# Clinical Analysis of Monozygotic Twin Pregnancies Following Assisted Reproductive Technology Treatment: Postprint

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## Abstract

Objective To investigate the occurrence, management, and pregnancy outcomes of monozygotic twinning (MZT) following assisted reproductive technology (ART) treatment. Methods A retrospective analysis was conducted on the basic data of patients who achieved clinical pregnancy after undergoing in vitro fertilization-embryo transfer (IVF-ET) and intracytoplasmic sperm injection-embryo transfer (ICSI-ET) at the Reproductive Medicine Center of Nanfang Hospital, Southern Medical University from January 2010 to June 2015. The incidence of MZT was statistically analyzed, and the management and pregnancy outcomes of 94 MZT cases were categorized and analyzed according to pure MZT, multifetal pregnancy with MZT, and ectopic pregnancy. Simultaneously, comparisons were made between pure MZT and non-MZT twin pregnancies during the same period, between multifetal pregnancy with MZT that underwent single fetus reduction versus double fetus reduction, and between multifetal pregnancy with MZT reduced to twins and non-MZT triplet pregnancies reduced to twins, in terms of miscarriage rate, live birth rate, preterm birth rate, and full-term birth rate. Results Among 6257 embryo transfer cycles that achieved clinical pregnancy, 94 were MZT pregnancies (1.5%, 94/6257). The incidence showed no statistically significant difference between IVF (1.8%, 47/2649) and ICSI (1.2%, 10/822) ($P=0.272$), nor between fresh embryo transfer cycles (1.6%, 57/3471) and frozen-thawed embryo transfer cycles (1.3%, 37/2786) ($P=0.310$). Among the 94 MZT cases, 45 were pure MZT, 43 were triplet pregnancies with MZT, 3 were quadruplet pregnancies with MZT, and 3 were ectopic pregnancies (including heterotopic pregnancy). Compared with non-MZT twin pregnancies during the same period, pure MZT pregnancies had lower full-term birth rate and live birth rate, and higher miscarriage rate and neonatal malformation incidence, with all differences being statistically significant ($P<0.05$). In comparing triplet

pregnancies with MZT that underwent single fetus reduction versus double fetus reduction, the double fetus reduction group had higher full-term birth rate and live birth rate, and lower miscarriage rate and preterm birth rate than the single fetus reduction group, but these differences were not statistically significant (P>0.05). Compared with non-MZT triplet pregnancies reduced to twins during the same period, triplet pregnancies with MZT reduced to twins had lower full-term birth rate, preterm birth rate, and live birth rate, and higher miscarriage rate, but none of these differences were statistically significant (P>0.05). Conclusion The incidence of MZT pregnancy after ART treatment is significantly higher than that in natural conception; the pregnancy outcomes of pure MZT are worse than those of dizygotic twins; for multifetal pregnancies with MZT, reduction of the MZT gestational sac may lead to better pregnancy outcomes.

## Full Text

## Preamble

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## Abstract

**Objective:** To investigate the incidence, management, and pregnancy outcomes of monozygotic twin (MZT) pregnancies conceived through assisted reproductive technology (ART). **Methods:** A retrospective analysis was conducted of clinical pregnancies following in vitro fertilization-embryo transfer (IVF-ET) and intracytoplasmic sperm injection-embryo transfer (ICSI-ET) at the Reproductive Medicine Center of Nanfang Hospital, Southern Medical University between January 2010 and June 2015. The incidence of MZT was calculated, and the management and outcomes of 94 MZT pregnancies were analyzed according to three categories: pure MZT, MZT with high-order multiple pregnancy, and ectopic pregnancy. Comparative analyses were performed between pure MZT and non-MZT twin pregnancies, between MZT triplet pregnancies reduced to singletons versus twins, and between reduced MZT triplet pregnancies and reduced non-MZT triplet pregnancies, focusing on miscarriage rates, live birth rates, preterm birth rates, and full-term birth rates. **Results:** Among 6,257 embryo transfer cycles resulting in clinical pregnancy, 94 were MZT pregnancies (1.5%, 94/6,257). The incidence did not differ significantly between IVF (1.8%, 47/2,649) and ICSI (1.2%, 10/822; P=0.272), nor between fresh embryo transfer cycles (1.6%, 57/3,471) and frozen-thawed cycles (1.3%, 37/2,786; P=0.310). Of the 94 MZT pregnancies, 45 were pure MZT, 43 were MZT triplets, 3 were

MZT quadruplets, and 3 were ectopic pregnancies (including heterotopic pregnancies). Compared with non-MZT twin pregnancies, pure MZT pregnancies had significantly lower full-term birth rates and live birth rates, and significantly higher miscarriage and neonatal malformation rates ($P<0.05$). Among MZT triplet pregnancies, those reduced to twins had higher full-term and live birth rates, and lower miscarriage and preterm birth rates compared with those reduced to singletons, though these differences were not statistically significant ($P>0.05$). Compared with non-MZT triplet pregnancies reduced to twins, MZT triplet pregnancies reduced to twins had lower full-term birth, preterm birth, and live birth rates, and higher miscarriage rates, but these differences were also not statistically significant ($P>0.05$). **Conclusion:** The incidence of MZT pregnancy after ART is significantly higher than in spontaneous conception. Pure MZT pregnancies have poorer outcomes than dizygotic twin pregnancies. For high-order multiple pregnancies with MZT, selective reduction of the MZT gestational sac may yield better pregnancy outcomes.

**Keywords:** assisted reproductive techniques; monozygotic twins; pregnancy outcome

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## 1. Materials and Methods

### 1.1 Study Population

We retrospectively analyzed 6,257 embryo transfer cycles resulting in clinical pregnancy after IVF-ET/ICSI-ET at the Reproductive Medicine Center of Nanfang Hospital, Southern Medical University between January 2010 and June 2015 (excluding cycles involving oocyte donation, sperm donation, lost to follow-up, or cycles with unclear fertilization methods including partial ICSI and rescue ICSI). Of these, 2,649 were IVF-ET cycles (42.3%, 2,649/6,257), 822 were ICSI-ET cycles (13.1%, 822/6,257), and 2,786 were frozen-thawed embryo transfer (FET) cycles (44.5%, 2,786/6,257). A total of 94 MZT pregnancies occurred: 45 pure MZT cases, 43 MZT triplet pregnancies, 3 MZT quadruplet pregnancies, and 3 ectopic pregnancies (including heterotopic pregnancies).

### 1.2 Study Methods

We calculated the incidence of MZT after ART and compared rates between IVF and ICSI, as well as between fresh and frozen-thawed transfer cycles. MZT pregnancies were categorized into three groups for analysis of management and outcomes: pure MZT, MZT with high-order multiple pregnancy, and ectopic pregnancy. We further compared clinical outcomes—including miscarriage rate, live birth rate, preterm birth rate, and full-term birth rate—between pure MZT and non-MZT twin pregnancies without reduction, between MZT triplet pregnancies reduced to twins versus singletons, and between reduced MZT triplet pregnancies and reduced non-MZT triplet pregnancies (MZT quadruplet and ectopic pregnancies were excluded from comparison due to small sample sizes).

### 1.3 Treatment Protocols

Personalized treatment protocols were selected based on patient age, body mass index (BMI), baseline hormone levels, antral follicle count, and medical history. Controlled ovarian stimulation was performed using exogenous gonadotropins (Gn). Follicular development was monitored by transvaginal ultrasound, with periodic measurements of serum estradiol (E2), luteinizing hormone (LH), and progesterone (P) levels; Gn dosage was adjusted according to ovarian response. When at least two follicles reached a mean diameter of 18 mm, human chorionic gonadotropin (HCG) 5,000–10,000 IU was administered to trigger final oocyte maturation. Oocyte retrieval was performed 36 hours later under transvaginal ultrasound guidance with anesthesia. Fertilization was achieved through IVF or ICSI based on semen parameters and prior fertilization history. Embryo transfer was performed on day 3 or day 5 post-fertilization, with supernumerary embryos cryopreserved using either slow-freezing or vitrification methods.

For frozen-thawed cycles, endometrial preparation was achieved through natural, hormone replacement, or ovulation induction protocols. Embryos were transferred on the day of or day after thawing. Luteal support with intramuscular progesterone 40–60 mg/day, with or without HCG, was initiated on the day of oocyte retrieval and continued until 8–9 weeks of gestation if pregnancy was achieved.

### 1.4 Pregnancy Outcome Determination

Serum or urine  -HCG was measured 12 days after embryo transfer. Transvaginal ultrasound performed 4 weeks after transfer confirmed clinical pregnancy upon visualization of a gestational sac. MZT was diagnosed by ultrasound when either: (1) the number of gestational sacs exceeded the number of embryos transferred, or (2) two fetal poles were observed within a single gestational sac.

### 1.5 Statistical Analysis

All data were analyzed using SPSS 20.0 software. Continuous variables were expressed as mean ± standard deviation and compared using t-tests. Categorical variables were expressed as percentages and compared using chi-square tests. $P<0.05$ was considered statistically significant.

## 2. Results

### 2.1 MZT Incidence

Among 6,257 clinical pregnancy cycles, there were 3,839 singleton pregnancies (61.4%), 2,014 twin pregnancies (32.2%), 148 high-order multiple pregnancies (HOMP) (2.4%), and 256 ectopic pregnancies (including heterotopic pregnancies) (4.1%). MZT occurred in 94 cases, yielding an incidence of 1.5% (94/6,257). The 94 patients had a mean age of 31.8±3.71 years and received 2.09±0.46 embryos per transfer. One case of monozygotic triplets combined with dizygotic twins (quintuplet pregnancy) occurred, giving a monozygotic triplet incidence of 0.02% (1/6,257). Of the 94 MZT cases, 47 occurred in IVF-ET cycles (1.8%, 47/2,649), 10 in ICSI-ET cycles (1.2%, 10/822), 57 in fresh embryo transfer cycles (1.6%, 57/3,471), and 37 in FET cycles (1.3%, 37/2,786). No significant differences were observed in MZT incidence between IVF-ET and ICSI-ET (1.8% vs. 1.2%, P=0.272) or between fresh and frozen-thawed cycles (1.6% vs. 1.3%, P=0.310).

### 2.2 Pregnancy Outcomes

**2.2.1 Pure MZT** None of the 45 pure MZT pregnancies underwent selective reduction. Among these, 14 resulted in full-term delivery (31.1%, 14/45), 20 in preterm delivery (44.4%, 20/45), and 11 in miscarriage (24.4%, 11/45; including 6 early and 5 late miscarriages). Live births occurred in 34 cases (75.6%, 34/45), delivering 62 surviving neonates (23 full-term, 39 preterm). One pure MZT pregnancy developed discordant fetal growth at 19 weeks, with intrauterine fetal demise of both twins, possibly due to twin-twin transfusion syndrome (TTTS). Another case developed hypertensive disorders of pregnancy, delivering two live infants at term, one of which had tricuspid regurgitation with pulmonary hypertension. Postnatal follow-up revealed one additional case of congenital duodenal atresia among the 62 surviving neonates, yielding a neonatal malformation rate of 3.2% (2/62).

Among 1,969 non-MZT twin pregnancies during the same period, 1,952 did not undergo selective reduction. These resulted in 935 full-term deliveries (47.9%, 935/1,952), 794 preterm deliveries (40.7%, 794/1,952), 216 miscarriages (11.1%, 216/1,952; including 96 early and 120 late miscarriages), 6 late-term terminations, and 1 termination for personal reasons. Live births occurred in 1,729 cases (88.6%, 1,729/1,952), delivering 3,055 surviving neonates (1,521 full-term, 1,538 preterm). Two non-MZT twin pregnancies developed hypertensive disorders: one resulted in late miscarriage; the other had severe preeclampsia with one fetus being a hydrops fetalis that died in utero, while the other was delivered preterm at 28+4 weeks as an extremely low birth weight infant who died after 13 days following withdrawal of care. Postnatal follow-up of 3,055 surviving neonates identified 4 cases of congenital heart disease, 1 hydrocephalus, 1 trisomy 21, 2 congenital megacolon, 1 intersex condition, and 1 polydactyly, yielding a malformation rate of 0.3% (10/3,055).

Comparison between pure MZT and non-MZT twin pregnancies without reduction revealed statistically significant differences in miscarriage rate, full-term birth rate, live birth rate, and neonatal malformation rate ($P<0.05$). No significant difference was observed in preterm birth rate between the two groups ($P>0.05$) .

**2.2.2 MZT with High-Order Multiple Pregnancy** Among 148 HOMP cases, 46 involved MZT. Ten MZT triplet pregnancies did not undergo reduction: one early spontaneous miscarriage, one termination for personal reasons, seven spontaneously reduced to twin pregnancies (six delivered twin live births, one delivered a single live birth with the other fetus dying in utero due to omphalocele), and one spontaneously reduced to singleton pregnancy delivering a single live birth.

Thirty-three MZT triplet pregnancies underwent selective embryo reduction (SER): 15 had the singleton fetus reduced, resulting in twin pregnancies; 18 had the MZT pair reduced, resulting in singleton pregnancies. All three MZT quadruplet pregnancies underwent SER at 7-8 weeks gestation, with the MZT pair reduced in each case, resulting in twin pregnancies. Among these, one delivered two live infants preterm, while two resulted in late spontaneous miscarriage. Additionally, one case of monozygotic triplets combined with dizygotic twins (quintuplet pregnancy) underwent reduction of the monozygotic triplets at 7 weeks, but later miscarried due to premature rupture of membranes in the late second trimester.

Among the 15 MZT triplet pregnancies where the singleton was reduced, outcomes included 3 full-term deliveries (20%, 3/15), 6 preterm deliveries (40%, 6/15), and 6 miscarriages (40%, 6/15; including 1 early and 5 late miscarriages). Live births occurred in 9 cases (60%, 9/15), delivering 18 surviving neonates (6 full-term, 12 preterm) with no malformations detected on follow-up.

Among the 18 MZT triplet pregnancies where the MZT pair was reduced, outcomes included 9 full-term deliveries (50%, 9/18), 3 preterm deliveries (16.7%, 3/18), 5 miscarriages (27.8%, 5/18; including 1 early and 4 late miscarriages), and 1 late-term termination. Live births occurred in 12 cases (66.7%, 12/18), delivering 12 surviving neonates (9 full-term, 2 preterm) with no malformations detected.

One case of TTTS occurred among the 43 MZT triplet pregnancies: after SER to preserve the MZT pair at 7 weeks, TTTS was diagnosed at 17+2 weeks. Radiofrequency ablation was performed at 18 weeks, but one twin was already dead on the day of procedure; both fetuses were lost by the following day, and the pregnancy was terminated at 18+2 weeks.

Comparison between MZT triplet pregnancies reduced to twins versus singletons showed no statistically significant differences in miscarriage rate, full-term birth rate, preterm birth rate, or live birth rate ($P>0.05$) . However, the group reduced to singletons had higher full-term and live birth rates, and lower miscarriage and preterm birth rates—differences exceeding 10% that are clinically meaningful.

During the same period, 101 non-MZT triplet pregnancies were managed; 61 underwent SER to twins, resulting in 21 full-term deliveries (34.4%, 21/61), 29 preterm deliveries (47.5%, 29/61), and 11 miscarriages (18.0%, 11/61; all late miscarriages). Live births occurred in 50 cases (82.0%, 50/61), delivering 97 surviving neonates (39 full-term, 58 preterm). One neonatal case of atrial septal defect was identified on follow-up. No cases of hypertensive disorders occurred during pregnancy in this group.

Comparison between MZT triplet pregnancies reduced to twins and non-MZT triplet pregnancies reduced to twins showed no statistically significant differences in miscarriage rate, full-term birth rate, preterm birth rate, or live birth rate ($P>0.05$) .

**2.2.3 Ectopic Pregnancy (Including Heterotopic Pregnancy)** Three cases of MZT with ectopic pregnancy occurred: (1) left cornual MZT combined with left tubal singleton pregnancy managed by laparotomy with left cornual wedge resection and right tubal ligation at 7+1 weeks; (2) intrauterine MZT combined with right tubal pregnancy managed by laparoscopic right salpingectomy at 6+4 weeks, followed by early spontaneous miscarriage of the intrauterine twins; and (3) intrauterine MZT combined with abdominal pregnancy managed by laparotomy for abdominal pregnancy removal at 7+3 weeks, followed by preterm cesarean delivery of two infants.

## 3. Discussion

### 3.1 MZT Occurrence in ART

Monozygotic multiple pregnancies are rare in spontaneous conception, with reported incidences of 0.4% for monozygotic twins and 0.004% for monozygotic triplets. The widespread application of ART has markedly increased the incidence of multiple pregnancies, with MZT rates exceeding those in natural conception by more than twofold. Our retrospective analysis identified 94 monozygotic twin pregnancies (1.5%) and one monozygotic triplet pregnancy (0.02%) among 6,257 clinical pregnancy cycles—both significantly higher than in spontaneous conception, consistent with previous reports. Since most cycles involved transfer of more than one embryo and some embryos may be lost intrauterinely, early ultrasound cannot reliably distinguish monochorionic dizygotic twins from monozygotic twins; definitive diagnosis requires DNA analysis, suggesting the actual MZT incidence may be even higher.

Since John Yovich et al. reported the first IVF-conceived MZT pregnancy in 1984, numerous studies have investigated the increased MZT incidence after ART. Edwards et al. suggested that components of artificial culture media may be associated with MZT occurrence. Derom et al. proposed that ovulation induction agents might alter zona pellucida structure, leading to MZT. Israeli scholar Blickstein hypothesized that ovarian stimulation may increase the number of oocytes with special self-splitting capacity, raising MZT rates. Skiadas and Haimov-Kochman et al. linked MZT to micromanipulation of the zona pellucida during ART (e.g., ICSI, assisted hatching, preimplantation genetic diagnosis). Other investigators have implicated extended in vitro culture time, suboptimal culture conditions, and cryopreservation-related low temperatures in zona pellucida hardening, potentially causing MZT. However, Taiwanese scholar Wu et al. found no significant association between MZT and ICSI, assisted hatching, or blastocyst transfer in their center' s data. A recent study suggested that the increased MZT incidence after ART is not related to the technology itself but rather to genetic factors, with ART facilitating splitting of embryos with superior reproductive potential. Our comparison of MZT incidence between IVF and ICSI, as well as between fresh and FET cycles, revealed no significant differences (P>0.05). While the precise mechanism of MZT remains unclear and may involve single or multiple factors, the documented increase in MZT after ART and its associated complications warrant serious attention.

### 3.2 Risks, Management, and Prognosis of MZT

Due to placental vascular anastomoses, MZT pregnancies carry higher morbidity and mortality than dizygotic twins. In addition to complications such as premature rupture of membranes and placenta previa, MZT is more prone to umbilical cord entanglement, congenital malformations, TTTS, and discordant fetal growth, making early identification and management crucial.

Given the placental architecture, selective reduction is generally not recommended for pure MZT because eliminating one fetus may cause retrograde hemorrhage from the surviving twin to the reduced twin, resulting in loss of both. None of our 45 pure MZT pregnancies underwent reduction; one resulted in late miscarriage possibly due to TTTS, and another developed hypertensive disorders with one twin having tricuspid regurgitation and pulmonary hypertension. Compared with non-MZT twin pregnancies, pure MZT showed significantly lower full-term and live birth rates and significantly higher miscarriage and neonatal malformation rates (P<0.05). The preterm birth rate was higher in pure MZT but without statistical significance (P>0.05), indicating poorer outcomes than dizygotic twins.

For triplet pregnancies with MZT, the high risk of obstetric complications necessitates selective reduction to optimize outcomes, with theoretical preference for reducing the MZT pair. However, some patients and families, hoping to retain twins, insist on reducing the singleton instead. In our cohort of 43 MZT triplet pregnancies, 33 underwent SER: 15 targeting the singleton and 18 targeting the MZT pair. One case of TTTS occurred in the group where the singleton was reduced. No neonatal malformations were observed in any of the 33 reduced MZT triplet pregnancies. While no statistically significant differences were found in pregnancy outcomes between reduction to singleton versus twin pregnancies (P>0.05), the singleton reduction group had higher full-term and live birth rates and lower miscarriage and preterm birth rates—differences exceeding 10% that are clinically meaningful. Therefore, we recommend reducing the MZT pair in high-order multiple pregnancies. With the universal two-child policy now in place, patient preference should no longer dictate retention of MZT. Compared with 61 non-MZT triplet pregnancies reduced to twins, the 15 MZT triplet pregnancies reduced to twins had lower full-term, preterm, and live birth rates and higher miscarriage rates, though without statistical significance (P>0.05).

In summary, ART significantly increases the incidence of MZT pregnancy compared with natural conception. Pure MZT pregnancies have poorer outcomes than dizygotic twins. For high-order multiple pregnancies with MZT, selective reduction of the MZT pair may achieve better outcomes than reducing the singleton. However, the exact mechanism of MZT remains unclear and complication risks are high. Therefore, clinical practice should emphasize early identification and accurate diagnosis, with active intervention for high-order multiple pregnancies involving MZT to reduce adverse outcomes and ensure maternal-fetal safety.

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*Note: Figure translations are in progress. See original paper for figures.*