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Case report

Monochorionic-triamniotic triplet pregnancy after intracytoplasmic sperm injection, assisted hatching, and two-embryo transfer: first reported case following IVF

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Abstract

Background: We present a case of monochorionic-triamniotic pregnancy that developed after embryo transfer following in vitro fertilization (IVF).

Methods: After controlled ovarian hyperstimulation and transvaginal retrieval of 22 metaphase II oocytes, fertilization was accomplished with intracytoplasmic sperm injection (ICSI). Assisted embryo hatching was performed, and two embryos were transferred *in utero*. One non-transferred blastocyst was cryopreserved.

Results: Fourteen days post-transfer, serum hCG level was 423 mIU/ml and subsequent transvaginal ultrasound revealed a single intrauterine gestational sac with three separate amnion compartments. Three distinct foci of cardiac motion were detected and the diagnosis was revised to monochorionic-triamniotic triplet pregnancy. Antenatal management included cerclage placement at 19 weeks gestation and hospital admission at 28 weeks gestation due to mild preeclampsia. Three viable female infants were delivered via cesarean at 30 5/7 weeks gestation.

Conclusions: The incidence of triplet delivery in humans is approximately 1:6400, and such pregnancies are classified as high-risk for reasons described in this report. We also outline an obstetric management strategy designed to optimize outcomes. The roles of IVF, ICSI, assisted embryo hatching and associated laboratory culture conditions on the subsequent development of monozygotic/monochorionic pregnancy remain controversial. As demonstrated here, even when two-embryo transfer is employed after IVF the statistical probability of monozygotic multiple gestation cannot be reduced to zero. We encourage discussion of this possibility during informed consent for the advanced reproductive technologies.

Background

Although the developmental mechanisms responsible monozygotic mutiple gestation are poorly characterized, some laboratory procedures altering the zona pellucida (*e.g.*, ICSI and assisted embryo hatching) have been proposed as important. In this report, we describe the first known case of monochorionic-triamniotic triplets after IVF, an unusual physiological event requiring the sequence of two properly timed, distinct embryo splitting events.

Case Report

A 21 year-old Caucasian nulligravida presented with her partner for assessment and treatment of primary male factor infertility of 24 months duration. Both were in good general health and the female's past gynecological history was unremarkable. The wife smoked approximately 1/2 pack of cigarettes daily, and used alcohol only rarely. Her only regular medication was a prenatal vitamin. Physical exam revealed no abnormality; BMI was 20 kg/m^2. Her blood type was O positive. Cervical cytology and laboratory tests results were all normal, and saline hysterography identified no intracavitary defects. The patient had never used ovulation induction agents or periovulatory hCG.

The partner was a non-smoker and took no regular medications. He had commissioned no pregnancies in a prior marriage, and had been diagnosed with azoospermia four years earlier. Urological evaluation found no anatomical abnormality. Karyotype was normal 46, XY and Y-chromosome microdeletion analysis via polymerase chain reaction method was negative for any known mutation. Obstructive azoospermia (rete testes obliteration) had been diagnosed after exploratory scrotal surgery four years before presentation. The couple declined intrauterine insemination with anonymous donor sperm for personal reasons. Given the male factor infertility diagnosis necessitating intracytoplasmic injection (ICSI), the couple elected in vitro fertilization utilizing surgically retrieved spermatozoa. Full informed consent was obtained, including a discussion of multiple gestation risk.

Following pituitary downregulation with intranasal nafarelin acetate (0.4 mg/d), controlled ovarian hyperstimulation commenced using a combined FSH+hMG protocol. Subcutaneous hCG was administered on stimulation day 11, when serum estradiol was 2110 pg/ml. Twenty-two oocytes were retrieved via ultrasound-guided transvaginal needle aspiration, undertaken in parallel with spermatozoa collection via testicular biopsy (both procedures performed under intravenous sedation). Utilizing ICSI, 10 oocytes advanced to the 2*pn* stage. Two embryos (8 and 9-cell stage) were transferred fresh on post-fertilization day three after assisted hatching via acid Tyrode's method. One blastocyst was cryopreserved. Serum hCG was 423 mIU/ml two weeks after embryo transfer.

Transvaginal 4 mHz sonogram (SDU400+, Shimadzu Corp; Kyoto, Japan) performed at eight weeks' gestation demonstrated a single 28 mm intrauterine gestational sac (chorion) with three distinct fetal poles, each with discrete cardiac activity. Amniotic membrane configuration could not be immediately determined. Follow-up transvaginal ultrasound one week later refined the diagnosis as monochorionic-triamniotic triplet pregnancy (Figure 1). Total daily folic acid dose was increased to 1 mg/d, and antenatal care was co-managed with periodic perinatal consultation. The obstetrical course was uncomplicated until ~19th gestational week, when cervical funneling became evident via transvaginal sonogram. Based on this finding, the patient was admitted to hospital and a McDonald (rescue) cerclage was placed without difficulty. The post-operative course was uneventful and the patient was discharged home the next day.

At 28 weeks' gestation, the patient was readmitted to hospital for mild preeclampsia with a blood pressure of 140/80. To promote fetal lung maturity, betamethasone (12 mg × 2 doses) was administered via intramuscular injection. The patient had no focal neurological signs and ALT and AST were elevated (97 and 121 u/l, respectively). Liver function tests normalized soon after admission. Platelet count was 157,000 and protein (325 mg) was present in 24 h urine collection. Multifetal biophysical profiles were performed every 48 h with reassuring results. At 30$^{5/7}$ weeks' gestation, serum uric acid was 7.8 mg/dl, platelets had fallen to 115,000, and the patient began to experience visual scotomata. Based on these findings consistent with worsening preeclampsia, the patient underwent a primary low-transverse cesarean delivery (estimated blood loss = 1,000 ml) resulting in the births of three viable female infants (1475, 1021, and 1021 g); one vertex and two breech presentations. Apgar scores were and 8/9, 9/9, and 8/9, respectively. A single three-umbilical cord placenta (weight = 639 g) was delivered without complication, and each umbilical cord was morphologically normal. The patient was discharged home after seven days in good condition, and two of the triplets were discharged home three weeks later. The other infant was discharged home at eight weeks age due to necrotizing enterocolitis that required surgery for intestinal obstruction. At six months follow-up, mother and babies continue to do well.

Discussion

Monozygotic twinning is thought to occur in 0.42% of all deliveries [1]. Not surprisingly triplet pregnancy in general accounts for even fewer births (1:6400), and the monochorionic triplet subset may occur only once in 100,000

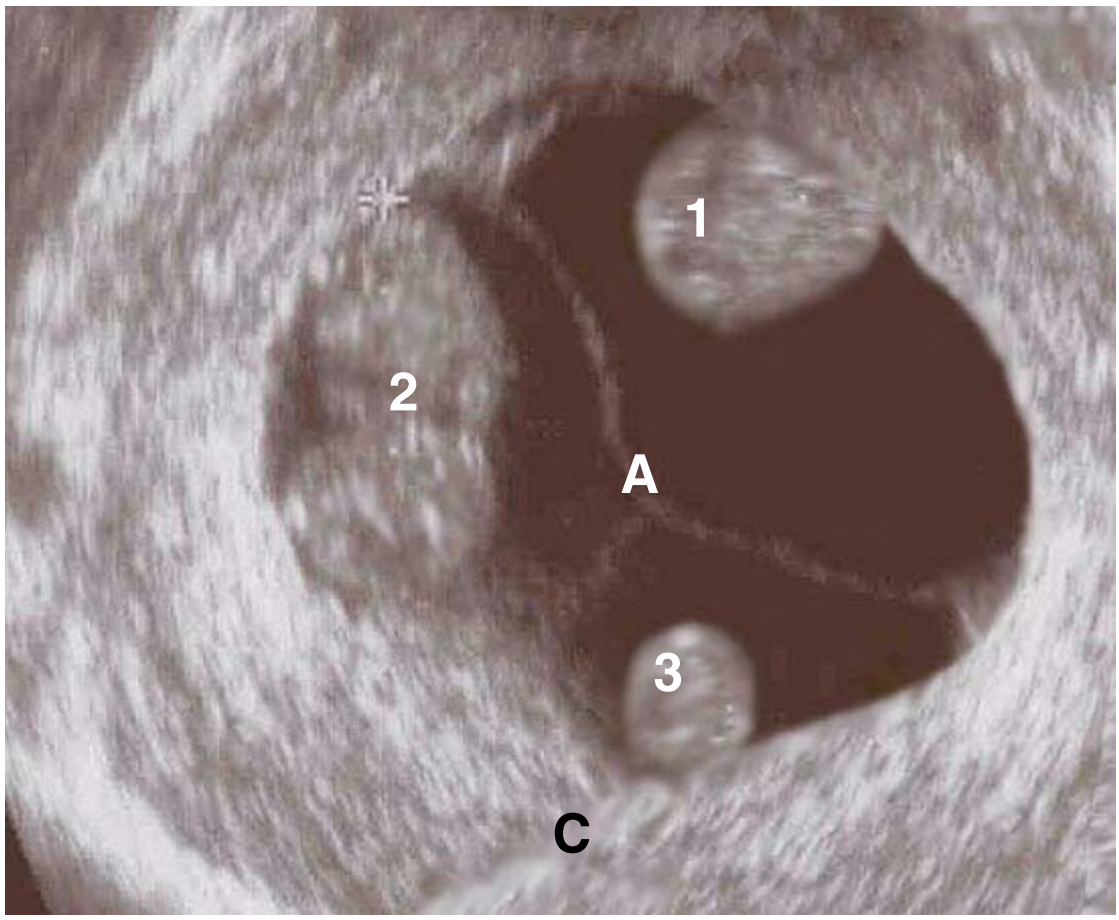

Figure 1
Transvaginal sonogram image of intrauterine monochorionic-triamniotic triplet pregnancy at 9 weeks' gestational age, demonstrating three separate embryos, distinct amniotic membranes (A), and unified chorion (C). The conception resulted from a two-embryo transfer following IVF+ICSI.

births. Monozygotic multiple gestations occur when a single fertilized ovum splits into genetically identical embryos (Figure 2). Exactly when this division occurs governs the configuration of chorion and amnion compartments, with later fission resulting in development of progressively greater shared tissue elements among conceptuses. While zygosity refers to the number of source zygotes comprising the gestational set (with profound life-long implications for a sibling cohort), the precise anatomical characterization of chorion and amnion for any multiple gestation is more important in intrapartum risk assessment and obstetrical management. Depending on time of embryo splitting, monozygotic multiplets may have separate chorions or placentas, yet monochorionic gestations must always develop in a monozygous context. Based on this relationship, the number of placentas correlates with embryo number for multizygotic gestations, while monochorionic multiplets without mosaicism must be uniformly classified as monozygotic. Here we present the first known case of monochorionic-triamniotic triplets after IVF, a highly unusual occurrence necessitating a sequence of two properly timed, distinct embryo splitting events.

Previous investigators [2–4] have suggested at least three factors could modulate development of monozygotic multiple gestations in the setting of the advanced reproductive technologies: ovulation induction *per se*, certain IVF culture conditions, and zona pellucida architecture/micromanipulation. As these three variables commonly occur together, multiple regression analysis to quantify the individual contribution of each intervention has

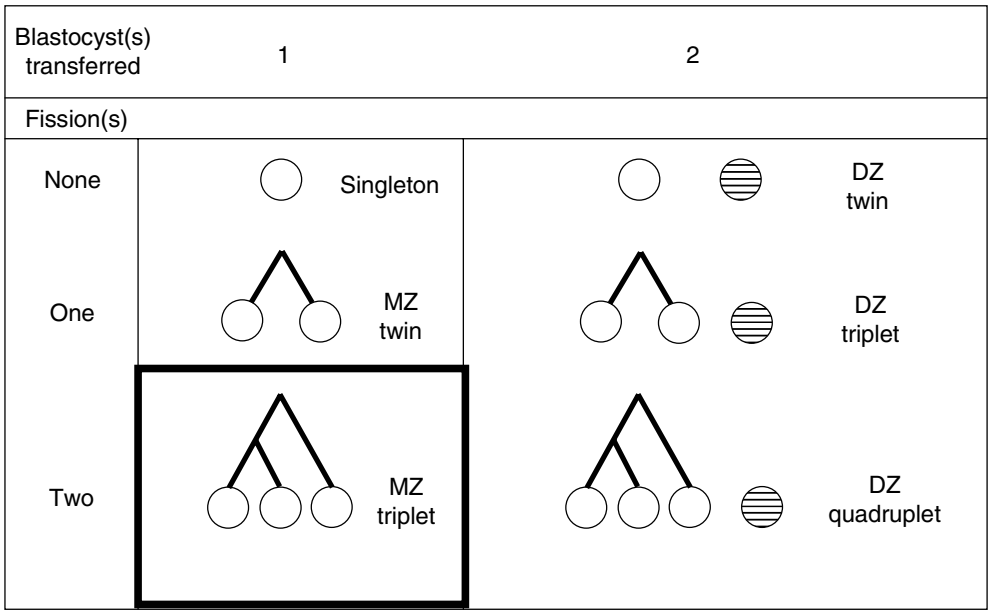

Figure 2
Derom's developmental hypothesis (1987) of multiple births as a function of embryo fission (splitting) events and blastocyst implantation. Delivery outcomes assume 100% implantation. The incidence of human MZ triplets (black box) is estimated at 1:6400 deliveries. DZ = dizygotic MZ = monozygotic.

proven difficult. The potential impact of such iatrogenic influences on monozygotic gestations is reviewed elsewhere [5]. Whether or not spontaneous embryo splitting or duplication might occur as another mechanism by which monozygotic/monochorionic multiplets develop remains controversial. Although rare, the *de novo* appearance of multiple inner-cell mass within a single embryo has been documented both in animals [6,7] and humans [8].

Intrapartum and neonatal challenges of triplet pregnancy include a higher risk of respiratory distress [9] and a known association between intratriplet birthweight discordance and overall neonatal mortality [10]. A recent Japanese study [11] revealed improved perinatal mortality rates among triplets over the interval 1980–1998, but birthweight <2000 g persisted as a poor prognostic indicator of outcome throughout the study period. While the societal cost of multiple births has proven more difficult to study, previous work has shown depression and divorce rates to be higher among parents of multiple births compared to those who have only singleton deliveries [12,13].

Epidemiologists have rightly called attention to the problem of higher-order multiple births when a larger number of embryos are transferred [14], and some authors have suggested that a reduction in multiple gestations can only be obtained by transfer of one embryo after IVF [15]. However, this monozygotic/monochorionic triplet pregnancy occurred in the context of a single blastocyst

implantation after day three transfer of two embryos with IVF, a practice we regard as clinically conservative.

While the largest published series of monozygotic twin sets from one center found the frequency of monozygotic twinning not statistically different between zona manipulated and zona intact subgroups [8], summary research collected from multiple institutions (and embracing an even larger pooled sample) has offered a contrasting view [16]. Indeed, it must be acknowledged that our present report describes a triplet conception and delivery following both ICSI and assisted embryo hatching. Yet whether these zona manipulations were causative or merely associative interventions cannot be fully determined from available data. Further observational studies will be necessary to characterize more completely the physiology of monozygotic/monochorionic multiple pregnancy.

Conclusion

The exact mechanism of monozygotic mutiple gestation is still poorly characterized. Procedures that modify the zona pellucida (*e.g.*, ICSI and assisted embryo hatching) have been suggested as important in the monozygotic multiple gestation hypothesis, yet a definitive relationship between any clinical intervention during IVF and the subsequent development of multiple monozygotic gestation remains speculative. Nevertheless, even when the number of embryos transferred is limited, the potential for spontaneous embryo duplication escapes prediction. Accordingly, counseling should disclose such information during informed consent and offer a full opportunity to discuss number of embryos to transfer.

Competing interests

None declared.

Author contributions

LMG was resident physician associated with the case and drafted the manuscript. MP was chief medical attending. MJT was senior embryologist responsible for cell culture and assisted fertilization. JHZ was the lead obstetrician and primary surgeon at delivery. DPE was consulting perinatologist. ESS conceived of the research, directed the resident, and coordinated manuscript revisions.

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