Monozygotic twinning after assisted reproductive techniques: a phenomenon independent of micromanipulation

Morey Schachter¹, Arieh Raziel, Shevach Friedler, Devorah Strassburger, Orna Bern and Raphael Ron-El

IVF and Infertility Unit, Assaf Harofeh Medical Center, Zerifin, The Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

¹To whom correspondence should be addressed at: IVF and Infertility Unit, The Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, Zerifin, Israel 70300. E-mail: ivfdoc@asaf.health.gov.il

A 3 year retrospective analysis was conducted of pregnancies achieved after various assisted reproductive treatment modalities in our infertility practice, to calculate and compare the rates of monozygotic twinning (MZT). A total of 731 pregnancies achieved after various assisted reproduction treatments were reviewed. Gonadotrophin therapy for induction of ovulation and controlled ovarian hyperstimulation (COH) yielded 129 clinical pregnancies. Conventional IVF yielded 139 pregnancies. IVF and intracytoplasmic sperm injection (ICSI) with or without assisted hatching (AH) yielded 463 pregnancies, all during the same time period. The rates of multiple pregnancy (monozygotic and dizygotic) twins and triplets were recorded. MZT was found in 1.5% of ovulation induction or COH pregnancies (2/129). The incidence of MZT after conventional IVF was 0.72% (1/139). After IVF–ICSI/AH, MZT was found in 0.86% (4/463). The overall rate of MZT was 0.95% (7/731). Five cases were dizygotic triplets and two cases were monozygotic twins. We found the rate of MZT after assisted reproduction treatment increased more than two-fold over the background rate in the general population. Dizygotic triplets were found more often than monozygotic twins. The rate of MZT was consistently increased, irrespective of treatment modality or micromanipulation. This may signify that the aetiology of increased MZT after assisted reproduction is the gonadotrophin treatment rather than in-vitro conditions, micromanipulation, or multiple embryo transfer.

Key words: gonadotrophin therapy/micromanipulation/monozygotic twins/multiple pregnancy/zona pellucida

Introduction

Factors predisposing to the production of dizygotic twins are known to include maternal age, parity, race, family history of dizygotic twinning and of course treatment with drugs that induce multiple ovulation and related assisted reproductive techniques. In contrast, the factors that affect the frequency of monozygotic twinning (MZT) have been poorly characterized. Previous reports have linked the use of ovulation induction drugs to MZT (Derom et al., 1987). Other reports (Edwards et al., 1986; Alikani et al., 1994; Slotnick and Ortega, 1996; Hershlag et al., 1999; Blickstein et al., 1999; Sills et al., 2000) have calculated an increased frequency of MZT in the setting of IVF, with or without zona manipulation. One hypothesis advanced in order to explain the higher incidence of monozygosity suggested that manipulation of the zona pellucida could encourage inner cell mass herniation during hatching. Another hypothesis (Sills et al., 2000) discussed the increased incidence of twinning as simply a function of the presence of more embryos in the uterine cavity after embryo transfer.

We report our experience with monozygotic twinning after ovulation induction or assisted reproduction treatment, from different clinical settings, and advance our hypothesis as to the evolution of this situation.

Materials and methods

A total of 731 pregnancies achieved after employing various assisted reproduction procedures over a 3 year period (1997–1999) at the Assaf Harofeh Medical Center IVF and Infertility Unit were reviewed. Induction of ovulation and controlled ovarian stimulation with gonadotrophins were administered in 480 cycles to 220 patients in this time period. The mean age of these patients was 30.2 ± 6.7 years. Tubal patency (at least one open tube) was demonstrated in all patients by hysterosalpingography prior to initiating gonadotrophins. Semen analysis carried out on all patients’ partners were within normal limits (85%) or suboptimal (15%). The indications for treatment included anovulatory infertility in 72% (158 patients) and unexplained infertility in 28% (62 patients). Gonadotrophin treatment was initiated after an initial unsuccessful trial utilizing clomiphene citrate in doses ranging from 50–200 mg/day for 5 days per cycle in 2–6 previous cycles per patient. The gonadotrophins used were human...
menopausal gonadotrophin (HMG, Pergonal®; Teva, Petah Tikva, Israel) in 365 cycles (76%) and purified urinary follicle stimulating hormone (pFSH, Metrodin®; Teva) in 115 cycles (24%). Doses ranged from 75 IU/day to no more than 225 IU/day for the duration of treatment ranging from 5–18 days per cycle (median 8 days). The final stage of ovulation was induced using human chorionic gonadotrophin (HCG, Chorigon®; Teva) in all cycles, when ovarian follicles with a diameter of ≥19 mm were identified by transvaginal ultrasound. The dose of HCG varied from 5000–10000 IU injected i.m. Serum βHCG was assessed in all patients who did not menstruate by day 16 after HCG administration. At 1, 2 and 3 weeks after a positive pregnancy test was returned, transvaginal ultrasound was carried out, and the number of gestational sacs and embryos recorded. Monochorionicity can only be proven in the case of a single embryo transferred, so in effect it is the chorionicity of the pregnancy that can be demonstrated by ultrasound. However, as all previous studies did not make this distinction, for the purpose of uniformity and clarity, we used the term monochoriality where chorionicity would have been more absolutely accurate. When more than one sac or embryo was visualized, the chorionicity/monochorionicity of the embryos was assessed, using the criteria of Townsend et al. (Townsend et al., 1988) and multiple pregnancies were followed in our unit until the 9th gestational week. In early pregnancy, the determination of chorionicity by ultrasound is based mainly on the inter-twin membrane thickness, as dichorionic septae have a mean thickness of 2.4 mm as opposed to 1.4 mm in monochorionic septae (Winn et al., 1989), and a 100% accuracy rate was reported by Monteagudo et al. using these criteria at 9 gestational weeks (Townsend et al., 1988; Monteagudo et al., 1994).

During the same time period, 602 pregnancies were achieved in our IVF programme. A total of 475 cycles of conventional IVF yielded 139 clinical pregnancies (29.3%). Conventional IVF was employed in patients with tubal factor infertility or unexplained infertility after gonadotrophin therapy with intrauterine inseminations had failed. Tubal factor infertility was determined by pathological hysterosalpingography or previous laparoscopy/laparatomy, and was so determined in 211 of these 299 patients, including 39 patients with endometriosis. Ninety-five patients were diagnosed as suffering from unexplained infertility. IVF was carried out after pituitary down-regulation with gonadotrophin-releasing hormone agonists (GnRHa, Decapeptyl® 3.75 controlled release; Ferring, Malmo, Sweden), or nasal Nafarelin® 400–600 µg/day (Synarel, Delpharm, France) and HMG, using a standardized long protocol. The administration of HCG, oocyte aspiration, laboratory procedures, semen preparation, embryo handling and transfer have been described in detail (Ron-El et al., 1991). Embryo transfer was achieved in 92.4% of attempted cycles, the average number of embryos replaced was 2.4 ± 0.8 embryos per cycle. Identification of pregnancy and subsequent ultrasound examinations were as for the ovulation induction group.

Micromanipulation was employed in our IVF programme in 1438 cycles in the same time period. Intracytoplasmic sperm injection (ICSI) was used for patients with male factor infertility—defined as severe oligoasthenoteratozoospermia—or in cases of failed fertilization in a previous conventional IVF cycle with a normal ovarian response. ICSI was also employed in cases of poor response on the part of the female partner (four oocytes or less aspirated per cycle) when two (or more) previous IVF attempts had been unsuccessful. Assisted hatching (AH) using acid Tyrode’s solution (Irvine Scientific, Santa Anna, CA, USA) was employed in 327 of the 1438 ICSI cycles (22.7%). All of these treatment cycles were initiated using the same standard drug protocols, criteria for HCG administration, oocyte aspiration, laboratory procedures and semen preparation as previously described. The ICSI procedure was carried out as described by Van Steirteghem et al. (Van Steirteghem et al., 1993). Embryo transfer was achieved in 94% of attempted cycles, the average number of embryos replaced was 2.3 ± 1.2. Identification of pregnancy and subsequent ultrasound examination were as for the previously mentioned groups. Micromanipulation (ICSI and/or AH) in 1438 embryo transfer cycles yielded 463 clinical pregnancies (32.2%), the proportion of AH in conception and non-conception cycles was similar.

Statistical analyses

Variables relating to the patient or treatment characteristics were examined and compared with the overall IVF patient population treated in our centre, using Fisher’s exact test to compare rates (drug type and dose distribution) and Mann–Whitney Rank Sum test, to compare non-parametric data (age, peak oestradiol and oocytes aspirated).

Results

A total of 731 pregnancies was achieved in the three assisted reproduction arms, of these, 126 clinical multiple pregnancies were identified (Table I), giving an overall multiple pregnancy rate of 17.2%.

In the ovulation induction/COS group, the multiple pregnancy group comprised 10.8% (14/129), of these, twins comprised ten sets of dizygotic twins and one set of monozygotic twins, triplets comprised two sets of trizygotic and one set of dizygotic triplets. Therefore, the overall rate of MZT in this group was 1.5% (2/129).

The multiple pregnancy rate in the conventional IVF arm was found to be 21.5% (30/139). Twins comprised 26 sets of 30 multiple pregnancies, all of which were dizygotic. Four sets of triplets were found in this group—three were trizygotic and one dizygotic. The overall rate of MZT in this group was 0.72% (1/139).

The addition of micromanipulation (ICSI with or without AH) to IVF resulted in 463 pregnancies. Of these, 82 were multiple pregnancies (17.7%). Twins comprised 63 of the 82 multiple pregnancies, 62 were dizygotic and one was monozygotic. Triplets were diagnosed in 19 of these 463 pregnancies, 16 were trizygotic and three dizygotic. The overall rate of monozygotic twinning in the IVF–micromanipulation group was 0.86% (4/463). If the rate of monozygosity is calculated by dividing the number of monozygotic twins found by the number of implanted embryos (and not the number of pregnant patients), the ‘true’ rate of monozygosity is slightly lower, as the denominator takes into account the dizygotic twins as two implantations, although this calculation was not made in previous studies (Table I).

Nearly 80% of the 126 sets of twins and triplets reached live delivery (99/126). Precise data on all placentae and membranes were not available.

Spontaneous reduction to singleton from twins or to twins from triplets occurred in five cases. Interventional reduction of triplets to twins in two cases of trizygotic triplets led to one late abortion of all three triplets, and to one ongoing twin pregnancy, which delivered at term. Analysis of the seven cases of MZT did not disclose any specific pattern, either in patient or in treatment variables, that could be construed as indicative of a monozygotic-prone profile. Patients’ ages and indication for treatment were...
Table I. Distribution and types of multiple pregnancies in 731 pregnancies after assisted reproduction treatment

<table>
<thead>
<tr>
<th>Zygosity</th>
<th>Ovulation induction</th>
<th>Conventional IVF</th>
<th>IVF and micromanipulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>All pregnancies</td>
<td>–</td>
<td>129</td>
<td>139</td>
</tr>
<tr>
<td>Twins</td>
<td>MZT</td>
<td>1 (0.77%)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Non-MZT</td>
<td>10 (7.7%)</td>
<td>26 (18.7%)</td>
</tr>
<tr>
<td>Triplets</td>
<td>MZT</td>
<td>1 (0.78%)</td>
<td>1 (0.72%)</td>
</tr>
<tr>
<td></td>
<td>Non-MZT</td>
<td>2 (1.55%)</td>
<td>3 (2.1%)</td>
</tr>
<tr>
<td>Total multiple pregnancies</td>
<td>–</td>
<td>14/129 (10.8%)</td>
<td>30/139 (21.5%)</td>
</tr>
<tr>
<td>True rate of monozygosity(a)</td>
<td>–</td>
<td>2/141 (1.4%)</td>
<td>1/168 (0.59%)</td>
</tr>
<tr>
<td>Monozygotic pregnancy rate(b)</td>
<td>–</td>
<td>2/129 (1.5%)</td>
<td>1/139 (0.72%)</td>
</tr>
</tbody>
</table>

\(a\) True rate of monozygosity refers to the ratio of monozygotic twins to the number of implanted embryos; dizygotic (non-MZT) twins are counted twice in the denominator.

\(b\) Monozygotic pregnancy rate refers to the ratio of monozygotic twins to the number of clinical pregnancies; dizygotic (non-MZT) twins are counted once in the denominator. This is the ratio reported in previous studies.

Table II. Patients’ clinical treatment and outcome data

<table>
<thead>
<tr>
<th>Patient age</th>
<th>Diagnosis</th>
<th>Tx.</th>
<th>Supp.</th>
<th>Stim. amp</th>
<th>Mat. foll.</th>
<th>Peak oest.</th>
<th>HCG dose</th>
<th>Oocytes fertil.</th>
<th>ET</th>
<th>Lat. suppl.</th>
<th>Type of preg.</th>
<th>Membrane config.</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (24 years)</td>
<td>Anovulation</td>
<td>OI</td>
<td>None</td>
<td>PFSH (8)</td>
<td>2</td>
<td>870</td>
<td>5000</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>None</td>
<td>Dizygotic triplet</td>
<td>Delivery 31 w</td>
</tr>
<tr>
<td>2. (28 years)</td>
<td>Unex. Infert.</td>
<td>OI</td>
<td>None</td>
<td>PFSH (13.5)</td>
<td>4</td>
<td>2300</td>
<td>5000</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>None</td>
<td>Monozyg. Monochor.</td>
<td>Ongoing</td>
</tr>
<tr>
<td>3. (25 years)</td>
<td>Oligo- spermia</td>
<td>ICSI</td>
<td>None</td>
<td>Dtrp6</td>
<td>HM (36)</td>
<td>3</td>
<td>687</td>
<td>10000</td>
<td>4</td>
<td>2</td>
<td>Dizygotic triplet</td>
<td>Dizygotic biamniotic</td>
<td>Delivery 26 w</td>
</tr>
<tr>
<td>4. (28 years)</td>
<td>Obstr. Azoosp</td>
<td>ICSI</td>
<td>Dtrp6</td>
<td>HM (59)</td>
<td>8</td>
<td>1054</td>
<td>10000</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>Dizygotic triplet</td>
<td>Dichorionic biamniotic</td>
<td>Late abortion 19 w</td>
</tr>
<tr>
<td>5. (37 years)</td>
<td>Oligospermia</td>
<td>ICSI</td>
<td>Nafer</td>
<td>HM (32)</td>
<td>10</td>
<td>2275</td>
<td>5000</td>
<td>15</td>
<td>13</td>
<td>3</td>
<td>Prog.</td>
<td>Dichorionic biamniotic</td>
<td>Late abortion 20 w</td>
</tr>
<tr>
<td>6. (27 years)</td>
<td>Oligospermia</td>
<td>ICSI</td>
<td>Dtrp6</td>
<td>PFSH (50)</td>
<td>9</td>
<td>1366</td>
<td>10000</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>Prog.</td>
<td>Monozyg. Monochor.</td>
<td>Delivery 36 w</td>
</tr>
</tbody>
</table>

\(Tx. = treatment; OI = ovulation induction; Supp. = suppression; Stim. = stimulation; amp = ampoules of gonadotrophins; Mat. foll. = mature follicles; Fertil. = fertilizations; ET = embryos transferred; Lat. suppl. = luteal supplementation; Dtrp6 = triptorelin; Nafer = naferelin (nasal); oest. = oestradiol pmol/l; HCG dose = international units; Prog. = progesterone in oil i.m.; NA = not applicable; prog. = pregnancy; config. = configuration; w = gestational weeks."

Discussion

The mechanism of MZT is as yet poorly understood. In the simplest terms, monozygotic twins arise from division of the fertilized ovum at various early stages of development of the embryo. When division of the embryonic cell mass occurs earlier than 72 h after fertilization, biamniotic bichorionic monozygotic twins will evolve. Division of the embryo after the inner cell mass has formed, between day 4 and day 8, will give rise to biamniotic monochorionic twins. Splitting after day 8 will lead to mono-amniotic monochorionic twins. This process probably begins with the protrusion of some tropho-ectoderm cells through a small opening in the zona pellucida (ZP). Some cells of the inner cell mass may then break off in utero to form monzygous twins (Malter and Cohen, 1989). Multiple gaps in the ZP may even lead to multiple herniation, possibly contributing to higher-order monozygotic pregnancies (Cohen and Feldberg, 1991).

Six of our cases showed a biamniotic monochorionic membrane configuration for the monozygotic twins and the seventh case was demonstrated to have a mono-amniotic–monochorionic membrane configuration for the twins, which demonstrates the time-frame of embryo splitting during hatching. Two of the seven cases demonstrated mono-amniotic twins in a dizygotic triplet (cases 2 and 3, Table II). This is rare event, and both patients had an extremely premature delivery.

Previous reports have described an increased incidence of MZT with assisted reproduction techniques (Table III). The incidence of MZT in the general population has been calculated...
Monozygosity independent of micromanipulation

Table III. Monozygosity after assisted reproduction – previous publications

<table>
<thead>
<tr>
<th>Author</th>
<th>Type of ART described</th>
<th>Type of MZT</th>
<th>No. of cases</th>
<th>Percent of all pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control population</td>
<td>None</td>
<td>Monozygotic twins</td>
<td>–</td>
<td>0.42-0.45%</td>
</tr>
<tr>
<td>Derom et al., (1987)</td>
<td>OI, CC DZ triplets and HMG</td>
<td>MZ twins</td>
<td>18/1485</td>
<td>1.2%</td>
</tr>
<tr>
<td>Edwards et al., (1986)</td>
<td>conventional IVF</td>
<td>MZ twins</td>
<td>9/600</td>
<td>1.33%</td>
</tr>
<tr>
<td>Steiner and Ojakangas (1994)</td>
<td>conventional IVF</td>
<td>DZ triplets</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Inion et al., (1998)</td>
<td>conventional IVF</td>
<td>DZ triplets</td>
<td>6/737</td>
<td>0.84%</td>
</tr>
<tr>
<td>Biljan et al., (1995)</td>
<td>conventional IVF</td>
<td>trizygotic quadruplet</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Salat-Baroux et al., (1994)</td>
<td>conventional IVF</td>
<td>trizygotic quintuplet</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Alikani et al., (1994)</td>
<td>micro-manipulation (AH, PZD)</td>
<td>MZ twins</td>
<td>5/143</td>
<td>3.49%</td>
</tr>
<tr>
<td>Slotnick and Ortega, (1996)</td>
<td>micro-manipulation (AH, PZD)</td>
<td>MZ twins</td>
<td>7/218</td>
<td>3.2%</td>
</tr>
<tr>
<td>Herschlag et al., (1999)</td>
<td>micro-manipulation (AH, SUZI, PZD)</td>
<td>MZ twins</td>
<td>8/674</td>
<td>1.2%</td>
</tr>
<tr>
<td>Behr et al., (1999)</td>
<td>IIIE–ICSI blastocysts</td>
<td>MZ twins</td>
<td>10/199</td>
<td>5%</td>
</tr>
<tr>
<td>Abusheikha et al., (2000)</td>
<td>IVF, ICSI</td>
<td>MZ twins</td>
<td>11/718</td>
<td>1.53%</td>
</tr>
<tr>
<td>Saito et al., (2000)</td>
<td>IVF, ICSI</td>
<td>MZ twins</td>
<td>12/475</td>
<td>2.52%</td>
</tr>
<tr>
<td>Sills et al., (2000)</td>
<td>IVF–ICSI</td>
<td>MZ twins</td>
<td>23/1911</td>
<td>1.2%</td>
</tr>
<tr>
<td>Schacter, (2000)</td>
<td>OI, IVF</td>
<td>MZ twins</td>
<td>7/731</td>
<td>0.95%</td>
</tr>
<tr>
<td>Total (excluding case reports)</td>
<td>All assisted reproduction</td>
<td>All monozygosity</td>
<td>120/7973</td>
<td>1.51%</td>
</tr>
</tbody>
</table>

Rate calculated in all studies as the number of monozygotic pregnancies per total number of pregnancies.

ART = assisted reproductive techniques; MZ, MZT = monozygotic twins; OI = ovulation induction; CC = clomiphene citrate; HMG = human menopausal gonadotrophin; DZ = dizygotic; AH = assisted hatching; SUZI = subzonal insemination; PZD = partial zonal dissection; ICSI = intracytoplasmic sperm injection.

to be ~1/250 (0.42%) live births, and could actually be higher if all clinical pregnancies were included (Bulmer, 1970; Saito et al., 2000).

A frequency of 1.2% for MZT after treatment with ovulation induction drugs (clomiphene citrate and gonadotrophins) was observed by Derom and colleagues, which was significantly higher than the expected frequency of 0.45% after spontaneous ovulation (Derom et al., 1987, 1993). Edwards and colleagues calculated the incidence of MZT in the population of IVF patients to be 1.33% (eight MZT in 600 conventional IVF pregnancies worldwide in 1986, the calculated 95% confidence interval 0.58–2.63%) (Edwards et al., 1986). The authors concluded that the artificial conditions of in-vitro media are the likely causes of increased incidence of MZT in this population. Recently, Blickstein and colleagues (1999) reported their experience in the setting of conventional IVF, in the special case of single embryo transfer, enabling an absolutely reliable diagnosis of MZT. They found the incidence of MZT in conventional IVF to be 5% (4/82) whereas no cases of MZT were found after ICSI (0/94) (Blickstein et al., 1999).

Alikani et al. (1994) reported their experience with MZT in 737 pregnancies achieved after IVF–embryo transfer, ~75% of these arose after various forms of zonal manipulation (Alikani et al., 1994). These included subzonal insemination (SUZI), partial zona dissection (PZD), zonal drilling, or AH of embryos. Six sets of MZT or triplets were described, resulting in an incidence of 0.84%, approximately twice the expected frequency. Here, the authors propound zonal manipulation as a possible cause of MZT.

Zonal manipulation was also cited (Slotnick and Ortega, 1996) as a major factor in the increased incidence of monoamniotic twins in the IVF–embryo transfer (micromanipulation) population. They found an incidence of 3.49% (5/143), more than seven times the background rate of twinning. Hershlag and colleagues (1999) noted a significantly increased rate of MZT after mechanical assisted hatching after conventional IVF, which reached a rate of 1.2% per embryo transfer (Hershlag et al., 1999). Schieve et al. (2000) calculated the odds ratio of monozygotic twinning after assisted hatching in assisted reproduction pregnancies to be 3.2–3.8, and they considered the contribution of AH to be significantly beyond that of the ovulation induction drugs (Schieve et al., 2000).

Wenstrom et al. (1993) counted seven monozygotic pregnancies of a total of 218 (3.2%). (Wenstrom et al., 1993)
These constituted 9.8% of all multiple gestations, whereby the type of assisted reproduction treatment had no effect on the incidence of monozygotic twinning. The authors theorized that assisted reproduction has an effect on the timing of embryonic events that lead to an increased incidence of MZT, regardless of the specific assisted reproduction used.

Additional reports have highlighted the possibility of higher-order monozygous pregnancies after assisted reproduction treatment. Three cases of dizygotic triplet pregnancy, in addition to those reported here, have been described in detail (Avrech et al., 1993; Steiner and Ojakangas, 1994; Inion et al., 1998). All three arose after IVF without micromanipulation. Avrech et al. (1993) reported good obstetric results, all three babies delivered alive, whereas Steiner and Ojakangas (1994) reported delivery at 28 weeks with subsequent neonatal death of two of the three infants. Inion et al. (1998) described monozygotic intrauterine twins and a tubal pregnancy after replacement of two embryos. This combination of heterotopic pregnancy and dizygotic triplets is extremely rare. A trzygotic quadruplet pregnancy has also been described (Biljan et al., 1995) after transfer of three grade I embryos after IVF, again without micromanipulation. Salat-Baroux and colleagues (1994) described trzygotic quintuplets (monoamniotic triplets with two additional sacs and embryos) after transfer of four grade I embryos after IVF without micromanipulation (Salat-Baroux et al., 1994). This patient aborted after an attempt at fetal reduction at 13 gestational weeks. These cases demonstrate that higher-order MZT may also be independent of micromanipulation.

Multicentric experience with MZT after blastocyst transfer has been reported (Behr et al., 1999). The incidence of MZT in this specific setting was calculated to be 5% (10/199), which is higher than the background incidence of MZT by a factor of 10. Abusheikha et al. (2000) reported on 11 monozygotic multiple pregnancies from two assisted reproduction centres of 718 pregnancies (Abusheikha et al., 2000). Six of the eleven occurred after ICSI.

Finally, Sills and colleagues reported on 23 sets of monozygotic twins from 1911 assisted reproduction pregnancies (1.2%) (Sills et al., 2000). Their data confirm that no correlation was found between zonal manipulative techniques and MZT. This group of investigators concluded that the most likely cause of MZT in the setting of assisted reproduction was the increased number of embryos transferred to the uterus. The rate of MZT was approximately three times the background rate, closely reflecting the number of embryos transferred per cycle.

A number of hypotheses may be advanced in an effort to explain the increased incidence of MZT in micromanipulation or blastocyst cycles. Alikani et al. (1994) suggested that artificially induced structural changes in the ZP may lead to complications in the normal process of zonal lysis (Alikani et al., 1994). The embryo might bypass its own mechanism of local zonal lysis and ‘choose’ to escape through the already established path. The dimensions of this gap could restrict the emerging embryo and encourage splitting, leading to MZT. A naturally thin or irregular zona pellucida may affect the hatching process in the same way, if during the blastocyst stage the zona disintegrates at more than one spot. This could be one of the mechanisms to explain the age-related increase of MZT, as zona thickness is more irregular with age (Cohen and Feldberg, 1991).

Edwards and colleagues discussed the incidence of MZT after IVF without zonal manipulation (Edwards et al., 1986). Importantly, they noted that most MZT arise spontaneously in vivo from a single embryo (monozygotic twins), conversely, many MZT arising from IVF were accompanied by one or more sibling embryos, i.e., monozygotic triplets or higher order twinning. They concluded that the nature of embryonic growth in vitro predisposes to twinning, as opposed to in-vivo growth after ovarian stimulation. A possible ‘hardening’ of the human ZP in vitro, after exposure to artificial media, as opposed to salpingeal or uterine secretions, could lead to increased fragility or brittleness and thence ‘fracture’ the ZP, or cell-to-cell adhesion might be disturbed after in-vitro culture. Sills et al. (2000) postulated that the increased rate of MZT is a simple reflection of an increase in the number of embryos transferred to the uterus, and that the rate of MZT per transferred embryo is not different from that found in the general population (Sills et al., 2000). This is not borne out by the data (Behr et al., 1999; Blickstein et al., 1999; Saito et al., 2000) which described increased rates of MZT in single replaced embryos and blastocysts. Derom et al. (1987) hypothesized that ovarian stimulation itself might cause hardening of the ZP, as observed in animals by Lungo (Lungo, 1981). If this hardening is not uniform, some weak spots could be formed through which the blastocyst might herniate. These authors also calculated a higher rate of monozygosity in ovulation induced triplets than in induced twins.

It is interesting that five of our cases were dizygotic triplets, whereas we identified in the same cohort of patients only two cases of monozygotic twins. Our literature search revealed 95 sets of monozygotic twins after assisted reproduction as opposed to 18 sets of dichorionic triplets or higher-order monozygotic pregnancies (Table III). The ratio of triplets to twins in nature is significantly less than that found in the setting of assisted reproduction. The mechanism responsible for the irregular distribution of MZT triplets after assisted reproduction is a matter of speculation, which warrants additional study.

Two possible biases limit our ability to draw firm conclusions from this study. Firstly, the low incidence of this phenomenon makes a large series difficult to accumulate. To be able to achieve conclusions with statistical significance, sample sizes of 10 000–20 000 cases are needed. These requirements necessitate multicentric cooperation or meta-analysis. Secondly, because we relied on the early diagnosis of pregnancy sacs and membranes to predict zygosity accurately, we might be underestimating the true incidence of MZT in cases of dichorionic biamniotic monozygotic twinning; our ultrasound criteria might mistakenly label these pregnancies as non-MZT, and accurate post-natal follow-up data on all same-sex multiple pregnancy children was not available. However, if these data are inaccurate, they only underestimate the true higher incidence of MZT.

The cumulative combined MZT rate of all published series

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was 1.51%, approximately three times the background rate in
the untreated population (Table III). It is significant that in all
series reported, the incidence of MZT was increased, regardless
of its being subsequent to ovulation induction, IVF and/or
micromanipulation—of any type—most often assisted hatching
or ICSI (Edwards et al., 1986; Wenstrom et al., 1993; Alikani
et al., 1994; Slotnick and Ortega, 1996; Hershlag et al.,
1997; Blickstein et al., 1999; Saito et al., 2000; Sills et al.,
2000)(Table III). This finding supports the surmise that the
ovulation induction stimulus could be a major influence pro-
moting MZT in assisted reproduction, as opposed to the current
thinking implicating zonal manipulation. The basic underlying
common denominator of these three situations is ovulation
induction therapy, which seems to predispose either to mono-
zygotic twinning or enhanced survival of monzygotic twins
after their formation. It is possible that improved endometrial
conditions after gonadotrophin therapy encourage monzygotic
implantation, or that the biochemical milieu of the uterine
cavity after gonadotrophin therapy encourages asymmetrical
ZP hatching, independently of zonal manipulation procedures
done in vitro. Each of our cases represents a different arm of
assisted reproduction therapies, gonadotrophin therapy, IVF–
embryo transfer and micromanipulation and embryo transfer.
This leads us to the same conclusion as Derom and his associ-
ates—that gonadotrophin treatment can increase the
incidence of MZT in all patients so treated (Derom et al.,
1987). The phenomenon of MZT, although uncommon, is
another factor to take into account during assisted reproduction
treatment. It would appear that the dizygotic triplet combina-
tion is even more common than one would expect, respective-
to the monzygotic twin. Ovarian stimulation for ovulation
induction or IVF–embryo transfer should be undertaken care-
fully, and the number of induced follicles limited, or the
number of embryos replaced limited, in order to minimize
multiple pregnancies. Extra consideration should be applied to
account for the increased multiple pregnancy rate, partially
due to an increased rate of MZT (Derom et al., 1993). The
problems of fetal reduction are also amplified in cases of
monozygosity, so that this modality may not always be
available to fall back on. This short series also emphasizes
the extremely poor obstetric outcome of monzygotic multiple
pregnancies, another reason to exercise caution in decision-
making in assisted reproduction treatment.

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