MONOZYGOTIC PREGNANCIES FOLLOWING ASSISTED REPRODUCTIVE TECHNOLOGY: A REVIEW

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ABSTRACT

Introduction: Assisted reproductive technology (ART) is associated with an increased risk of monozygotic twinning. This narrative review attempts to summarise the known literature regarding the aetiology, incidence, risk factors, diagnosis, and prognosis of monozygotic twinning following ART.

Aetiology: Monozygotic twinning is caused by the splitting of the early embryo during the peri-implantation phase. According to the classical hypothesis, the timing of the split determines the chorionicity and amnionicity, however this has been questioned in recent literature.

Incidence and risk factors: The incidence of monozygotic twinning in natural conception appears to be independent of extrinsic factors such as ethnicity and age. The incidence of monozygotic twinning is increased from 0.4% of natural conceptions to around 0.9–2.24% of pregnancies following ART. The available literature supports a role of ovarian stimulation and extended culture to the blastocyst stage in increasing the risk of monozygotic twinning. The impact of maternal age and micromanipulation techniques such as assisted hatching and intra-cytoplasmic sperm injection appear to depend on the stage of the embryo being transferred leading to significant heterogeneity between studies.

Diagnosis: The gold standard for diagnosing monozygotic twinning is genetic testing but its cost precludes it from routine widespread use. Most epidemiological studies utilise statistical estimates such as Weinberg's differential rule and tailored questionnaires. Most studies from ART units have utilised transvaginal sonography for counting the number of gestational sacs or assessing the chorionicity.

Prognosis: The prognosis of twins appears to be dependent on the chorionicity and amnionicity and is largely independent of the zygosity.

Keywords: Monozygotic, monochorionic, twins, assisted reproductive technology (ART).

INTRODUCTION

Assisted reproductive technology (ART) has been associated with an epidemic of multiple pregnancies.[1] Increasing awareness of the risks posed by higher order multiple pregnancies has led to recommendations and legislation limiting the number of embryos being transferred.[2] The availability of efficient cryopreservation techniques has also obviated the need to transfer multiple embryos in a fresh cycle by enabling cryopreservation of supernumerary good quality embryos which could be sequentially transferred in frozen cycles.[3] This sequential approach gives equivalent cumulative pregnancy rates whilst minimising the risk of multiple pregnancy.[4]

Most twin pregnancies following ART are dizygotic, yet monozygotic twins account for 0.9–2.24% of pregnancies following ART.[5,6] Monozygotic twinning in ART is increasingly coming under focus due to two factors: 1) adoption of a single embryo transfer policy has resulted in a steady decline in the number of dizygotic twins and hence monozygotic twins will increase in their relative proportion; 2) increasing patient awareness of the risks of

multiple pregnancy leads to the diagnosis of a monozygotic twin pregnancy following a single embryo transfer coming as a shock to the patient. Hence healthcare professionals need to be equipped with up-to-date information regarding the aetiology, risk factors, diagnosis, and prognosis of monozygotic twin pregnancies in ART to allow them to offer tailored evidence-based management. This narrative review summarises the known literature regarding the aetiology, incidence, risk factors, diagnosis, and prognosis of monozygotic twinning following ART.

AETIOLOGY

Twins can either be dizygotic (developing from two different eggs fertilised by two different sperms) or monozygotic (formed by splitting of a single fertilised egg). Dizygotic twins, also known as 'fraternal' twins, have different genetic compositions just like any other sibling pair. Monozygotic twins, also known as identical twins, share the same genetic composition. In natural conceptions, dizygotic twins are much more common than monozygotic twins. In addition, the rate of dizygotic twinning appears to vary according to extrinsic factors such as increasing maternal age, ethnic variation, and previous obstetric or family history of twinning. In contrast, the rate of monozygotic twinning through natural conception appears to be constant across ethnic groups and different age groups.[7]

Monozygotic twinning is caused by splitting of the early embryo during the peri-implantation period, but the mechanisms that operate to cause this split are yet to be clarified. Various theories include splitting of the inner cell mass, repeated cycles of blastocoel collapse and re-expansion, and alterations in the zona pellucida leading to abnormal hatching have been postulated.

Transfer of an embryo with a split inner cell mass has been noted to result in monozygotic twinning in a case report.[8] In the above case, two blastocysts were transferred: one with a normal inner cell mass and other with a double inner cell mass resulting in a trichorionic, triamniotic gestation. Since the trophectoderm was apparently single at the time of transfer, the authors speculated that the splitting of the chorion indicated that either the trophectoderm cells were destined to split after transfer or the splitting could not be visualised at the time of transfer. Repeated cycles of blastocoel collapse and re-expansion has been speculated to lead to partial adherence of the inner cell mass to the opposing trophectoderm leading to splitting of the inner cell mass. This has been noted using time-lapse photography.[9] A study in murine embryos has shown that *in vitro* culture could predispose to the splitting of the inner cell mass.[10] Alterations in the zona pellucida could occur due to extended culture[11] and micromanipulation techniques such as intra-cytoplasmic sperm injection (ICSI) and assisted hatching.[12] This can lead to abnormal hatching and consequently to partial or complete pinching off of a section of the inner cell mass with or without the trophectoderm, leading to monozygotic twinning.

The classical theory holds that chorionicity and amnionicity of monozygotic twins are based on the timing of the split.[13] A split prior to 3 days causes dichorionic diamniotic twinning, between 3 and 8 days causes monochorionic diamniotic twins, between 8 and 13 days causes monochorionic monoamniotic twins, and splitting beyond Day 13 leads to conjoined twins.

Table 1: Complication profile with the different types of twins.

Zygosity	Dizygotic	Monozygotic			
Timing of split (classical theory)	N/A	<3 days	3–8 days	8–13 days	>13 days
Characteristics	Dichorionic diamniotic	Dichorionic diamniotic	Monochorionic diamniotic	Monochorionic monoamniotic	Conjoined twins
Risks	INCREASING RISK OF PREMATURITY (SPONTANEOUS AND INDUCED)		TWIN-TO-TWIN TRANSFUSION SYNDROME	CORD ENTANGLEMENT	SHARING OF MAJOR ORGANS

The risk of obstetric complications also increases along the timing of the split, with dichorionic twins having the lowest and conjoined twins having the highest risk of complications (summarised in Figure 1). However, this long-held theory has now come under question, with evidence from ART centres that splitting at the morula or the blastocyst stage can lead to dichorionic twinning.[14,15]

INCIDENCE AND RISK FACTORS

The incidence of monozygotic twin pregnancies following natural conception is around 0.4%.[16] In ART conceptions, the incidence of monozygotic twinning appears to be increased with quoted figures in studies ranging from 0.9–2.24%.[5,6] This increased risk appears to be mediated by a multitude of factors which have been reviewed in the literature.[5,6,17]

Maternal Age

Monozygotic twinning rates in natural conception appear to be largely independent of extrinsic factors such as maternal age.[7] However, the effect of maternal age on monozygotic twinning in ART has been controversial, with some studies quoting an increased risk in women >35 years of age,[18,19] some quoting a reduced risk in older women,[17,20] and others showing no significant difference with age.[12] A recent large retrospective cohort study by Kanter et al.[6] in 2015 appeared to suggest that there may be a differential impact of maternal age with respect to the day of embryo transfer. In this study, there was a significant increase in monozygotic twinning rate in cleavage stage transfers if the maternal age was <30 years and a significant decrease in monozygotic twinning rate with blastocyst transfers if the maternal age was >35 years.[6] The underlying mechanism of this opposite impact of age on cleavage stage and blastocyst stage transfers remains unknown. Donor oocytes also appear to be a risk factor for monozygotic twinning but this could be secondary to a confounding effect of age.[20]

Ovarian Stimulation

Ovarian stimulation could cause hardening of the zona pellucida and other alterations in the embryonic development which would predispose to monozygotic twinning.[21] This is supported by data showing an increased risk of zygotic splitting in the East Flanders Prospective Twin Study following the use of clomiphene citrate.[22] A similar effect has been hypothesised following gonadotropin stimulation during ART cycles.[23]

Micromanipulation

Micromanipulation techniques such as ICSI, assisted hatching, and pre-implantation genetic diagnosis cause a breach in the integrity of the zona pellucida which can lead to abnormal hatching of the embryo. This abnormal hatching could lead to bisection of the inner cell mass with or without the trophectoderm leading to monozygotic splitting. Retrospective studies evaluating whether micromanipulation techniques cause an increase in monozygotic twinning are conflicting with some supporting an association[12,18,24-28] and others refuting it.[11,23,29-34] Lending further credence to the impact of micromanipulation on monozygotic twinning is a case report where abnormal herniation has been observed *in vitro* to result in premature splitting of the inner cell mass and trophectoderm, creating two half blastocysts and leading to a monozygotic pregnancy.[35] A Cochrane review in 2012[36] has suggested a non-significant increase in monozygotic twinning with assisted hatching from 0-0.8%.[36] However, only 6 out of 31 trials in this review reported on monozygotic twinning. In addition, the rates of monozygotic twinning reported by these trials are quite low compared with large-scale retrospective studies which suggests an under-reporting of this diagnosis. The same review also reported a significant increase in multiple pregnancy rates with assisted hatching (odds ratio: 1.38, 95% confidence interval: 1.11–1.70) including data from 14 trials and the authors of the review have suggested that this could be partly attributed to an increase in monozygotic twinning. A subsequent randomised controlled trial involving 160 vitrified-warmed blastocyst cycles by Ren et al.[37] in 2013 which compared assisted hatching near the site of the inner cell mass and opposite the inner cell mass did not show any change with monozygotic twinning rates (3.9% versus 5.6%). One large retrospective cohort study suggests that the impact of assisted hatching on increasing monozygotic twinning rates might primarily be on cleavage stage embryo transfer and not on blastocyst transfers.[6] This would make physiological sense as a zona breach at the Day 2–3 stage would be a greater deviation from the natural *in vivo* state, whereas the zona pellucida undergoes dissolution after reaching the uterine cavity for implantation at the blastocyst stage. This would also explain the significant

heterogeneity between studies. Future studies looking at the impact of micromanipulation techniques should control for the stage of embryo transfer.

Blastocyst Culture

Extended culture to the blastocyst stage has been shown to be associated with increased monozygotic twinning in a recent meta-analysis.[38] Extended culture could lead to zona hardening[11] and sub-optimal culture conditions could lead to splitting of the inner cell mass[39] or blastocoel collapse,[9] all of which could explain the increased monozygotic twinning rates after blastocyst transfer. There is evidence from an 8-year follow-up study at an ART unit that improvement in culture techniques can reduce monozygotic twinning after blastocyst transfer.[40] There is also evidence of a possible synergistic effect between ICSI and blastocyst culture on monozygotic twinning.[12]

DIAGNOSIS

The gold standard for diagnosis of monozygotic twinning is genetic testing.[41] However, these techniques are expensive and hence in epidemiological practice, alternatives such as questionnaires and statistical methods incorporating fingerprinting and blood groups are used.[42-44] These alternative techniques cannot provide a confirmatory diagnosis in all cases. Large-scale epidemiological studies on the population prevalence of monozygotic and dizygotic twin pairs utilise Weinberg's differential rule, which is based on the premise that among dizygotic twins, the numbers of unlike-sexed twins and like-sexed twins are equal. Weinberg's rule has been validated in large scale prospective studies such as the East Flanders Prospective twin study.[45] Some studies utilise a combination of questionnaires and genetic testing to estimate the prevalence of monozygotic twinning.[17]

Most retrospective studies on monozygotic twinning following ART have relied on an ultrasound diagnosis of monochorionic placentation or when the number of gestational sacs was greater than the number of embryos transferred. Chorionicity can be diagnosed through antenatal ultrasound examination and be confirmed through postpartum placental examination. Antenatal ultrasound diagnosis of chorionicity relies on the thickness of the inter-twin membrane[46,47] and the 'twin peak' sign.[48] However, since up to one-third of monozygotic twins can be dichorionic, relying on chorionicity alone risks underestimating the prevalence of monozygotic splitting.[49] Estimating the number of gestational sacs may also underestimate monozygotic twinning unless a strict single embryo transfer policy is in place. A recent large retrospective cohort study including 28,596 elective single embryo transfers between 2003 and 2012 reported to the National ART Surveillance System calculated the incidence of monozygotic twinning based on the number of pregnancies with more than one fetal cardiac activity seen on transvaginal scanning.[6] However, a limitation of using the number of gestational sacs or fetal hearts as a surrogate marker is the possibility of a concurrent natural conception alongside a single embryo transfer. This has been noted in a recent study to account for up to one in five twin pregnancies following a single embryo transfer.[50]

PROGNOSIS

Multiple pregnancy places a greater strain on the health of both the mother and the offspring, resulting in greater maternal and neonatal morbidity. Couples undergoing fertility treatment do express a desire for twins in order to complete their family in one attempt[51] but follow-up studies indicate an increased risk of postpartum depression[52] and parenting difficulties among parents of twins conceived through ART.[53] ART twin pregnancies carry a higher risk of caesarean section, preterm delivery, and low birth weight than their naturally conceived counterparts.[54]

Dichorionic Diamniotic Twins

Chorionicity rather than zygosity appears to be a main determinant of obstetric and perinatal risks. Monozygotic twins with a dichorionic diamniotic placentation appear to have a similar risk profile as dizygotic twins.[55] In addition, ART-conceived dichorionic twins do not appear to be at increased risk of adverse obstetric and neonatal outcomes when compared with spontaneously conceived dichorionic twins.[56,57]

Monochorionic Diamniotic Twins

Unlike their dichorionic counterparts, monochorionic twins share a single placenta and are thus at risk of growth discordance due to unequal placental sharing and unequal vascular anastomoses, leading to twin-to-twin transfusion syndrome.[58,59] This leads to a higher risk of

intrauterine fetal demise, neonatal death, and discordant birth weight as noted in a large Dutch twin cohort study.[60] Data from the Southwest Thames Obstetric Research Collaborative (STORK) multiple pregnancy cohort indicate that the early fetal loss rate is significantly increased in monochorionic compared with dichorionic twins.[61] Data from the STORK cohort also indicated that a discordance between crown-rump lengths in early pregnancy was highly predictive for single fetal loss.[62] As monochorionic twins share a single placenta, the surviving co-twin of a monochorionic twin pair has an increased risk of abnormal cranial imaging and neurodevelopmental morbidity after a single fetal demise than their dichorionic counterparts.[63] A recent study compared 483 spontaneously conceived monochorionic twin pregnancies with 25 ART-conceived monochorionic twins and 320 ART-conceived dichorionic twins.[64] ART-conceived monochorionic twins had an increased risk of prematurity and very low birth weight leading to an increased neonatal mortality rate. ART-conceived monochorionic twins were also at increased risk of prematurity and low birth weight compared with ART-conceived dichorionic twins.

Monochorionic Monoamniotic Twins

Monochorionic monoamniotic gestations, in addition to sharing a placenta, also share an amniotic cavity. This increases the risk of cord entanglement and necessitates intensive surveillance.[65] An intensive regimen of ultrasound surveillance, medical amnioreduction with sulindac, and elective caesarean delivery at 32 weeks gestation has been suggested to improve the outcomes of monochorionic monoamniotic gestations.[66] A recent Cochrane review to assess the role of early delivery in improving outcomes for monoamniotic gestation failed to identify any eligible trials.[67] However, current literature based on expert opinion recommends elective delivery for monochorionic diamniotic twins between 34 and 37 weeks and monochorionic monoamniotic twins between 32 and 34 weeks gestation to reduce the risk of intrauterine fetal demise.[68] The exact prevalence of monoamniotic twinning following ART is unknown, but a few case reports suggest an association with micromanipulation procedures of the zona such as ICSI or assisted hatching.[69,70] This correlation needs to be explored in larger studies.

Conjoined Twins

Conjoined twins are very rare, with an incidence of 1 in 50,000 to 1 in 100,000 live births in natural conception.[71] Conjoined twins have a more adverse outcome than other types of monozygotic splitting and survival depends on the presence of other congenital anomalies, the extent of sharing of organ systems, and the timing of appropriate medical and surgical interventions. Due to its rarity, the exact prevalence of conjoined twins following ART is unknown, and literature is limited to case reports and reviews, most of which have shown an association with zona manipulation.[72]

Monozygotic Triplet and Quadruplet Pregnancies

Literature regarding monochorionic triplet pregnancies following ART is limited to case reports.[33,73-80] The prognosis appears to be poor unless selective fetal reduction to twins is conducted through cord ligation.[74] Only one case of monochorionic quadruplet pregnancies following ART has been reported in the literature which was managed through selective fetal reduction of two fetuses and the delivery of the surviving two fetuses at 35 weeks and 6 days gestation through caesarean section.[81]

CONCLUSION

Clinicians need to have a greater understanding of the difference in prognosis associated with monozygotic twinning following ART as they will be increasingly encountering patients with this diagnosis due to the rising use of ART to treat infertility. Although dizygotic twins are the most common type of twins following ART, the risks that dizygotic twins face cannot be extrapolated to monozygotic twins. Monozygotic twins appear to carry a poorer prognosis and the prognosis appears to be largely dependent on the type of chorionicity and amnionicity. There remains a need to further assess the risk factors for monozygotic twins, both to provide couples with an individualised risk assessment for monozygotic twinning and to identify strategies to reduce its prevalence.

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