Monozygotic triplets and monozygotic twins after ICSI and transfer of two blastocysts: case report

Solvejg Unger, Markus Hoopmann, Rainer Bald, Dolores Foth and Frank Nawroth

Department of Obstetrics and Gynecology, University of Cologne, Kerpener Str. 34, 50931 Cologne, Germany

1To whom correspondence should be addressed: Frank.Nawroth@medizin.uni-koeln.de

There are data regarding the possible influences of extended embryo culture to the blastocyst stage as well as zona pellucida manipulation on the incidence of monozygotic multiples. This is interesting, as one aim of extended culture with embryo selection is to minimize the multiple pregnancy rate. We report, to our knowledge, on the first case of monozygotic twins and monozygotic triplets after ICSI and the transfer of two blastocysts. Monozygotic multiples after ICSI and blastocyst transfer and the resulting problems are another reason to encourage the transfer of only one blastocyst. Theories about risk factors and the pathophysiology of monozygotic multiples will be discussed both in terms of this case report and of the literature. In our opinion, the incidence of 5.9–8.9% monozygotic multiple occurrence after ICSI and blastocyst transfer reported in the literature requires that patients are informed of the uncertainties until this phenomenon and its risk factors are better understood.

Key words: blastocyst culture/ICSI/monozygotic triplets/monozygotic twins/zona pellucida manipulation

Introduction

It has long been a topic of discussion as to whether ovulation induction (Derom et al., 1987), all assisted reproductive techniques (Saito et al., 2000; Schachter et al., 2001), blastocyst culture (Peramo et al., 1999; Da Costa et al., 2001) or assisted hatching (Schieve et al., 2000) leads to a higher rate of monozygotic twins. The possible influences of an extended embryo culture to the blastocyst stage on the incidence of monozygotic multiples could be interesting, as one reason for performing this effective form of embryo selection is to minimize the multiple pregnancy rate. In Germany in 2001 the multiple pregnancy rate after assisted reproductive techniques was 40% (Felberbaum, 2003). A further decrease is problematic as the German embryo protection law does not allow embryo selection, only selection at the pronuclear stage. Only as many pronuclear stage oocytes are allowed to be selected as are planned to be transferred in the same cycle. After pre-selection of, for example, three pronucleated oocytes, these three must be transferred and a second selection process is not allowed (Ludwig et al., 2000). Therefore some German patients undergo IVF or ICSI in other countries to benefit from blastocyst culture with increased implantation and decreased multiple pregnancy rates.

We report, to our knowledge, on the first published case of monozygotic twins and monozygotic triplets after ICSI and the transfer of two blastocysts. We discuss the literature on the possible connection between monozygotic multiples, ICSI and blastocyst transfer.

Case report

An ICSI cycle was planned for a 38 year old patient due to andrological subfertility. Due to the long distance between the home-town and the foreign therapeutic centre with its resulting logistic problems, ejaculated sperm from the man were frozen before starting ovarian stimulation. The stimulation was performed with recombinant FSH and hMG in a down-regulated cycle according to the short protocol. Ten oocytes were aspirated during follicular puncture, of which eight were in metaphase II. A further decrease is problematic as the German embryo protection law does not allow embryo selection, only selection at the pronuclear stage. Only as many pronuclear stage oocytes are allowed to be selected as are planned to be transferred in the same cycle. After pre-selection of, for example, three pronucleated oocytes, these three must be transferred and a second selection process is not allowed (Ludwig et al., 2000). Therefore some German patients undergo IVF or ICSI in other countries to benefit from blastocyst culture with increased implantation and decreased multiple pregnancy rates.

We report, to our knowledge, on the first published case of monozygotic twins and monozygotic triplets after ICSI and the transfer of two blastocysts. We discuss the literature on the possible connection between monozygotic multiples, ICSI and blastocyst transfer.
Discussion

The main focus of attention in IVF treatment over the last few years has been on the reduction of multiple pregnancies. One possibility is to decrease the number of embryos transferred. The selection of one or a maximum of two embryos after prolonged culture for 5–6 days is one way to achieve an acceptable pregnancy rate with this strategy. It has been published that in ‘good prognosis’ patients the single blastocyst transfer is an efficient way of avoiding multiple pregnancies without decreasing the pregnancy rate (Gardner et al., 2000; Veiga, 2003). Additionally, the single transfer could avoid the risk of heterotopic pregnancies with the often delayed diagnosis of an extrauterine pregnancy (Hoopmann et al., 2003).

Recently a discussion started regarding the possible increased risk of monozygotic twinning following IVF (Alikani et al., 2003) especially after prolonged culture and blastocyst transfer in comparison to the transfer of 4–8 cell stage embryos (Da Costa et al., 2001). This would be in contrast to one aim of extended culture: the selection of one blastocyst to achieve a single pregnancy. Monozygotic twinning with an estimated incidence of 4.3% after blastocyst transfer (Racowsky et al., 2000) was first described by Peramo et al. (1999).

There are also reports of an increased monozygotic twinning rate without prolonged culture (Schachter et al., 2001). It was postulated that culture conditions and not prolonged culture time are responsible for monozygotic twinning (Ménézo et al., 2003). Belaisch-Allart et al. (1995) reported on a monozygotic triplet pregnancy following the transfer of frozen–thawed embryos. Salat-Baroux et al. (1994) described trizygotic quintuplets (monoamniotic triplets with two additional fetal sacs) after IVF and the transfer of four embryos without zona pellucida manipulation or extended culture. The patient aborted after fetal reduction. A dichorionic–quadramniotic pregnancy after ICSI, assisted hatching and embryo transfer was recently published (Wehbe et al., 2003).

While Behr et al. (2000) described an increased incidence of monozygotic twins (5%) after IVF and blastocyst transfer, Abusheika et al. (2000) and Tarlatzis et al. (2002) compared IVF and ICSI and found a significantly higher rate only after ICSI and blastocyst transfer (8.9 versus 0%; 5.9 versus 0%). The increased incidence after ICSI and blastocyst transfer could be the result of changes in the zona pellucida during extended culture or of the artificial opening of the zona pellucida during ICSI (Kolibianakis and Devroey, 2002; Tarlatzis et al., 2002).

Another form of zona pellucida manipulation which could lead to an increased rate of monozygotic multiples is assisted hatching (Hershlag et al., 1999; Schieve et al., 2000). In the case of our patient the oocytes were fertilized with ICSI but assisted hatching was not performed.

A single artificial zona pellucida opening (ICSI and/or assisted hatching) may alter the hatching procedure and could cause constriction following bisection of the trophoblast and

Figure 1. The monozygotic (monochorionic, triamniotic) triplets and one of the monozygotic (monochorionic, diamniotic) twins in the 11th week.
the inner cell mass, which results in twinning. Repeated manipulation could promote multiple herniation, resulting, for example, in monozygotic triplets due to an unexpected splitting of the inner cell mass (Tarlatzis et al., 2002). It has not been proven that monozygotic twinning is really age-related but zona pellucida changes during ageing (for example, decreased flexibility and/or increased thickness) could influence hatching.

In contradiction to the above-mentioned studies, Sills et al. (2000) did not find a connection between the incidence of monozygotic twinning and assisted hatching, ICSI or the transfer of cryopreserved embryos.

The timing of monozygotic twinning is probably not fixed and the mechanism varies (Scott, 2002). Tarlatzis et al. (2002) reported only on monochorionic–diamniotic twins. As this type is formed after the splitting of the inner cell mass 4–8 days after fertilization, they concluded that the embryonic division happens closer to the time of implantation. Our patient’s pregnancy was also monochorionic–triamniotic and monochorionic–diamniotic respectively, which promotes the suggested mechanism. Recently an interesting case was published demonstrating that monochorionic twins can be dizygotic (Souter et al., 2003). There is only speculation regarding the underlying embryological event. In our case an amniocentesis of the triplets showed three females and the twins were also two female fetuses. The overall micromanipulation data together with the unexpected placentation data suggest that zona-mediated embryo splitting is not the only mechanism of twinning following assisted reproduction (Alikani et al., 2003).

Familial monozygotic twinning is very rarely seen and can be transmitted by both the father and the mother (Hall, 2003). Steinman (2003) examined the possible role of inheritance in monozygotic twinning and found an influence of genetics and familial clustering on the monozygotic twinning rate, especially in IVF procedures.

It can be summarized from the literature that prolonged culture as well as manipulations of the zona pellucida (ICSI and/or assisted hatching) could lead to an increased rate of monozygotic multiples. To minimize the resulting risks such as increased abortion rates, increased perinatal morbidity and mortality (Al-Nuaim and Jenkins, 2002), assisted hatching in combination with ICSI and blastocyst culture should be critically performed. The aim of therapy should be the transfer of only one blastocyst in as many patients as possible. In our opinion the incidence of 5.9–8.9% monozygotic multiples after ICSI and blastocyst transfer reported in the literature requires the prior informing of patients of the uncertainties until this phenomenon and its risk factors are better understood.

Acknowledgement
The authors would like to thank Eva Kortstegge for her careful translation of the paper.

References


Racovsky C, Jackson KV, Cekleniak NA, Fox JH, Hornstein MD and Ginsburg ES (2000) The number of eight-cell embryos is a key determinant for selecting day 3 or day 5 transfer. Fertil Steril 73,558–564.


Submitted on July 22, 2003; accepted on October 1, 2003.