Human Genetics & Embryology
ISSN: 2161-0436

Al Dibouni et al., Human Genet Embryol 2016, 6:1
DOI: 10.4172/2161-0436.1000132

Research Article Open Access

Incidence, Sex Ratio and Perinatal Outcomes of IVF and ICSI Monozygotic Twin Pregnancies Following either Cleavage or Blastocyst Stage Embryo Transfer

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Abstract

To determine if prolonged time in embryo culture has an effect on the rate, sex ratio, and perinatal outcomes of monozygotic twins (MZT) following either cleavage stage or blastocyst embryo transfer after assisted conception. This is a retrospective study of 2,316 consecutive clinical pregnancies resulting from cleavage stage transfer (CT) and blastocyst transfer (BT). Criteria examined included (i) incidences (ii) sex ratios (iii) gestational age and birth weight; (iv) perinatal outcomes of these pregnancies from cleavage stage and blastocyst transfer procedures. Monozygotic twin pregnancies were identified by (i) presence of a gestational sac containing more than one fetal pole with cardiac activity, (ii) the number of gestational sacs or fetal hearts exceeds the number of embryos transferred and (iii) twin pregnancies following a single embryo transfer.

Overall the incidence of twinning was 1.64% (38 out of 2,316 pregnancies). The frequency of twinning was 2.3 × higher following BT (18 out of 649) compared to CT (20 out of 1,667). IVF techniques skewed the sex ratio in favour of males while ICSI significantly favoured females. There was no statistically significant difference between transfer type and gestational age, birth weight and perinatal outcome. All pregnancies resulted in the birth of 86 infants. In our experience, BT more than doubles the chances of conceiving a monozygotic twin pregnancy, however IVF techniques lead to a greater likelihood of male birth(s) if twins are conceived. Appropriate pre-conception counselling should be given to advise the potential risks associated with both types of transfer as well as using alternative methods such as single embryo transfer to reduce the risk of multiple gestations.

Keywords: Blastocyst; Cleavage; IVF/ICSI; Monozygotic twinning; Perinatal outcomes

Introduction

The manner by which division of a single fertilized embryo during the premature phases of embryonic development (monozygotic twinning (MZT)) leads to the formation of two separate individuals is dependent on the timing of embryonic cleavage after conception [1]. If embryonic cleavage occurs within the first three days of conception dichorionic diamniotic (individual chorionic and amniotic sacs) twins can result. Between days 4 and 8 it is likely that a monochorionic diamniotic (twins in separate amniotic sacs sharing a placenta) pregnancy will arise, while between days 9 and 12 a monochorionic monoamniotic (twins share amniotic sac and placenta) twin pregnancy can ensue [2]. The gold standard for the identification of MZT pregnancies is DNA fingerprint analysis, [3] but they can also be identified by Weinberg's method of diagnosis, which calculates the approximate rate of monozygosity from the numeral value of unlike-sex twins. Ultrasound examination between 6 and 13 weeks of gestation, placental histology, blood grouping, [4] and the number of fetal hearts exceeding the number of embryos transferred are additional identification methods. The true incidence of MZT from assisted reproductive technology (ART) is not clear and the possible mechanisms involved in the twinning process are poorly understood. Zona manipulation and the use of ovarian induction and embryo culture are currently the three most cited explanations reasons as to why MZT may occur. On the other hand, there is little evidence to support an association with maternal age, paternal age, number of cycles, level of peak estradiol and progesterone at time of human chorionic gonadotropin (HCG), number of embryos transferred [5], race, family history, parity [6] or laboratory experience of the embryologist [7]. The clinical consequences of multiple pregnancies play an important role in society in part because of the economic burdens but due to both maternal and fetal complications that can arise. In economic terms, more specialist antenatal equipment is needed to ensure infant survival, along with hospitalisation of newborns and mothers being longer, due to a higher frequency of preterm births and procedures such as caesarean sections. Maternal complications can include gestational hypertension, pre-eclampsia [1], postpartum bleeding, polyhydramnios, gestational diabetes, maternal anaemia and antepartum bleeding [8]. Fetal risks include twin-to-twin transfusion syndrome (TTTS) [9] premature birth, low birth weights, [10] congenital abnormalities including cloacal anomalies, neural tube defects, congenital heart defects and limb reduction defects [11]. With the ongoing debate of the relative merits of embryo transfer at cleavage stage vs. blastocyst stage, the incidence of twinning and subsequent perinatal outcomes becomes an important consideration. The purpose of this study therefore was to test the hypothesis that there is a differential rate of twinning, a differential sex ratio, different gestational ages at birth, different birth weights and different clinical outcomes of MZT depending on whether embryos were transferred at cleavage or blastocyst stage.

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Received October 20, 2015; **Accepted** January 27, 2016; **Published** February 03, 2016

Citation: Al Dibouni Z, Shah T, Wheat S, Griffin DK, Thornhill AR (2016) Incidence, Sex Ratio and Perinatal Outcomes of IVF and ICSI Monozygotic Twin Pregnancies Following either Cleavage or Blastocyst Stage Embryo Transfer. Human Genet Embryol 6: 132. doi:10.4172/2161-0436.1000132

Materials and Methods

This is a retrospective analysis of patients treated at The London Bridge Fertility, Gynaecology and Genetics Centre, between January 2007 and July 2012, who achieved a monozygotic twin (MZT) pregnancy after cleavage stage or blastocyst embryo transfer. The University of Kent Research and Ethics Committee approved this analysis. A total of 7,375 patients who conceived a pregnancy after assisted conception between January 2007 and July 2012 were analysed. In order to confirm a clinical pregnancy a transvaginal ultrasound was performed in all patients with ascending βHCG titres; presence and number of intrauterine gestational sacs were assessed, as well as the presence of a fetal pole and cardiac activity. Pregnancy outcomes were obtained directly from patients or from obstetrician reports with perinatal mortality, miscarriages, number of new borns per delivery, gender of new-borns, gestational age and birth weights also recorded. The exclusion of patients was carried out in a two-part process and is summarised in Figure 1. Part 1 excluded patients who did not present with a positive HCG, clinical pregnancy or fetal heart activity. Part 2 selected those remaining patients against criteria used to identify MZT:

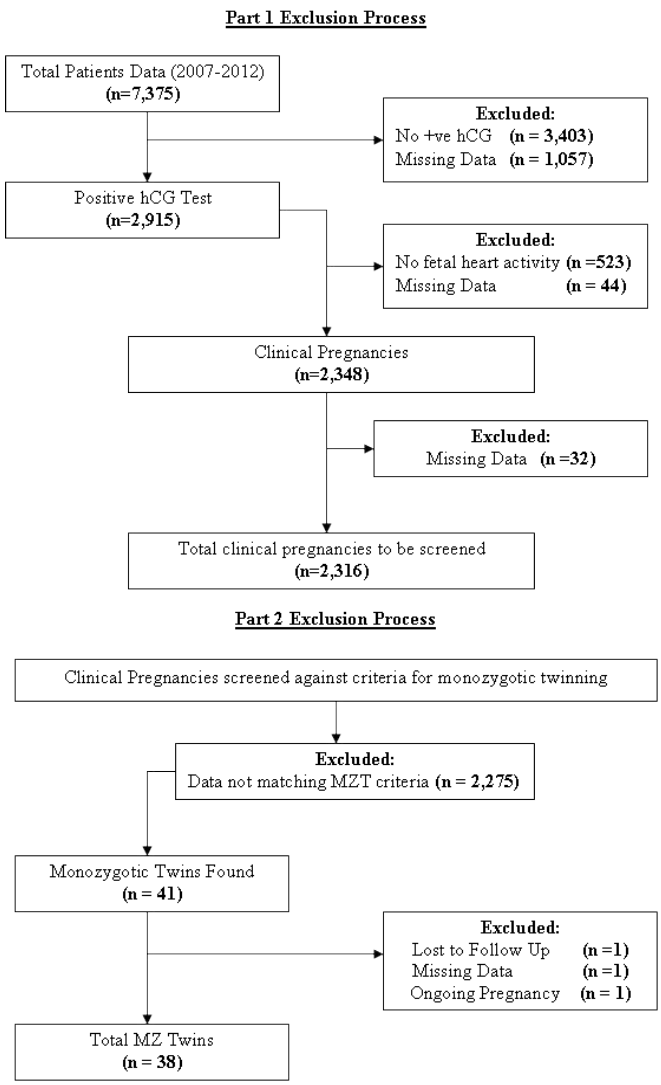

Figure 1: Two part exclusion process.

(i) presence of a gestational sac containing more than one fetal pole with cardiac activity, (ii) the number of gestational sacs or fetal heart activity exceeds the number of embryos transferred and (iii) twin pregnancies diagnosed following a single embryo transfer (SET). To determine if the respective outcomes in terms of MZT differed depending on whether embryos were transferred at cleavage or blastocyst stage (i.e. whether or not prolonged time in culture affected the twinning rate, the sex ratio, gestational age, birth weight and perinatal outcomes, we analysed 1,667 and 649 cleavage stage and blastocyst transfer pregnancies respectively. In order to establish statistically significant differences on our analyses statistical package for the social sciences (SPSS) software was used. Statistical significance was set at $P \leq 0.05$, to analyse the data, descriptive statistics tests were used to gain mean values and standard deviation values for maternal age, gestational age and birth weights. Birth weight parameters were defined as follows: very low birth weight (32 and The association between gender and embryo transfer type was assessed using a Chisquare test to evaluate the null hypothesis that embryo transfer does not have any effect on the outcome of the neonates' gender. Sex ratios were calculated by dividing the number of male infants to the number of female infants to give a decimal value, two sample t-tests established if embryo transfer type had an effect on gestational age and birth weight, while a bivariate correlation test was used to determine if there was any statistical significance between gestational age and birth weight.

Results

Of the 7,375 patients, 2,348 achieved clinical pregnancies, of which 32 were excluded due to incomplete data resulting in a total of 2,316 clinical pregnancies being screened against the MZT criteria (Figure 1). Blastocyst transfer pregnancies accounted for 28% (649 out of 2,316) of the data set whereas the remaining 72% (1,667 out of 2,316) originated from cleavage stage transfer. After screening against the above criteria 41 MZT pregnancies were acknowledged. Excluded from this data set were 3 individual cases due to an ongoing pregnancy, lost to follow up, and incomplete data, resulting in 38 MZT pregnancies being examined. Analyses of total data set demographics: The incidence of twinning was calculated at 1.64% (38 out of 2,316 pregnancies), of which only 1 patient underwent assisted hatching. Overall, 86 infants were born (live and still births). Here not all births resulted in 2 infants being born as some twins were born as part of a triplet pregnancy, producing 41 males and 45 females who were born to mothers with a mean (± standard deviation) maternal age of 33.76 ± 3.64 years, gestational age of 32.46 ± 4.04 weeks and a mean birth weight of 1782.08 ± 682.41 grams. Incidence: A total of 18 incidences of MZTs out of 649 pregnancies (2.77%) conceived via blastocyst transfer compared to the 20 out of 1,667 (1.19%) seen for cleavage stage embryo transfer. There were a total of 1,002 IVF cycles and 1,314 ICSI procedures carried out. In the cleavage group, the incidence of MZT was not increased by techniques that manipulated the zona such as ICSI (12 of 1,314, 0.91%) compared to traditional IVF methods (8 of 1,002, 0.79%). Similarly, in the blastocyst group ICSI did not increase the incidence of MZT either (11 of 1,314, 0.83% for ICSI and 7 of 1,002, 0.69% for IVF). (iii) Sex ratio: By comparing sex ratios between IVF and ICSI pregnancies, we identified that IVF skewed the sex ratio in favour of males (20 males and 9 female infants born (sex ratio 2.2) compared to ICSI who favoured females (21 males and 36 female infants (sex ratio 0.58). Statistical analysis confirmed that there was a significant association (p=0.004) between the type of technique used and the outcome of the neonates' gender. (iv) Perinatal Outcomes: An overview of perinatal outcomes for blastocyst and cleavage stage embryo transfer is given in Table 1. In the cleavage

Abnormality Classification	Blastocyst	Cleavage
Cardiac	2	5
Thyroidal	1	-
Pulmonary	1	-
Multiple Abnormalities	-	1*
Chromosomal	-	2**

* Infant presented with micrognathia, hyperfelorism, congenital left facial palsy, trachealmalacia and hypotonia.
** Twins missing part of chromosome 11.

Table 1: Blastocyst v cleavage stage perinatal outcomes.

	IVF		ICSI	
	Cleavage	Blastocyst	Cleavage	Blastocyst
	n=8	n=7	n=12	n=11
Number of infants born**	12	17	27	30
Male	11	9	11	10
Female	1	8	16	20
Mean maternal age (years)***	35.75 ± 3.28	35.00 ± 2.70	32.08 ± 4.01	33.36 ± 3.41
Mean gestational age (weeks)***	35.50 ± 3.08	33.57 ± 4.03	31.27 ± 4.90	31.27 ± 2.72
Mean birth weight (Kg)***	2.2 ± 0.66	2.04 ± 0.63	1.66 ± 0.85	1.69 ± 0.44

**Value includes stillborns. ***Values given at ± standard deviation.

Table 2: Summary of demographics IVF v ICSI.

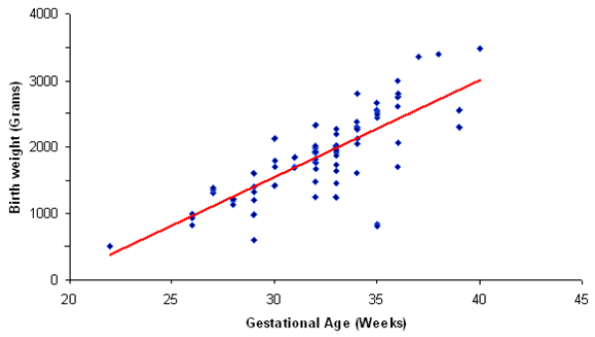

Figure 2: Scatter plot relationship between gestational age and birth weight.

stage group, 20.5% of infants (8 out of 39) where born with congenital abnormalities/birth defects. These infants presented with triplets with heart defects, one infant with micrognathia, hyperfelorism, congenital left facial palsy, trachealmalacia and hypotonia, 2 infants with parts of chromosome 11 missing, and 2 infants with heart defects (cases 4,9,12, and 34 correspondingly). When compared to the blastocyst group only 8.5% (4 out of 47) exhibited birth defects-an infant with an underactive thyroid, triplets; one presenting with pulmonary stenosis with the other two infants having heart defects (cases 17 and 39 respectively). Statistical analysis concluded that the type of embryo transfer has no effect on whether or not the child is born with a congenital abnormality/ birth defect (p=0.109)

(v) Gestational age and birth weight: An overview of IVF and ICSI cleavage and blastocyst embryo transfer demographics is given in Table 2. Results of the two sample t-test concluded that there was no statistical differences between embryo transfer type and gestational age (p=0.668) as well as the birth weight of infants (p=0.636). Women in the cleavage stage group demonstrated a mean maternal age and gestational age of 33.55 ± 4.08 years and 32.76 ± 4.72 weeks compared to their blastocyst counterparts (34.00 ± 3.18 years and 32.17 ± 3.38 weeks), whereas infants born from cleavage stage transfer had a mean birth weight of 1741.92 ± 830.29 grams, compared to the 1815.40 ± 537.14 grams seen for blastocyst infants. The bivariate correlation test between gestational age and birth weight concluded that there was a positive correlation (p=0.000) between the two, demonstrating a direct linear relationship (Figure 2), with infants of a higher gestational age being born heavier than those of a low gestational age.

Discussion

In this study the overall incidence of MZT pregnancies was 1.64%, resulting in 86 infant births (45 females and 41 males). In general, patients who underwent assisted conception resulting in a multiple pregnancy were in their early thirties (33.76 ± 3.64 years), managed to reach the third trimester of their pregnancy (32.46 ± 4.04 weeks) and delivered infants of a low birth weight (1782.08 ± 682.41 grams). MZT births developed in both groups by using ICSI and IVF were comparable and suggestive that zona manipulating techniques do not have any effect on the incidence. This is also supported by a study conducted by Saito, et al. [12], who shows in their study that the incidence for ICSI and IVF were similar. Previous publications support the notion that zona manipulation and assisted hatching to be among the factors that may increase the risk of twinning [12-15], however our data suggests that twinning rates, which were comparable in IVF and ICSI cleavage and blastocyst groups, may not be a factor. Interestingly, the one case that did have assisted hatching conducted belonged to the IVF cohort, which is also suggestive that assisted hatching may not be a factor for twinning. This is reinforced by findings of Dennis, et al. [16] who suggested that the incidence of monozygotic pregnancies is not increased by assisted hatching. Similarly, data collected by Franasiak, et al. [17] failed to show any association between treatments type (IVF or ICSI) as well as assisted hatching on the incidence of MZT. In finding that the incidences of MZT following blastocyst transfer was 2.77%, compared to cleavage stage transfer of 1.19%, our data shows that twinning rate in blastocyst transfer is 2.3 times higher than in cleavage stage transfer. In researching earlier studies our findings are confirmed by work carried out by former studies imply that the incidence of twins is higher for blastocyst transfer [4,18]. This is also strengthened by Knopman and Franasiak et al. [17], who put forward the proposition in their articles, that prolonged culture exposure increases the risk of MZTs. The exact underlying mechanisms as to why the incidence is higher in blastocyst stage transfer are still unclear. It has been theorised that techniques which manipulate the zona such as ICSI and assisted hatching may cause MZ twins to form due to the construction of an aberrant opening that allows spontaneous herniation of the blastocyst [19]. The zona is considered to act as a barrier that limits the degree in which the inner cell mass can divide and disruption of this barrier may contribute to the twinning process [20]. Knopman et al. state that the delayed implantation time seen for blastocyst transfer may contribute to the increased risk whereas Carrillo-Vadillo et al. [21] and Franasiak et al. [17] declare that the prolonged culture exposure may cause a decline in the integrity of the zona pellucida leading to an increased chance of the embryo dividing due to the herniation of the blastomeres. Milki et al. [22] suggests from animal studies, that biochemical mechanism such as low calcium levels in the blastocyst before implantation could possibly lead to the weakening of the inner cell mass making splitting of the embryo more likely. Also from animal models, Franasiak et al. [17], has suggested that insulin-like growth factor may play a role in the development of MZT pregnancies by altering signalling mechanism and changing the polarity within the embryo. It is thought that insulin growth factor II ligand is produced before implantation occurs, and blastocyst that have a high expression of the appropriate receptor can lead to the mitogenic mechanisms of the embryo being altered. Another interesting finding in our study was the sex ratio observed.

IVF methods seemed to favour males (IVF cleavage stage: 11 males: 1 female, IVF blastocyst: 9 males: 8 females), whereas ICSI techniques favoured females (ICSI BT: 10 males: 20 females, ICSI CT: 11 males: 16 females). Analysis of these values confirmed that there was a statistically significant difference between the treatment type and the gender of the new-born. Furthermore this finding was confirmed by vetting existing publications such as Hentemann et al. [23] who found a sex ratio skewed in favour of females being born from ICSI. Looking at our results it can be seen that IVF blastocyst sex ratio skewed in favour of males with Kausche et al. also corroborating this finding in their study. However, if looking at the entire cohort it can be seen that cleavage stage embryo transfer favoured males whereas blastocyst transfer favoured females. Change et al. have hypothesised, based on bovine and murine models that male embryos divide faster than female embryos and so suggest that the sex ratio for blastocyst transfer should be skewed in favour of males rather than females. This statement disagrees with our findings, (blastocyst skewed in favour of females), however they do go on to say it is probable that slower developing embryos are given more time to develop when opting for blastocyst transfer, which implies that a sex ratio for cleavage stage transfer would be skewed in favour of males, which is what we see in our study. There were a large variety of different maternal and fetal complications observed during gestation in the data set. Patients belonging to the cleavage stage transfer group seemed to have the most complications. This group had a total of six mothers having miscarriages, while there was one case of TTTS as well as one infant presenting with micronathia, hyperfelorism, congenital left facial palsy, trachealmalacia and hypotonia. Five infants were born with heart defects while two infants were born with parts of chromosome 11 missing [24-26]. Out of the 39 infants born via cleavage stage embryo transfer two did not survive bring the mortality rate for this group to be 5.1%. When compared to the blastocyst embryo transfer cohort, the most serious complications presented were: an underactive thyroid, two infants with heart defects and one with pulmonary stenosis. There was a possible case of VTS with one pregnancy showing two fetal hearts in one gestational sac, which resulted in a singleton, birth [27-29]. The mortality rate for infants in this group was calculated to be 2.1% as only one infant was stillborn out of the 47 that were born. The overall combined mortality rate for monozygotic pregnancies conceived by assisted reproduction in this study is 3.5%. However, looking at all the infants that presented with a congenital abnormality or birth defect in the two groups, no statistical significance was found between them, suggesting that embryo transfer type has no effect on birth outcome. The retrospective nature of this study could be consider a limitation, due to the fact that this type of study does not differentiate between many demographics, such as BMI, parity, smoking, and previous health problems which all can influence perinatal outcome. Additionally it is a possibility that the incidence of MZT may have been underreported due to the overlooking of liked-sexed MZTs as a result of the identification methods implemented [3]. Another limitation of this study may be the small sample size, however Kawachiya et al. suggests that all retrospective analyses on MZTs are limited by their sample size. Indeed our studies are in line with many MZT studies. To conclude, the incidence for blastocyst transfer was 2.3 times higher than cleavage stage transfer. IVF techniques skewed the sex ratio in favour of males whereas ICSI favoured females, with no statistical significance found between embryo transfer type and gestational age, birth weight and perinatal outcome. Patients undergoing both sets of transfer should be appropriately counselled with alternative methods such as SET being implemented to avoid multiple gestations [30,31].

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