most of the studies reviewed

![Figure 4 Monozygotic twins in natural conception (Weinberg method).](image)

*Figure 4* Monozygotic twins in natural conception (Weinberg method).

Pooled proportion (fixed effects) = 0.0040 (95% CI = 0.0040–0.0040); Pooled proportion (random effects) = 0.0044 (95% CI = 0.004–0.004); Cochran Q = 0.33; P = 0.8448; I² (inconsistency) = 0% (95% CI = 0–72.9%).
had shown increased incidence of monozygotic twinning after blastocyst transfer. However, there is great variation between the studies in the reported incidence of monozygotic twinning. Apart from the sample size, we could not find any other reason to explain the difference in monozygotic twinning rate between these studies. The population-based survey by Wright et al., (2004) reported the lowest rate of monozygotic twinning after blastocyst transfer. Other authors (Moayeri et al., 2007) compared the monozygotic twinning rate after blastocyst transfer with previously published results from the same institute (Milki et al., 2003), at the time when blastocyst transfer had just been introduced into their practice. They showed a significant reduction in the monozygotic twinning rate from 5.6 to 2.3% and explained this reduction in monozygotic twinning rate by a possible improvement in culture systems.

One of the limitations of this meta-analysis is that most of the included studies used first trimester ultrasound data to identify cases of monozygotic twinning based on the number of fetuses relative to the number of embryos transferred, which might have led to underestimation of monozygotic twins. There is no other method to diagnose monozygotic twin pregnancies in women who had more than one embryo transferred. Using post-natal DNA studies to confirm zygosity will lead to further underestimation as all pregnancies ending in miscarriage or fetal death in utero will be missed. The percentages of presumed monozygotic twinning reported in this review should not be used to assume absolute risk for monozygotic twinning.

In conclusion, monozygotic twinning is an uncommon phenomenon in both natural and assisted conceptions. The rate of monozygotic twins is 2.25 times higher in assisted conceptions than natural conceptions. The incidence of monozygotic twinning in natural pregnancy is likely to be underestimated even though the rate of monozygotic twinning from SET is higher than the rate of monozygotic twinning in natural conceptions. Some assisted reproductive techniques such as blastocyst transfer and ICSI seem to carry higher risk of monozygotic twinning than the others. Larger studies reporting on monozygotic twinning following SET or after post-natal confirmation of zygosity with DNA analysis are warranted before definitive conclusions can be drawn and guidelines produced. In order to provide adequate preconception counseling, it is important to monitor the incidence of monozygotic twins after natural and assisted conception. Henceforth, we suggest building a national multiple pregnancy database based on accurate diagnosis of zygosity in early pregnancy and its confirmation after the delivery.

**Supplementary material**

Supplementary material is available at HUMUPD Journal online.

**Funding**

This work was not supported financially by any internal or external source.

**References**


Monozygotic twins after assisted reproductive techniques


Rijnders PM, van Os HC, Jansen CAM. Increased incidence of monozygotic twinning following the transfer of blastocysts in human IVF/ICSI. Fertil Steril 1998; 70:513–516.


Submitted on April 13, 2008; resubmitted on September 2, 2008; accepted on September 16, 2008.