Monozygotic twin delivery following reduction from quadramniotic-dichorionic gestation established after ICSI and embryo transfer: Case report

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We report the delivery of healthy monozygous (MZ) twins in a 31 year-old nulligravida following gonadotrophin ovulation induction, ICSI, assisted hatching and fresh embryo transfer. Although a sonogram on day 35 confirmed that two of four transferred embryos had implanted, a second transvaginal sonogram 1 week later showed each gestational sac had two conceptuses—all four were associated with distinct amniotic compartments. Cardiac activity was observed in all four embryos. At 12 weeks and 5 days gestation, chorionic villus sampling was performed on fetuses 1 and 2 which were euploid for chromosomes 13, 18, 21, X and Y via fluorescence in-situ hybridization analysis. Subsequent KCl injection into sacs 3 and 4 resulted in asystole for these fetuses, while cardiac activity in sacs 1 and 2 remained unchanged after reduction. A twin vaginal delivery occurred at 36 weeks gestation, resulting in the birth of two male infants and one placenta. This case represents the first known report of human quadruplet pregnancy consisting of two MZ twin sets conceived by assisted reproductive techniques. Our report reviews proposed mechanisms for explaining twinning, with special emphasis on zona pellucida micromanipulation and subsequent MZ twin induction.

Key words: delivery/IVF/monozygotic/quadramniotic/twins

Case report

A 31-year-old non-smoking Caucasian nulligravida was referred for infertility consultation. Her physical examination was normal and body mass index (BMI) was 20 kg/m². Day 21 serum progesterone levels and a biphasic basal body temperature pattern supported ovulatory cycles. Cervical cytology was negative and her blood type was A+. Regular intrauterine contours and bilateral tubal patency were confirmed at hysterosalpingography. The partner’s semen analysis was normal.

The patient had already received four unsuccessful ovulation induction cycles with clomiphene (100 mg/day for 5 days) before referral. We estimated ovarian reserve as reported previously (Perloe et al., 2000), with cycle day 3 serum FSH and estradiol (E₂) levels at 6.3 mIU/ml and 35.4 pg/ml respectively. All other laboratory tests including thyroid stimulating hormone and serum prolactin were normal. The provisional diagnosis of unexplained infertility was made and the couple requested IVF. The initial IVF cycle resulted in the transfer of three embryos (3, 7 and 9 cells/embryo); neither ICSI nor assisted embryo hatching (AH) was done. No pregnancy was established.

After 5 months, a second IVF cycle including mid-luteal pituitary down-regulation with GnRH agonist, and controlled ovarian hyperstimulation using a combined FSH + hMG protocol (Sills et al., 1998) was commenced. Following a 9 day follicular recruitment phase, the patient’s terminal serum E₂ was 2950 pg/ml. Thirteen oocytes were retrieved via transvaginal sonogram-guided needle aspiration, and all advanced to the 2 pronuclei (PN) stage after ICSI (Palermo et al., 1992). AH was accomplished with acid Tyrode’s solution (Tucker et al., 1994). On post-fertilization day 3, four embryos (7, 7, 7 and 8 cells/embryo) were transferred using a co-axial Frydman catheter (Laboratoire CCD, Paris, France) under transabdominal sonographic guidance. Embryo morphology was compromised by moderate fragmentation (15–22%); some blastomere irregularity/asymmetry was also noted. The patient received 10 mg diazepam p.o. 1 h before transfer. None of the non-transferred embryos was considered suitable for cryopreservation. On the evening of embryo transfer, supplemental progesterone was administered transmucosally (Sills et al., 2002). When measured 14 days post-transfer, the serum hCG value was 753 mIU/ml. The patient returned to our centre at 35 days gestation and transvaginal sonogram (probe
of pregnancy, biophysical profile score for both twins was 8/8 and the placenta was grade 1. Fetal biometrics confirmed appropriate growth for twin A, but twin B was ‘borderline small for gestational age’. No sonographic evidence of pathological vascular anastomosis between the twins was evident. At 36 weeks gestation, presentation was vertex/vertex and the patient underwent induction of labour. An uncomplicated vaginal twin delivery occurred within 24 h. The weights at birth were 2495 and 2239 g. For both twins, 1 and 5 min Apgar scores were 8 and 7 respectively. Inspection of the single placenta confirmed monozygosity. Mother and twins were discharged home in good condition on post-partum day 2 and were continuing to do well 3 months later (see Note added at proof).

Discussion

When a single fertilized ovum splits into two genetically identical embryos, MZ twins are believed to develop (Sills et al., 2000a). Such ‘identical’ twins are curiosities that have captivated the imagination since antiquity; portraits of these unusual births—sometimes celebrated, sometimes feared—form an intriguing part of ancient human culture (Margalith, 1994). In modern times the understanding of human twinning has advanced with technical gains in embryology and genetics, but much remains to be learned. Even the identical genome once thought common between MZ twins (Azuma et al., 1989) has been questioned (Machin, 1996).

MZ twins account for ~0.4% of all births (Bulmer, 1970), and early studies found MZ twinning frequency independent of race, age, parity or family history. However, other investigators (Harvey et al., 1977; Shapiro et al., 1978) have suggested that MZ twinning may be affected by an inheritable component. The roles of ovulation induction, in-vitro culture conditions, and zona pellucida tampering in the MZ twinning process also remain controversial and have been reviewed elsewhere (Sills et al., 2000b; Schachter et al., 2001).

Our patient elected to undergo multifetal reduction after she considered the risks associated with higher order multiple gestation. The possibility of a spontaneous reduction was also discussed (Dickey et al., 2002) but the couple found expectant management unacceptable. In such settings, the role of supportive counselling cannot be overstated (Garel et al., 1995). The reassuring chromosomal status present in one MZ twin pair via CVS and FISH analysis reflects current recommendations for genetic testing before multifetal reduction is performed (Eddleman et al., 2000). While the overall pregnancy loss rate after multifetal reduction is influenced by operator experience (Evans et al., 1993), most referral centres have sufficient proficiency with the procedure to make the risk acceptable.

Several investigators have called attention to the increased incidence of higher-order multiple gestations following IVF, but such outcomes are almost always related to the number of embryos transferred rather than physiological twinning events. Nevertheless, exceptional cases have been documented. A trizygotic quadruplet pregnancy after IVF and transfer of only three embryos (Biljan et al., 1995) illustrates the need to
consider natural twinning events in assisted reproductive settings. Another report of quadrichorionic-quadruplet pregnancy (after IVF with implantation of two transferred embryos) found two additional gestational sacs from a concurrent pregnancy (after IVF with implantation of two transferred embryos) and another report of quadrichorionic-quadruplet pregnancy after assisted hatching. The challenge of harmonizing conflicting observations is an unanswered challenge when the number of MZ twin sets reported no significant increase in MZ twinning after AH, ICSI or frozen embryo transfer (Sills et al., 2000). How to reconcile our anecdotal report of ‘double monozygosity’ after AH and ICSI with these conflicting observations is an unanswered challenge inviting continued research. The possibility of spontaneous MZ twinning, while still a remote risk, should be included in IVF patient counselling until more is known.

It is acknowledged that this double MZ twin-quadruplet pregnancy after AH and ICSI appears congruent with some studies where a connection between MZ twinning and zona pellucida tampering was hypothesized (Sheiner et al., 2001; Tarlatzis et al., 2002). However, a larger series of more than 1900 IVF cycles including 23 MZ twin sets reported no significant increase in MZ twinning after AH, ICSI or frozen embryo transfer (Sills et al., 2000a). How to reconcile our anecdotal report of ‘double monozygosity’ after AH and ICSI with these conflicting observations is an unanswered challenge.

Note added at proof
Mother and twins were discharged home in good condition on post-partum day 2 and were continuing to do well 6 months later.

References


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