Objective: To describe a case of monozygotic triplet pregnancy following egg donation and the transfer of a single frozen-thawed embryo.

Design: Case report.

Setting: District general hospital and regional department of fetal medicine in northeast England.

Patient(s): A 38-year-old woman with a 2-year history of primary infertility due to severe endometriosis and poor ovarian reserve who conceived after egg donation and transfer of a single frozen-thawed embryo.

Intervention(s): Transfer of a single frozen-thawed embryo and delivery of three identical female infants by emergency caesarean section because of preterm labor at 32 weeks’ gestation.

Main Outcome Measure(s): Review of effect of assisted conception on monozygotic twinning rates.

Result(s): Healthy outcome for all three infants.

Conclusion(s): Assisted reproductive treatments may lead to disturbances in zona pellucida architecture and an increase in monozygotic twinning rates. Couples need to be informed of this and the increased risks associated with these pregnancies before they begin with treatment. (Fertil Steril 2008;89:1260.e9–12. ©2008 by American Society for Reproductive Medicine.)

Key Words: Monozygotic triplets, ART, zona pellucida
Regular fetal surveillance was carried out with serial growth scans, umbilical artery Doppler studies, and liquor volume assessments. The results remained in the normal range for all three fetuses with no evidence of twin–twin transfusion. Dexamethasone was administered every 2 weeks from 24 weeks’ gestation to accelerate fetal lung maturity at a dose of 24 mg in two divided doses, 12 hours apart. The aim was to try to progress the pregnancy to 34 weeks’ gestation and then deliver by caesarean section.

The antenatal course was complicated by the presence of anti-c and anti-E antibodies in the maternal serum, probably secondary to blood transfusion during the patient’s previous surgery. Serial titers performed during the pregnancy revealed the levels of antibody remained very low and did not require further intervention.

Impaired glucose tolerance was diagnosed at 30 weeks’ gestation and was managed by dietary modification.

The patient presented at 32 weeks’ gestation in advanced labor and was delivered by emergency caesarean section. Three healthy female babies were delivered, weighing 1695 g, 1560 g, and 1500 g, respectively. A single placenta was also delivered. All babies were in good condition at birth with normal arterial blood gases. Each cord was normal containing three vessels. A microscopic examination of the placenta and membranes confirmed that the placenta was normal with a triamniotic monochorionic configuration.

**DISCUSSION**

The desirable increase in total pregnancy rates after assisted reproductive techniques (ART) has been associated with a less desirable increase in the rate of multiple pregnancies. The majority of cases of multiple pregnancies in IVF occur as a result of successful uterine implantation after the transfer of more than one embryo to ensure a pregnancy (2). As multiple pregnancies are associated with a higher perinatal mortality, reducing the number of embryos transferred has been suggested as a method of reducing the incidence of multiple pregnancy in IVF (3, 4). With the development of prolonged culture and blastocyst transfer, it has been shown that in “good prognosis” patients the transfer of a single blastocyst is an efficient way of avoiding multiple pregnancies without decreasing the pregnancy rate (5). However, this will not always be the case, as the patient we describe illustrates: even limiting the number of embryos transferred to one embryo does not exclude the possibility of multiple pregnancy. Monozygotic twinning after blastocyst transfer was first described in 1999 by Peramo et al. (6) and has an estimated incidence of 4.3% (7).

Monozygotic multiple pregnancies are also known to occur more frequently after ART than following spontaneous pregnancies. This phenomenon was first reported in 1984 (8). The incidence of monozygotic twinning in the general population has been calculated to be approximately 1 in 250 (0.42%) (9, 10). A recent review of the published literature suggested a monozygotic twinning rate of 1.51% after ART (11).

Intrapartum and neonatal challenges of triplet pregnancy include a higher risk of respiratory distress and a known association between intratritplet birthweight discordance and overall neonatal mortality. Monozygotic multiple pregnancies have an increased risk of adverse outcome as compared with dizygotic multiple pregnancies. This increased risk arises from congenital malformations, cord entanglement, and the complications associated with monochorionic placentation; fetal loss between 10 and 24 weeks of gestation occurs in about 12% of monochorionic twin pregnancies, compared with 2% for dichorionic twins or singletons (12). Much of the excess second trimester mortality in monochorionic pregnancies is due to the twin-to-twin transfusion syndrome, where one twin shunts blood to the other through vascular connections in the placenta. These vascular anastomoses, which are in about 96% of monochorionic twin pregnancies (13), are also responsible for the neurodevelopmental problems seen in up to 28% of the survivors following in-utero single fetal death (14).

Monozygotic triplets have been previously reported following transfer of frozen-thawed embryos (15), but we believe this is the first reported case of monozygotic triplets arising as a result of egg donation and transfer of a single frozen-thawed embryo.

The chorionicity of a monozygotic multiple pregnancy depends on the time of splitting of the embryo. Early splitting (within 72 hours of fertilization) will lead to diamniotic, dichorionic multiple pregnancy; later splitting (after day 8) may give rise to monoamniotic, monochorionic multiple pregnancy.

The timing of monozygotic twinning is probably not fixed, and the mechanism varies (16). In 2002, Tarlatzis et al. (17) reported only on monochorionic-diamniotic twins. As this type is formed after the splitting of the inner cell mass 4 to 8 days after fertilization, they concluded that the embryonic division happens closer to the time of implantation.

In the case we describe, the possibilities are that the embryo split initially between day 4 and day 8 and that one of the resulting embryos split again soon after giving rise to monochorionic, triamniotic triplets, or that multiple fragmentation of the initial embryo led to the formation of the triplets.

How ART leads to an increase in monozygotic multiple pregnancy is not fully understood. It has been suggested that the increased incidence of monozygotic multiple pregnancy is merely a reflection of the number of embryos transferred and that the rate of monozygotic twinning per embryo transferred is not significantly different from that in the general population (18). However, the case reported here and other case reports of monozygotic multiple pregnancy arising after transfer of a single embryo would not substantiate this hypothesis.
Previous investigators (19, 20) 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. It is speculative whether these three factors are involved in the causation of monozygotic multiple pregnancy or whether the added impact of prolonged freezing process (as in our case) is implicated. Disturbance in the architecture of the zona pellucida (21) may arise as a result of exposure to artificial media, which may lead in turn to abnormal zona hardening with subsequent increased zona fragility. Abnormal zona consistency could theoretically lead to abnormalities in blastocyst hatching and a subsequent increase in monozygotic multiple pregnancies. The longer an embryo is exposed to artificial culture media, the more likely that the zona will be affected; this may be a relevant factor in the increased incidence of monozygotic twinning reported after transfer of the embryo at the blastocyst stage (22).

Mechanical breaching of the zona occurring during micromanipulation techniques such as intracytoplasmic sperm injection (ICSI) or assisted hatching may further affect the integrity of the zona pellucida, which could further increase the rate of monozygotic twinning. The evidence to support this is conflicting. Sills et al. (18) reported that the increased rate of monozygotic twinning following ART appeared to be independent of micromanipulation, but others have reported increased rates of monozygotic twinning following IVF/ICSI (23). A major problem in clarifying this issue relates to the small numbers of cases involved. To achieve conclusions with statistical significance, very large sample sizes are required.

The structure of the zona pellucida changes with advancing age of the woman, leading to more irregularity in zona thickness; this may account for the age-related increase in monozygotic twinning rates. In the case described here, although the patient was 43 years of age, the egg donor was only 27, so age is unlikely to have been an important factor.

In the case described, the pregnancy arose after cryopreservation of the embryo. If disturbances of the zonal architecture do contribute to increased monozygotic twinning rates, it is possible that the process of cryopreservation and thawing of the embryo may affect the integrity of the zona pellucida. Ovulation induction also has been theoretically linked to abnormalities in blastocyst hatching and a subsequent increase in monozygotic multiple pregnancies (20). This suggests that exposure to ovulation induction drugs such as clomiphene citrate and gonadotropins also could be an important factor.

Due to the increased risks associated with monozygotic multiple pregnancies, it is important that the diagnosis be made early. Fortunately, scans performed early in the first trimester after successful ART allow an early diagnosis. It is also easier to assess the type of placentation from an early scan, though as was evident in this case it is not always easy to visualize the thin dividing amnion.

In high-order multiple pregnancy, fetal reduction has an important role to play. However, selective feticide in monochorionic multiple pregnancies is problematic. Potassium chloride injected into the heart of one fetus might enter the circulation of the other fetuses through placental vascular connections. Umbilical cord ligation or diathermy is less likely to cause immediate co-fetal demise, but there is concern regarding the potential for acute exsanguination into the placenta of the dead fetus(es).

It is important that steps are taken during ART to reduce the risks of multiple pregnancy. The process by which suitable patients are selected for transfer of a single high quality embryo at the blastocyst stage is likely to reduce the overall rate of multiple pregnancy after ART. However, this may come at the expense of increasing the relative incidence of monozygotic multiple pregnancy, with all its attendant risks. All couples should be informed of the potential risk of a high-order multiple pregnancy after IVF, even when only one or two embryos are transferred.

**REFERENCES**

7. Racowsky C, Jackson KV, Cekleniak NA, Fox JH, Hornstein MD, Ginsburg ES. The number of eight-cell embryos is a key determinant for selecting day 3 or day 5 transfer. Fertil Steril 2000;73:558–64.