Successful monozygotic twin delivery following in vitro maturation of oocytes retrieved from a woman with polycystic ovary syndrome: Case Report

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The incidence of monozygotic twinning (MZT) appears to be increasing within the field of assisted reproductive technology (ART), although the factors contributing to the phenomenon are still far from being identified. On the contrary, in vitro maturation (IVM) of oocytes is becoming more accepted and more and more babies have been born worldwide using this procedure. Assessing its safety and impact on monozygotic twinning (MZT), and following up the health of these babies, is essential. We report here a first case of successful monozygotic (MZ) twin delivery following IVM.

The patient was a 28-year-old Japanese female, referred to the IVF clinic for primary infertility. Several previous cycles of ovarian stimulation had resulted in ovarian hyperstimulation syndrome (OHSS). The patient received norethisterone–mestranol to initiate the menstruation, and oocyte retrieval was performed 36 h after hCG. A total of 22 immature oocytes were obtained. Following incubation for 24 h in IVM medium, 50% of the oocytes were matured to the metaphase II (MII) stage. Nine oocytes were fertilized after ICSI with the husband’s sperm. Three day 3 embryos were transferred into the uterus on the fourth day following oocyte retrieval. Three weeks after embryo transfer, a single gestational sac was visualized in the uterus. At 7 weeks of gestation, two fetal poles with cardiac activity were seen in the single gestational sac. Serial ultrasound examinations revealed a MZ, monochorionic diamniotic pregnancy. After intensive perinatal monitoring, two healthy male infants were delivered by Caesarean section at 35 weeks of gestation.

Key words: IVF-ET/in vitro maturation/monozygotic twinning/PCOS/prolonged in vitro culture

Introduction

Monozygotic (MZ) twins are considered to develop when a single fertilized ovum splits into two genetically identical embryos (Sills et al., 2000a). However, the precise mechanism responsible for this division is poorly understood. Monozygotic twinning (MZT) is a rare phenomenon in humans and occurs in just approximately 0.4% of spontaneous pregnancies (Bulmer, 1970; MacGillivray, 1986). Early studies reported that the frequency of MZT was independent of race, age, parity or family history. However, there have been many reports that assisted reproductive technology (ART) significantly increases the incidence of MZT. The effects of ovulation induction (Derom et al., 1987), extended in vitro culture (Milki et al., 2003), zona pellucida manipulation (Tarlatzis et al., 2002) and multiple embryo transfer (Sills et al., 2000b) in the MZT process have been reviewed elsewhere (Sills et al., 2000a; Schachter et al., 2001) but still remain controversial. Two of the growing candidates linked to this phenomenon are culture conditions and prolonged in vitro culture.

In vitro maturation (IVM) of immature oocytes has been proposed as a potential alternative for conventional IVF treatment. The first report of a successful pregnancy following IVM of immature oocytes in a woman with anovulatory infertility was made in 1994 (Trounson et al., 1994). This procedure is performed in only a limited number of institutes worldwide. As the number of births from IVM is still relatively small, prospective and extensive follow-up is mandatory so as to assess its safety and the impact on embryonic development.

In this case report, we describe the first successful delivery of MZ twins resulting from IVM oocytes derived from an unstimulated ovary.

Case report

A 28-year-old nullgravida Japanese female was referred to the IVF NAMBA CLINIC with primary infertility. She had been married for 3 years and received several courses of ovulation induction with urinary FSH, resulting in repeated incidences of
oocyte-handling procedures were conducted in a mini-chamber. A portable aspiration pump was used with a pressure between 160 and 180 mmHg. The aspirates were collected in 10 ml tubes containing pre-warmed heparinized human tubule fluid (HTF) medium. The follicular aspirates were filtered (70 μm mesh, Falcon 1060; Becton Dickinson, NJ, USA) and washed with pretreatment medium (IVM-LAG; MediCult, Copenhagen, Denmark) to remove erythrocytes and small cellular debris. The remaining cells were then re-suspended in the medium, and the oocytes were isolated under a stereomicroscope. All oocytes were suspended in the medium (IVM®; MediCult) at 37.5 °C in an atmosphere of 5% CO₂, 5% O₂ and 90% N₂. The medium was supplemented with 10% patient’s serum collected from the patient 24 h after HCG administration. Patient blood was collected in a sterilized glass tube and allowed to coagulate at room temperature and then centrifuged at 1600 g for 5 min. Supernatant was collected and inactivated for 30 min at 56°C in a water bath, then added to the medium. Twenty-six hours post collection, the oocytes were denuded with hyaluronidase (Sigma Chemical, St. Louis, MO, USA) and mechanical pipetting. Mature oocytes [metaphase II (MII)] were identified by the presence of the first polar body.

Sperm were prepared by 90% Percoll separation at 300 g for 20 min. After Percoll separation, motile sperm were collected using a swim-up method with 10 ml of culture medium (universal IVF; MediCult). All MII oocytes were fertilized by ICSI. Zygotes were then cultured in the culture medium (universal IVF; MediCult), and fertilization was assessed 16–18 h after ICSI for the appearance of two distinct pronuclei and two polar bodies. Fertilized zygotes were transferred into culture medium (IVC-1, In Vitro Care, MD, Frederick, USA). Day 2 embryos with sufficient viability were exposed to protease solution (1 μg/ml Protease type XIV; Sigma Chemical) for 24 h (biochemical-assisted hatching; Lee et al., 1997; Fukuda et al., 2001b). Day 3 embryos were transferred on the fourth day after oocyte retrieval.

A total of 22 immature oocytes were obtained, and 11 oocytes reached the MII stage after 24 h of culture. Nine oocytes were fertilized after ICSI and cleaved. Three embryos (two eight-cell embryos of grade 1 and one nine-cell embryo of grade 1) were transferred 3 days after fertilization. On the day of embryo transfer, the endometrial thickness was 10 mm on ultrasound. Supernumerary high-grade embryos were cryopreserved by vitrification. Progesterone (Proge depot® 125 mg, Mochida, Tokyo, Japan) was administered i.m. on the day of embryo transfer. Transcutaneous estradiol (Estraderm, Kissei Pharm, Matsumoto, Japan; four sheets every 2 days) and transvaginal progesterone (400 mg of progesterone suppository daily, made in our pharmacy) were administered until 9 weeks of gestation.

Two weeks after embryo transfer (4+3/7 weeks of gestation), the serum hCG concentration was 294 IU/l and pregnancy was confirmed. Three weeks after embryo transfer (5+3/7 weeks of gestation), transvaginal ultrasound revealed a single intrauterine gestational sac (Figure 1). Follow-up ultrasound evaluation was performed every week. At 6 weeks of gestation, a single growing gestational sac was recognized, but it contained two distinct fetal poles, both with cardiac activity, suggesting a MZ twin pregnancy. The following week, two fetal poles in a single gestational sac were identified by two other physicians and a MZ twin pregnancy was diagnosed (Figure 2). At 10 weeks of gestation, this was reconfirmed and a thin septum between the two fetuses helped in the definite diagnosis of a monochorionic diamniotic twin pregnancy (Figure 3). A month later with continuing ultrasound, the patient was referred to an obstetrician. The obstetrical course was closely observed for complications, particularly those related to MZT such as twin–twin transfusion syndrome (TTTS). Mild discordancy of estimated fetal birthweight was recognized at 30 weeks of gestation by ultrasonography. Intensive examinations of fetal umbilical cord blood flow, amniotic fluid volume and cardiotocography were continued. At 35+4/7 weeks of gestation, selective Caesarean section was performed and two healthy male infants were delivered with 5 min Apgar scores of 8 and 10, respectively. The weights at birth were 2470 g and 1840 g, respectively. Monochorionic diamniotic placenta was confirmed by macroscopical inspection.

![Figure 1](https://academic.oup.com/humrep/article-abstract/21/7/1777/2938553/1778)

**Figure 1.** Transvaginal ultrasound at 5+3/7 weeks of gestation. A single gestational sac 5 mm in diameter was visualized in the uterus.
successful twin births after ivm

Figure 2. Transvaginal ultrasound at 7+3/7 weeks of gestation. Two fetuses with cardiac activity were identified in the single gestational sac, and a monozygotic pregnancy was suspected.

Figure 3. Transvaginal ultrasound at 10+3/7 weeks of gestation. A thin septum between two fetuses led to the definite diagnosis of a monochorionic diamniotic twin pregnancy.

Discussion

The incidence of MZT appears to be increasing within the field of assisted human reproduction. Many hypotheses have been proposed in relation to this phenomenon. The East Flanders Prospective Twin Study first reported an increased risk of MZT after fertility treatment with ovulation induction (Derom et al., 1987). Another study found that the MZT rate was higher after ovulation induction than other forms of ART and that perhaps exposure to gonadotropin might be the cause of the increase (Schachter et al., 2001). Associations with zona pellucida micromanipulation (assisted hatching, ICSI) and zona hardening (due to prolonged in vitro culture, blastocyst transfer, frozen embryo transfer and so on) have been described by several authors (Peramo et al., 1999; Behr et al., 2000; Van Langendonckt et al., 2000; da Costa et al., 2001; Steiner et al., 2001b) in up to 5% of ongoing pregnancies. The ‘zona shearing’ hypothesis following these zona altering treatment has been most emphasized, where the inner cell mass (ICM) is trapped by an altered zona pellucida during the hatching process and divides into two identical embryos (Sills et al., 2000). But it is unlikely to provide the full answer to MZ outcomes after fertility treatment because recent studies reported no significant increase in MZT after zona micromanipulation (Sills et al., 2000b; Schachter et al., 2001), and even monozygosity can arise independently of the zona pellucida (Frankfurter et al., 2004). Growing evidence supports the existence of polarity within mammalian oocytes, and culture conditions could increase the risk of MZ by altering polar gradient formation within the oocyte and early embryo (Scott, 2002). It has been suggested that culture conditions can lead to MZT, that is, that excessive amount of glucose or free radicals induce apoptosis (Ménézo and Sakkas, 2002) or lower calcium activity by chelators included in the culture medium, which weakens interblastomeric bonds (Steinman and Valderrama, 2001). Factors contributing to this phenomenon might include prolonged in vitro culture, which is considered to be one of the main factors linked to MZT.

IVM treatment has several potential advantages including lower treatment costs (less drugs and monitoring), reduced health risks (reduced incidence of OHSS) and increased convenience to the patient (fewer blood tests and ultrasonographic monitorings and no daily injections) compared with conventional IVF treatment. However, the main difference between IVM and conventional IVF is a longer period of in vitro culture before insemination and one more day of culture of immature oocytes. If MZT is mainly a phenomenon linked to prolonged culture, IVM might be one of the factors that increase its incidence. In our case, embryos were cultured in vitro for 4 days (1 day before fertilization and 3 days after ICSI), a period 1 day shorter than blastocyst stage transfer in ordinary IVF.

It is unknown whether biochemical-assisted hatching has any effect on MZT. As far as we know, there is no report of protease exposure affecting monozygosity and this is our first case of MZT after this procedure. Prospective follow-up is necessary to assess its impact on embryo development.

To date, 165 pregnancies from IVM oocytes have been reported in Europe (Medicult IVM network, http://www.medicult.com/). We have obtained 41 healthy babies from IVM oocytes since our first success in 1999 (Fukuda et al., 2001a). In addition, IVM has been actively performed in countries such as Korea and Canada, and obstetric outcomes have been evaluated (Cha et al., 2005; Le Du et al., 2005; Mikkelsen, 2005).

Approximately 500 live births following IVM are estimated to occur worldwide now (Chian, 2004), and this is the first reported case of MZT following IVM. The incidence is not as high as the MZT rate in ART (1–5%), which demonstrates that IVM itself does not increase the risk of MZT. However, due to the small number of babies born following IVM worldwide, it is still not possible to categorically state that IVM does not have an impact on MZT.

In conclusion, we report here a first case of MZT from IVM oocytes and successful delivery after intensive perinatal care. To elucidate the incidence of MZT following IVM, we recommend the worldwide experience of IVM be collated by a central body so as to fully assess the impact of this procedure on MZ twinning, fetal development and the health of the babies born.
References


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