Case Report

Monozygotic Triplets and Dizygotic Twins following Transfer of Three Poor-Quality Cleavage Stage Embryos

Reshef Tal, Dmitry Fridman, and Richard V. Grazi

Division of Reproductive Endocrinology and Infertility, Maimonides Medical Center, No. 1355, 84th Street, Brooklyn, NY 11219, USA

Correspondence should be addressed to Reshef Tal, resheft@gmail.com

Received 16 November 2012; Accepted 12 December 2012

Academic Editors: G. Capobianco, D. Hochner-Celnikier, I. MacKenzie, and E. Shalev

Copyright © 2012 Reshef Tal et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Assisted reproductive technology has been linked to the increased incidence of monozygotic twinning. It is of clinical importance due to the increased risk of complications in multiple pregnancies in general and in monozygotic twins in particular.

Case. A 29-year-old female, nulligravida underwent her first IVF cycle. Three poor-quality cleavage stage embryos were transferred resulting in monochorionic triamniotic triplets and dichorionic diamniotic twins. Selective embryo reduction was performed at 12 weeks leaving dichorionic twins. The patient underwent emergency cesarean section due to preterm labor and nonreassuring fetal heart tracing at 30 weeks of gestation.

Conclusion. Our case emphasizes that even embryos with significant morphological abnormalities should be considered viable and the possibility of simultaneous spontaneous embryo splitting must be factored into determining number of embryos to transfer.

1. Introduction

Although assisted reproductive technology (ART) has improved the chances of many subfertile couples to achieve pregnancy, it has increased the occurrence of multifetal pregnancies, most of which are dizygotic twins due to the transfer of more than one embryo. ART has also been linked to the increased incidence of monozygotic twinning, which is of clinical importance due to the increased risk of complications in multiple pregnancies in general, and in monozygotic twins in particular. Multifetal pregnancies are characterized by increased risk of both fetal and maternal complications. Fetal complications include restricted growth, pregnancy loss, preterm delivery, and perinatal mortality. Maternal complications include increased nausea and vomiting, gestational hypertension, and preeclampsia. In addition, monozygotic twin pregnancies carry specific risks, depending on their amnionicity, including twin to twin transfusion syndrome and cord entanglement. Numerous studies indicate that monozygotic twinning rates following ART procedures are between two and twelve times higher than the natural occurrence of 0.4% [1–4]. Several cases of monozygotic triplets as a result of ART procedures have also been reported [5–10]. The reason for the increased incidence of monozygotic twinning after ART has been a matter of debate for a long time and there is no definite explanation. However, various factors have been shown to be associated with monozygotic twinning. These include techniques in which there is zona pellucida manipulation such as intracytoplasmic sperm injection (ICSI) [11, 12] or assisted hatching [13, 14], and also after frozen embryo transfer [1] and/or blastocyst transfer [15, 16].

Here, we report a case of quintuplet pregnancy consisting of monozygotic (monochorionic triamniotic) triplets and dizygotic (dichorionic diamniotic) twins following transfer of three cleavage stage embryos. Remarkably, it occurred despite the poor quality of the transferred embryos and in the absence of any of the aforementioned risk factors for monozygotic twinning.

2. Case Report

An IVF cycle was planned for a 29-year-old nulligravida patient with unexplained infertility. Prior to this, she had one failed intrauterine insemination (IUI) and three...
failed IUI cycles with clomiphene citrate. The stimulation was performed with recombinant FSH and hMG using a short protocol with GnRH antagonist. Fourteen oocytes were aspirated during follicular puncture. Seven oocytes were fertilized and cultured. The cultured embryos were of relatively poor quality and the grading of the three embryos selected for transfer on day 3 was 6-cell, grade C; 4-cell, grade B; 4-cell grade C [17]. About 4 weeks later, three intrauterine gestational sacs were noted by transvaginal ultrasound (Figure 1). At 7 weeks of gestation, a quintuplet pregnancy with monozygotic (monochorionic, triamniotic) triplets and dizygotic (dichorionic diamniotic) twins was sonographically confirmed (Figure 2). All five embryos demonstrated cardiac activity and were discordant. The patient was counseled and referred for selective fetal reduction of the monochorionic triamniotic triplets. This was performed without complications on the 12th week of her pregnancy. Amniocentesis of the triplets during fetal reduction showed normal chromosomes (46, XX) and no structural abnormalities. The pregnancy was subsequently complicated by preterm labor followed by emergency cesarean delivery due to nonreassuring fetal tracing resulting in delivery of healthy female and male babies at 30 weeks of gestation. Both babies were born in 2006 and are currently doing well.

3. Discussion

A major current focus in the field of ART has been on the reduction of multifetal gestation rates. One way of achieving this is the selection of one or a maximum of two embryos after prolonged culture for 5-6 days. It has been shown that in “good prognosis” patients, the rate of dizygotic twinning can be substantially reduced while maintaining robust pregnancy rates by adapting a single embryo transfer (SET) policy [18]. However, accumulating data suggest increased risk of monozygotic twinning following IVF, especially after prolonged culture and blastocyst transfer [1, 19]. This lies in contrast with the aim of extended culture: the selection of a single blastocyst to achieve a single pregnancy.

Monozygotic twinning is an uncommon phenomenon, the etiology of which is still unclear. Monozygotic twinning rates following ART procedures are between two and twelve times higher than the natural occurrence of 0.4% [1-4]. Monozygotic twins carry additional risks above those of multiple pregnancies including higher perinatal morbidity and mortality, and increased risk of developmental anomalies, discordant growth, and prematurity. The incidence of monozygotic triplets is much more rare (≈0.000023%) [20].

In addition to prolonged culture and blastocyst transfer, monozygotic twinning has been associated with several other risk factors. Disruption of the zona pellucida that occurs during ICSI was shown to lead to increased rate of monozygotic multiples in several studies [11, 12, 19]. Another form of zona pellucida manipulation which could lead to an increased rate of monozygotic multiples is assisted hatching [13].

While there is some disagreement as to the impact of maternal age on monozygotic twinning rate, a 12%-22% increase in MZ twinning has been reported in women over the age of 35 compared with women younger than 25 [21]. More recent reports indicate a trend of increasing monozygotic twinning following IVF associated with advanced maternal age [19, 22]. On the other hand, other studies found no association between maternal age and monochorionic twinning [12, 23]. These limited data indicate that increasing maternal age may contribute to an increased incidence of MZ twins, but the contribution is likely minimal in ART cases.

Another factor that has been linked to monozygotic twinning is transfer of frozen embryos [8, 9]. A recent meta-analysis study showed a monozygotic twinning rate of 3% following frozen embryo transfer but the small number of cases precluded the authors from reaching statistical significance [1].

In this case paper, we describe the occurrence of a quintuplet gestation consisting of monozygotic (monochorionic triamniotic) triplets and dizygotic (dichorionic diamniotic) twins following transfer of three poor-quality cleavage stage embryos. Previously, several cases of monozygotic triplets following IVF have been reported in which ICSI [5, 7, 10], assisted hatching [5, 7], blastocyst transfer [6, 9], or frozen embryo transfer [8, 9] were the risk factors for the monozygotic triplet occurrence. It is noteworthy that...
contrast to these case reports, in our case monozygotic splitting into triplets occurred without any of the known risk factors previously discussed. One similar case report to ours was found in the literature. Salat-Baroux et al. described a case of trizygotic quintuplets (monoamniotic triplets with two additional fetal sacs) following IVF and the transfer of four grade 1 embryos without zona pellucida manipulation or extended culture [24]. Our case differs from their case report in that we transferred a smaller number of embryos and they were all of poor quality.

The etiology of the quintuplet gestation described here is difficult to determine. Edwards et al. [2] suggested that the nature of embryonic growth in vitro predisposes to twinning. A possible “hardening” of the human zona in vitro after exposure to artificial media, as opposed to salpingeal or uterine secretions, could lead to increased fragility or brittleness of the zona pellucida, or cell-to-cell adhesion might be disturbed after in vitro culture. In agreement with this, Cassuto et al. showed that improved culture media may reduce the incidence of monozygotic twins [25]. Alternatively, Schachter et al. [26] suggested that improved endometrial conditions after gonadotropin therapy may encourage monochorionic implantation, or that the biochemical milieu of the uterine cavity after gonadotropin therapy encourages asymmetrical ZP hatching, independent of zona manipulation procedures done in vitro.

In conclusion, the current paper emphasizes that monozygotic triplets are possible consequence of ART even in cycles where the known risk factors for monozygotic twinning do not exist, and it is, therefore, necessary to identify additional predictive factors for their occurrence. This can be accomplished by performing large case-control studies that will allow us to counsel patients appropriately. In addition, the present case demonstrates that even cleavage stage embryos with significant morphologic abnormalities should be considered viable with implantation potential, underlining the importance of minimizing the number of embryos transferred and moving towards single embryo transfer in IVF.

Consent

Written informed consent was obtained from the patient for publication of this paper and any accompanying images.

References


Dichorionic twins and monochorionic triplets after the transfer of two blastocysts

Marcelo Ferreira · Adriana Bos-Mikich · Marcos Höher · Nilo Frantz

Abstract
Purpose To describe a unique case of MZ dichorionic twins and MZ monochorionic triplets in a quintuplet gestation after intracytoplasmatic sperm injection (ICSI) and blastocyst transfer.

Methods Case report. A 24-year-old woman underwent ICSI and received two blastocysts transferred. A quintuplet gestation was established. Transvaginal ultrasonography was performed sequentially during early pregnancy.

Results Three intrauterine gestational sacs were revealed at about 5th week. At the 7th week, five gestational sacs presenting heart beats were detected and a quintuplet pregnancy consisting of two monozygotic (MZ) dichorionic twins and three MZ monochorionic triplets was determined. At the 10th week, a single gestational sac with heart beats was detected. The prenatal course was uneventful. A healthy baby was born at 36th week.

Conclusion Few other reports have described the occurrence of a quintuplet gestation after the transfer of two blastocysts generated by ICSI. Our case is unique in that the two blastocysts underwent two different splitting processes, which occurred possibly at a similar time giving rise to MZ dichorionic twins and MZ monochorionic triplets.

Keywords Quintuplets · Monozygosity · ICSI · Blastocysts

Introduction
Extended embryo culture to the blastocyst stage represents an effective means of embryo selection, aiming to reduce the number of embryos to be transferred and the rate of multiple pregnancies [1]. However, it seems that the extended period of time that embryos spend in culture to reach the blastocyst stage may be responsible for an observed increase in monozygotic (MZ) twinning after ART [2–4]. In addition, there are suggestions of an association between the breach of the zona pellucid (ZP) for ICSI [5–9] and an increased rate of MZ multiple gestations.

Monozygotic twins arise from the splitting of an embryo from the pronuclear to the blastocyst stage. Depending on the stage of embryonic development that the embryo splits, monozygotic multiplets may have individual chorions and amnions or a single chorion and separate multiple amnions [10]. We report, to our knowledge, the first case of a quintuplet pregnancy consisting of two MZ dichorionic twins and three MZ monochorionic triplets achieved after the transfer of two blastocysts.

Case report
A 24-year-old woman underwent her first ICSI attempt due to andrological subfertility, in February 2009. The patient was stimulated with 225 IU of recombinant FSH (Gonal F, Serono) and 75 IU of HMG (Menopur, Ferring) in a down-regulated cycle using GnRH agonist.
(Nafarelin, Pfizer) according to the long protocol, starting on midluteal phase of the previous cycle. Twelve cumulus-oocyte complexes were collected 36 hrs after an injection of 10,000 IU of hCG (Choragon, Ferring). Eleven MII oocytes were injected, resulting in 11 two-pronuclei zygotes. After three days of culture in Early Cleavage medium (Irvine), embryos were placed in MultiBlast Medium (Irvine) and two blastocysts, grade G1 and G2 [11] were transferred (Fig. 1) and two others were cryopreserved. The luteal phase was supported with 800 mg of micronized progesterone daily. Fourteen days after transfer the patient had a positive β-hCG exam (121 ng/ml). The clinical pregnancy was defined as the presence of three intrauterine gestational sacs, at about five weeks gestational age. Two to three weeks later, the ultrasonographic exam was repeated and five gestational sacs presenting heart beats were detected. A quintuplet pregnancy, consisting of two MZ (biamniotic) dichorionic twins and three MZ (triamniotic) monochorionic triplets was then determined (Fig. 2). At the 9-10th week of gestation a new transvaginal sonography revealed one gestational sac with a single beating heart. The prenatal course was uneventful. A normal healthy baby girl was born at the 36th week of gestation by spontaneous vaginal delivery (weight 1.800 g, apgar 8/9).

Discussion

This case report describes the occurrence of a quintuplet gestation after the transfer of two blastocysts generated by ICSI. One blastocyst split into MZ dichorionic twins and the other split into three MZ embryos with a single chorion.

Monozygotic twinning is a rare phenomenon among humans. Its incidence among natural conception is about 0.4% of births [12, 13]. There seems to be an increased rate of MZ twinning following ART, especially after embryo culture till blastocyst [14]. Culture to the blastocyst stage was envisaged as a means of embryo selection, to reduce multiple gestations. However, higher-order multiple pregnancies have been reported following the transfer of two blastocysts [9, 15, 16]. The etiology of the quintuplet gestation here described is difficult to know. Culture conditions may affect embryonic metabolism, favoring the splitting of the inner cell mass (ICM) before or during hatching [17]. This was our first experience, in which the number of gestational sacs and fetal heart beats exceeds the number of blastocysts transferred. Two other similar cases were found in literature. One described a quintuplet pregnancy consisting of monochorionic diamniotic and monochorionic triamniotic multiples [15]. The other report described a gestation consisting of MZ triplets (monochorionic, triamniotic) and monozygotic twins (monochorionic diamniotic) [9]. The striking fact of the present case is that the quintuplets, two MZ dichorionic and three MZ monochorionic were possibly generated by splitting processes that happened in each embryo at a similar time. Our case differs from the other reports [9, 15] in that the patient was significantly younger. This agrees with results from a review on MZ twinning after IVF [4] showing a higher incidence of this condition among young women undergoing ART. Also, in none of the previous reports and in our case, assisted hatching or pre-implantation genetic diagnosis (PGD) were performed.

The monochorionic triplets may have resulted from multiple herniations of the ICM, due to several ICSI openings in the ZP [8]. However, we have no records of difficulties during ICSI of that cycle and a single opening in the ZP should have been made. Other studies have also
found no relationship between the incidence of MZ twinning and micromanipulation techniques [3, 18–20].

In conclusion, we believe that multiplet pregnancies after the transfer of one or two blastocysts are a rare, unwanted outcome of ART that should not discourage blastocyst transfers [21]. However, the possibility of MZ twinning after blastocyst transfers should be disclosed to the couples and single embryo transfer should be considered. Despite the existence of guidelines, legislation in Brazil does not limit the number of embryos to be transferred, however the present case prompt us to question the transfer of more than one blastocyst to young patients (<30 yrs). Also, predictive factors that may promote MZ twinning should be identified from the available literature to avoid its recurrence and unwanted results.

References


Monozygotic twinning: an eight-year experience at a large IVF center

Jaime Knopman, M.D., Lewis C. Krey, Ph.D., Jennifer Lee, M.S., Mary Elizabeth Fino, M.D., Akiva P. Novetsky, M.D., and Nicole Noyes, M.D.

New York University Fertility Center, New York University–Langone Medical Center, New York, New York

Received January 6, 2009; revised February 21, 2009; accepted March 13, 2009; published online May 5, 2009.

Objective: To characterize incidence, chorionicity, amnionicity, and pregnancy outcome for monozygotic twin pregnancy (MZT) after IVF.

Design: Retrospective review.

Setting: University-based fertility center.

Patient(s): Autologous and oocyte donation IVF cycles eventuating in 4,976 clinical gestations from 2000 to 2007.

Intervention(s): None.

Main Outcome Measure(s): MZT incidence, chorionicity, zygosity, pregnancy outcome.

Result(s): Ninety-eight MZTs were diagnosed after first-trimester ultrasound evaluation (2% incidence). The incidence in cycles transferring autologous oocytes was 1.7% but was 3.3% with donor oocytes; however, women <35 years old using their own oocytes displayed a similar rate (3.1%) to women using donor oocytes. Eighty MZTs occurred after fresh day-5 transfer; only 14 followed fresh day-3 transfer (2.6% vs. 1.2%). The MZT incidence in day-3 transfers without hatching was not different from those with hatching (1.3% vs. 1.1%). In addition, MZT incidence did not differ significantly whether or not ICSI was performed (2.4% vs. 2.0%). Four MZTs occurred after frozen-thawed embryo transfer (0.8% incidence). Ninety-five percent of all placentral arrangements were confirmed as monochorionic-diamniotic on obstetric ultrasounds.

Conclusion(s): These findings confirm a higher incidence of MZT after IVF. Monochorionic-diamniotic implantations were increased, whereas monochorionic-monoamniotic were not. The MZT risk factors included young age and extended culture, but not zona penetration or cryopreservation. (Fertil Steril 2010;94:502–10. ©2010 by American Society for Reproductive Medicine.)

Key Words: Monozygotic pregnancy, chorionicity, amnionicity, blastocyst, twinning, high risk pregnancy

Assisted reproductive technologies (ART) are widely used and highly successful for the treatment of infertility. In 2005, 52,041 infants were born as a result of IVF treatments performed in the United States (1). However, despite success and widespread use, some concerns remain regarding the overall safety of ART, particularly its contribution to the rise in multiple gestations. In nature, monozygotic twinning (the splitting of a single fertilized oocyte into two or more fuses) is a rare event, estimated to occur in 3–4 per 1,000 live births (2). However, the East Flanders Prospective Twin Survey in Belgium estimates that monozygotic twin (MZT) pregnancies account for ~40% of all naturally conceived same-sex twin pregnancies (3, 4). With the advent of ART, these proportions have changed.

Although the majority of ART-related multiple gestations are thought to be dizygotic (derived from two or more fertilized eggs), numerous reports suggest an increase in MZT pregnancies (5–10). Certainly, the primary explanation for the increase in twinning with modern fertility treatment is the in vivo release of multiple oocytes following ovulation induc-
Acknowledgments: The authors acknowledge the other physicians on the New York University Fertility Center team, Dr. James Grifo, Dr. Alan Berkeley, Dr. Fredrick Licciardi, and Dr. Lisa Kump-Checcio, as well as the members of Embryology Laboratory. They also acknowledge the physicians who provided the follow-up data and, most importantly, the patients who endured the added hardships, risks, and worries associated with a monozygotic twin pregnancy.

REFERENCES

Monochorionic triamniotic triplets following conventional in vitro fertilization and blastocyst transfer

Sumana Gurunath, Adinarayana Makam, Sriprada Vinekar, and Reeta H. Biliangady

Abstract

Multiple pregnancy in in vitro fertilization (IVF) is on the decline with a reduction in number of embryos transferred. But the risk of monozygotic splitting persists. The risk of monozygotic twinning in women undergoing IVF is reported to be twice that of natural conception, and monochorionic triplets are even rarer at 100 times more than natural conception. We report a case of monochorionic triamniotic (MCTA) triplets following conventional IVF and blastocyst transfer without zona manipulation. This report highlights the possibility of zygotic splitting in IVF in young couples with no family history, in centers with good experience with blastocyst transfer. MCTA triplets carry a high risk of perinatal mortality and morbidity and need multidisciplinary care. Prevention and prediction of zygotic splitting ought to be realized with better reporting and identification of possible risk factors.

KEY WORDS: Blastocyst transfer, in vitro fertilization, monochorionic triamniotic triplet, zygotic splitting

INTRODUCTION

A significant decline in the incidence of higher order multiple pregnancy has been achieved worldwide with the reduction in the number of embryos transferred during assisted reproduction. Despite this, the prevalence of zygotic splitting and monozygotic multiple pregnancies persists. The risk of monozygotic twinning in women undergoing in vitro fertilization (IVF) is reported to be twice that of natural conception (0.9% vs. 0.4%, respectively). The incidence of monochorionic triplets is even rarer at 0.048%, which is 100 times more than natural conception.[1]

Various theories have been proposed to explain the increased risk of monozygotic splitting after assisted reproduction. They include advanced maternal age, ovarian stimulation, culture media, prolonged in vitro culture and blastocyst transfer, zona manipulation such as intracytoplasmic sperm injection (ICSI) and assisted hatching. Monochorionic triamniotic (MCTA) triplets are rather uncommon and highly challenging to manage as they are associated with much greater chances of obstetric and perinatal morbidity and mortality. Most of the cases of MCTA triplets reported to date are consequent to some form of zona manipulation either ICSI or assisted laser hatching. To the best of our knowledge, there have been 20 reports of MCTA triplets published in the literature. 11 of these reports are following some form of zona manipulation (ICSI or Assisted hatching) and 8 are following conventional IVF (2 frozen embryo transfer [FET]; 3 day 3 transfer and 3 day 5 transfer) [Table 1].

Table 1
Published case reports of MCTA triplets following IVF

In this publication, we report a case of MCTA triplets following IVF in a young couple following blastocyst transfer. This is the fourth reported case following blastocyst transfer and conventional IVF.

CASE REPORT
Monozygotic twinning associated with assisted reproductive technologies: a review

K I Aston\textsuperscript{1,2,3}, C M Peterson\textsuperscript{4} and D T Carrell\textsuperscript{1,2,3,4}

\textsuperscript{1}Andrology and IVF Laboratories, Departments of \textsuperscript{2}Surgery, \textsuperscript{3}Physiology and \textsuperscript{4}Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, Utah 84108, USA

Correspondence should be addressed to D T Carrell at Andrology and IVF Laboratories, University of Utah School of Medicine, 675 Arapeen Drive, Ste 205, Salt Lake City, Utah 84117, USA; Email: douglas.carrell@hsc.utah.edu

Abstract

Twin birth rates have increased markedly in developed countries since the 1970s for two primary reasons: increasing maternal age and the advent and increasing use of fertility treatments. In addition, monozygotic (MZ) twin pregnancies have been reported to occur at a significantly higher rate following assisted reproductive technologies (ART) procedures compared with the natural incidence. Twin pregnancies are of concern due to a dramatically increased risk of associated complications. Monozygotic twin pregnancies carry a 10–20\% risk of twin–twin transfusion syndrome, and monoamniotic monochorionic twins are additionally at risk for cord entanglement. While the mechanisms and contributory factors for dizygotic twinning are well established, very little is known about the mechanisms involved in MZ twinning or the factors that contribute to its occurrence. In this review, we will discuss a number of potential mechanisms involved in MZ twinning and explore factors that may be contributing to the increased incidence of ART-associated MZ twins. An improved understanding of the factors that contribute to increased MZ twinning associated with ART will help to elucidate the poorly understood mechanisms involved in the process and will further aid in reducing the overall incidence of multiple pregnancies with their associated risks following ART procedures.


Introduction

In humans, twins generally result from one of two scenarios. Dizygotic (DZ) twins result from the ovulation and fertilization of two oocytes with the resultant twins sharing the same genetic relationship as common siblings. Monozygotic (MZ) twins, on the other hand, are the result of a single fertilized embryo splitting at some stage of early embryo development to form two separate embryos derived from one zygote. DZ twinning is associated with increased maternal serum follicle-stimulating hormone associated with maternal genetics (White & Wyshak 1964), age (Beemsterboer et al. 2006), parity (Rao 1978), and other factors (Hall 2003). The occurrence of DZ twinning varies widely between populations ranging from about 6/1000 in Asia to 10–20/1000 in the United States and Europe and as high as 40/1000 in Africa (Hall 2003).

While the genetic contribution to DZ twinning is well established, genetic associations with the occurrence of MZ twins have been described infrequently, and penetrance for this trait seems to be extremely low (Parisi et al. 1983, Steinman 2003, Hamamy et al. 2004). Monozygotic twin occurrence is universally about 0.4–0.45\% following non-stimulated \textit{in vivo} conception (MacGillivray 1986, Derom et al. 1987). The universally low incidence of MZ twinning independent of environmental factors has been suggested to support the notion of a genetic rather than an environmental contribution (Bortolus et al. 1999). Overall, MZ twin pregnancies account for \sim 30\% of all naturally conceived twin pregnancies.

With the relatively recent advent and growing popularity of assisted reproductive technology (ART) procedures, the occurrence of both DZ and MZ twins has increased. Monozygotic twinning following \textit{in vitro} fertilization (IVF) was first reported in 1984 (Yovich et al. 1984), and since that time numerous studies have demonstrated an increased occurrence of MZ twins associated with ART procedures. Following 127 977 ART procedures in the United States in 2004, 32\% of live birth deliveries were twin or higher order pregnancies (Wright et al. 2007). In Europe, summaries of 365 103 ART cycles in 2003 indicated that twin and triplet pregnancies accounted for 23\% of all deliveries (Andersen et al. 2007).

The United States and European summaries do not include data on the zygosity of twin births, but other published reports indicate that MZ twinning rates following ART procedures are between two and twelve times higher than the natural occurrence of 0.4\%. 

© 2008 Society for Reproduction and Fertility
ISSN 1470–1626 (paper) 1741–7899 (online)
DOI: 10.1530/REP-08-0206
Online version via www.reproduction-online.org
number of mammalian species, including cattle (Ozil et al. 1982), mice (Nagashima et al. 1984), sheep (Chesne et al. 1987), pigs (Reichelt & Niemann 1994), and rhesus monkeys (Mitalipov et al. 2002).

Though MZ twinning occurs rarely if ever in mice (McLaren et al. 1995), it has been demonstrated that in vitro culture of mouse embryos can induce MZ twin formation in $\sim 1\%$ of embryos cultured (Hsu & Gonda 1980). The twinning was induced when hatched blastocysts attached to the culture dish antipolar to the ICM, and embryonic growth coupled with physical growth restrictions resulted in subdivision of the ICM. Another study reported the occurrence of double ICMs at a rate of 0.6 and 3.1% respectively for in vivo and in vitro fertilized mouse embryos prior to hatching (Chida 1990).

Two cases from human IVF suggest that double ICMs account for some MZ pregnancies. One report exists on the day 5 transfer of two blastocysts, including one with a double ICM, resulting in a triplet pregnancy (Meintjes et al. 2001). Unexpectedly, the MZ twins were DCDA, indicating the trophectoderm was presumably split in the embryo. In another IVF laboratory, time-lapse photography of developing human IVF embryos documented the formation of two distinct ICMs in two of twenty-six embryos analyzed. The formation of double ICMs was believed to result from ectopic adhesion and subsequent proliferation of ICM cells on the opposing trophectoderm wall following blastocoel collapse and re-expansion (Fig. 1B-2; Payne et al. 2007).

The role of the zona on induction of MZ twinning by mechanical splitting or ICM disruption of the hatching blastocyst has also been proposed (Edwards et al. 1986; Figs 1A-2, B-3, C-2 and 2). Factors such as thickening or hardening of the zona have been reported to contribute to twinning (Edwards et al. 1986, Tarlatzis et al. 2002).

While the zona may be involved in some cases of MZ twinning, it is certainly not the exclusive mechanism as MZ twinning has been demonstrated to occur following transfer of zona-free blastocysts (Frankfurter et al. 2001). Together, the multiple mechanisms of MZ twinning observed and induced experimentally along with the occurrence of multiple placentational arrangements in ART-associated MZ pregnancies suggest that MZ twinning is likely associated with a variety of factors that may include delay in fertilization, embryo development, or implantation, mechanical disruption of the early embryo, and alterations in culture conditions. Indeed, a combination of factors is likely involved.

**Association of MZ twinning with ART**

Over two decades ago, it was observed that MZ twinning frequency was greater following ART procedures (Edwards et al. 1986) and artificial induction of ovulation (Derom et al. 1987). These early reports have been followed by numerous additional corroborating studies cited below; however, a large Danish cohort study recently published compared MZ and DZ twin rates between natural conceptions and conceptions following infertility treatment and found no association between fertility treatment and MZ twin deliveries (Zhu et al. 2007). Owing to the relatively limited number of individuals in the study undergoing infertility treatment and the small number of MZ twins in the group, the authors were not able to analyze MZ twinning rates based on treatment type. As a result, ICSI, IVF, intrauterine insemination, and hormone treatment cases were all analyzed together.

In the context of ART, there exists a great deal of debate over the factors that contribute to increased MZ twinning as well as the mechanisms involved. Maternal age, ovarian stimulation, zona manipulation, temperature effects, and in vitro embryo culture have all been suggested as contributory factors in ART-associated MZ twinning. Table 1 summarizes reports investigating the impact of some of these factors on MZ twinning rates.

**Maternal age**

While there is some disagreement as to the impact of maternal age on MZ twinning rate (Steinman 2001), a 12–22% increase in MZ twinning has been reported in women over the age of 35 compared with women younger than 25, while parity has been shown to have little or no effect (Bulmer 1970). On the other hand, an epidemiological review of a number of studies concluded that the rate of MZ twinning is not affected by maternal age (Bortolus et al. 1999). More recent work in the context of IVF/ICSI treatment reported an increased risk of MZ twinning in women over the age of 35 (Abusheikha et al. 2000), although this conclusion was...