Perinatal Outcomes in Monozygotic Pregnancies Resulting From Assisted Reproductive Techniques: A Single-Center 6-Year Experience Base on a Large Cohort of Pregnancies

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Abstract

Purpose: Monozygotic twin (MZT) pregnancies increase the risk of maternal and infant mortality and reveal many complications. In the present study, we aimed to share our assisted reproductive techniques (ART) through the analysis of perinatal outcomes in MZT pregnancies.

Materials and Methods: In this retrospective clinical cross-sectional study, 1159 cycles in the In vitro fertilization (IVF) program between October 2014 and December 2019 were retrospectively evaluated and perinatal outcomes and general clinical conditions were analyzed.

Results: 16 cycles with MZT were achieved with IVF, resulting in a MZT incidence of 1.38%. The MZT incidence aged ≤ 35 and >35 years were 0.2% and 1.1%, respectively. Eight MZT pregnancies resulted in a live birth, while 5 MZT resulted in an abortus. A significant positive correlation was found between the number of trials and the age of female ($r:0.674$; $p=0.004$) and male ($r:0.657$; $p=0.006$). Cumulus-Oocytes Complexes (COC) ($r:0.635$; $p=0.008$), Metaphase II Oocyte (MII O) ($r:0.627$; $p=0.009$), Pronucleus Oocyte (PO) ($r:0.585$; $p=0.017$) were correlated with serum AMH levels. Number of MZT was positively correlated with the male age ($r:0.527$; $p=0.036$), while negatively correlated with embryo transfer day (ETd) ($r: -0.548$; $p=0.028$).

Conclusion: The pregnancies that we identified and achieved with IVF had a similar incidence of MZT with the literature, although its risk was more for women >35 years. MZT pregnancies can become a problem as the increasing number of IVF applications continue due to its potential risks for both maternal and fetus.

Introduction

The infertility term, generally known as the failure to perform a reproductive function, is a worldwide health problem in all societies (1). Although infertility treatments vary depending on the source of the problem, age, ovarian difficulty in egg release, alcohol or smoking, intense physical or mental stress are the known causes (2, 3). Assisted reproductive techniques (ART), which is the strongest way in treatment today, includes all infertility treatments that ensure the fertilization of eggs and sperm outside the human body (4, 5). These are techniques such as pre-implantation genetic screening, embryo cultures, intracytoplasmic sperm injection, fresh or frozen embryo transfer, and in vitro fertilization (IVF) with donor oocytes (6).

ART has been practiced for nearly forty years, and more than a million babies have been born as a result of IVF, and the use and effectiveness of IVF have increased over time (7). Especially frozen embryo transfer and intracytoplasmic sperm injection in this method contribute strongly to these results (8). More than 30% of pregnancies with ART are multiple pregnancies, and more than half of these newborns are the product of multigestations (9). This causes prematurity and other complications and, consequently, babies born from multiple pregnancies have higher rates of hospitalization and mortality compared to single (10).

The frequency of anomalies in multiple pregnancies has been reported higher than single pregnancies (11). Except for some problems that can be treated antenatally, non-vital anomalies cause new problems for both obstetricians and parents in multiple pregnancies (12). Although twin births are 2% of all births, 8% of perinatal deaths occur in multiple pregnancy cases (13). Mortality is 4 times higher in multiple pregnancies than single pregnancies and perinatal mortality is 19–32% (14). Specific problems associated with high mortality in multiple pregnancies are common in monochorionic twins (15). Monochorionic placentation is shown as an increase in the frequency of congenital anomalies in monozygotic twin (MZT) pregnancies. Compression-related deformations and lower extremity deformities occurring in the last stages of pregnancy and related to intrauterine posture position are not uncommon in multiple pregnancies (16).

Considering that MZT pregnancies increase the risk of maternal and infant mortality and reveal many complications, sharing rates in ART treatment facilities constitutes importance to ensure a certain standard and to closely monitor the perinatal risks. In the present study, we aimed to share our ART experience through the analysis of perinatal outcomes in MZT pregnancies.

Materials And Methods

Study Design

We retrospectively evaluated 1159 cycles that were included in the ICSI program at Department of Obstetrics and Gynecology, Faculty of Medicine, between October 2014 and December 2019. We analyzed the perinatal outcomes and general clinical conditions of all these Monozygotic pregnancies. This retrospective clinical cross-sectional study was conducted following the Bahcesehir University Ethics Committee approval with the date and number: 17.07.2020-0003. All the experiment protocol for involving humans was in accordance to guidelines of national/international/institutional or Declaration of Helsinki. Informed consent was obtained from all subjects and all the data collected included the basic clinical conditions of the patients, delivery results, type of conception and pregnancy outcomes. Recorded data belonging to the patients were obtained from the retrospective examination result, and was completed by using inpatient treatment charts from our institution. Patient data included in the study were reached with incomplete or inconsistent participants. We evaluated all the cases included in the study in terms of the average age of pregnant women, average gestational week, delivery type, birth weight, presence of EMR, gender, cesarean indications, abortion, fetal problems, fetal reduction, discordance between fetuses, pregnancy anemia, blood transfusion to mother, cerclage, abortion imminens, emesis gravidarum, BMI.

Inclusion & Exclusion Criteria

Women were identified through the use of an electronic data. As a result of all clinical and sonographic analyzes, we included MZT pregnancies that reached pregnancy after 16 weeks. All other pregnancy or related conditions were excluded from the present study. In detailed sonographic analysis, pregnancies were accepted as monochorionic according to the presence of placenta and the absence of twin peak

mark expressed as "lambda sign". Clinical assessment and ultrasound analyzes of the patients were performed by the experienced gynecologist team in obstetric sonography and IVF.

Statistical Analysis

The SPSS program for Windows (v16.0, SPSS Inc. Illinois,USA) was used to evaluate the study results. Descriptive statistics outputs were given as mean $\pm$ standard deviation for continuous numerical variables or median (minimum-maximum) and percentage for categorical variables. Comparative analysis was done with the Mann-Whitney U test for continuous data and the χ^2 test for categorical data. $P < 0.05$ was considered statistically significant for all data assessment.

Results

In the 1159 cycle, a total of 16 cycles with MZT were achieved with IVF, resulting in a MZT incidence of 1.38%. The MZT incidence aged ≤ 35 and > 35 years were 0.2% and 1.1%, respectively. Given in Table-1, the main reason for infertility was male-related factors (n:7). Other reasons were endometriosis (n:3), Duchenne muscular dystrophy (n:2), tubal (n:1), and unexplained (n:3). The mean female age was 29.8 ± 3.4 , while it was 33.7 ± 3.2 for the male age. The BMI was found as $24.2 \pm 2.3 \text{ kg/m}^2$. The mean value of the trial frequency was 2.1 ± 2.2 , and the mean AMH level was 2.4 ± 1.7 .

Eight MZT pregnancies resulted in a live birth. Of those, 2 pregnancies were triplet pregnancy (%25). Five MZT pregnancies resulted in an abortus. Of those, 2 pregnancies were triplet pregnancy (%40). Three MZT pregnancies, which were twin pregnancy, resulted in partial abortion. All the patients' details about cycle characteristics were given in Table-2.

A significant positive correlation was found between the number of trials and the age of female ($r:0.674$; $p = 0.004$) and male ($r:0.657$; $p = 0.006$). Similarly, Cumulus-Oocytes Complexes (COC) ($r:0.635$; $p = 0.008$), Metaphase II Oocyte (MII) ($r:0.627$; $p = 0.009$), Pronucleus Oocyte (PO) ($r:0.585$; $p = 0.017$) were correlated with serum AMH levels. Number of MZT was positively correlated with the male age ($r:0.527$; $p = 0.036$), while negatively correlated with embryo transfer day (ETd) ($r: -0.548$; $p = 0.028$).

Discussion

Today's career planning individuals' approach to marriage at an advanced age and the tendency to delay pregnancy has increased by IVF application worldwide. Although a rare event, previous studies have reported that the incidence of MZT has increased more than four times as a result of IVF procedures, with an increased risk of obstetric complications and poor pregnancy outcomes (17–19). For these reasons, MZT pregnancies can become a problem as the increasing number of IVF continue. In that term, the present study will contribute to the MZT numbers resulting from the application of IVF in our country and the perinatal clinical outcomes of MZT.

Despite the problematic effects of MZT caused by pregnancies, which we frequently see, it still transfers multiple embryos in many hospitals in our country and around the world to increase the chance of pregnancy (20). The reduction in fertility may be mainly due to oocyte aging rather than poor endometrial receptivity, as older women produce fewer oocytes and have lower implantation rates, suggesting that follicles are less responsive to exogenous hormones and fewer retrieval high-quality oocytes (21). In Sotiroska's study (22), the pregnancy at highest rates were reported in who received ET on 5th day. However, they observed a strong decrease in delivery/pregnancy rates in the higher age group (age > 36 years) compared to the younger age group. This suggests that older women have a lower chance of conceiving through assisted reproductive technology, despite having low basal FSH levels. In the present study, the number of MZT was positively correlated with the male age, while negatively correlated with embryo transfer day. A significant positive correlation was found between the number of trials and the age of female and male.

In recent studies, various aspects of IVF techniques, parental age, hormone levels, and anatomical problems have previously been discussed concerning MZT incidence. According to Knopman et al. (13), the likelihood of MZT was related to the fact that younger individuals exhibited their possible superior reproductive potential as a result of finding healthier oocytes. Even though this approach is very logical, it is still very uncertain and more data is needed. Embryo derived from younger oocyte is transferred at a blastocyst stage of the advanced levels. This because age factor may not be an independent risk factor for MZT, but rather a representative of blastocyst transfer (23). In the study by Sills et al, the MZT incidence was 1.3%, comparable to reported results, 3 times higher than a natural pregnancy at 0.4%(24). Vega et al., in the largest study of IVF cycles evaluating multiple pregnancies, dizygotic, and discordant twinning rates, reported the rate of MZT in women < 35 years of age is 1.7%(25). Osianalis et al. analyzed a large single-institution database and determined that cycles carried a 2.3% risk of multiple pregnancies (26). In our study with the 1159 cycle, 16 cycles with MZT were achieved with IVF, resulting in a MZT incidence of 1.38%. The MZT incidence aged ≤ 35 and > 35 were 0.2% and 1.1%, respectively.

As a result, high-risk results in terms of maternal and infant health can occur in IVF. Shevell et al. detected an increased abnormal placentation rate in IVF and it was assumed that it may cause these complications during pregnancy. Romundstad et al. found that placenta previa occurs six times more frequently in singleton pregnancies after assisted reproduction. Zhu et al. reported abnormal placental cord placement among women who gave birth after ART and matched controls of spontaneous pregnancies. Considering these studies, it has been suggested that MZT may be the cause of poor maternal and stillbirth outcome events resulting from inadequate or abnormal placental development. In our study, we observed that 8 MZT pregnancies resulted in a live birth. Five MZT pregnancies resulted in an abortus. Three MZT pregnancies, which were twin pregnancy, resulted in partial abortion. These MZT results support increasing risks for maternal health in both our study and literature.

The effect of low AMH levels on oocyte quality is known. However, the possibility that the association between AMH and the presence of oocyte defects may result from decreased granulosa secretion in poor quality oocytes remains an important issue that continues to be investigated. AMH levels may influence

the determination of the dominant follicle through the inhibitory effects of AMH during the primary follicle collection process from the primordial pool (27) and regulation of FSH sensitivity in ovarian tissue (28). Borges et al. (29) and Fanchin et al. (30), they examined whether follicular AMH production was positively associated with oocyte and embryo development and demonstrated its significant effect. Ebner et al. showed that AMH levels are directly and strongly associated with oocyte quality (31). While Loh and Maheshwari previously explained that AMH was unable to predict the likelihood of pregnancy (32). La Marca et al. reported that extremely low levels of AMH were associated with the inability to conceive (33). Lamazou et al. found reasonable pregnancy rates following extremely low serum AMH levels (34). Borges et al. showed that the probability of pregnancy and the number of embryos obtained, high-quality embryos, and the number of embryos transferred were positively correlated with the AMH level (29). Similarly, in the present study, we found that cumulus-oocytes complexes, metaphase-II oocyte, pronucleus oocyte were strongly correlated with serum AMH levels.

The present study had its limitations. All the patients' data about risk factors for MZT in ART-related pregnancies could not be reached in this retrospective study. Another issue is the fact that women who gain pregnancy with IVF and conceive naturally may vary according to demographics that may be associated with the possibility of MZT. Since being a young women is directly connected with the MZT in ART, we cannot exclude the possibility of underestimating the risk. Generally, this limitation could be seen in all clinical observational analysis in different medical fields. In the present analysis, however, this should be noted that although some confounders cannot be ruled out, the magnitude of the risk tends to support a causal effect.

Conclusion

The pregnancies that we identified and achieved with IVF had a similar incidence of MZT with the literature, although its risk was more for women > 35 years. Strong positive correlation was found between the number of trials and the age of female and male. The number of MZT was positively correlated with the male age, while negatively correlated with embryo transfer day. As a result, MZT pregnancies can become a problem as the increasing number of IVF applications continue due to its potential risks for both maternal and fetus.

Declarations

Conflict of Interest: The authors have declared no conflict of interest for the present study.

Ethics approval and consent to participate: The study was approved by Ethics Committee of Bahcesehir University with the date and number: 17.07.2020-0003. Informed consent was obtained from all subjects

Consent for publication: Not Applicable (17 July 2020).

Availability of data and materials: All data generated or analysed during the present study are included in this published article. The datasets analysed during the current study available from the corresponding

author on reasonable request.

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Authors' contributions: N.D.G. and T.G. planned the study, conducted and wrote the main manuscript text. All authors reviewed the manuscript.

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Tables

Table-1. Demographic details of the patients

Female Age	Male Age	BMI	Trial	AMH	Reason
26	30	28	0	3	Genetic-DMD
36	39	22	8	1	Male-Related
28	31	23	0	0,7	Unexplained Infertility
29	34	23	2	1	Unexplained Infertility
25	35	24	3	0,7	Male-Related
27	36	24	0	5	Male-Related
25	27	22	2	2	Male-Related
31	34	27	4	1	Tubal
27	30	25	0	4	Genetic-DMD
31	33	22	2	5	Endometriosis
31	38	24	3	1	Unexplained Infertility
30	32	23	0	1	Endometriosis
32	35	26	2	1	Male-Related
36	38	27	5	5	Male-Related
30	33	20	0	3	Endometriosis
32	34	27	2	4	Male-Related

Abbreviations. DMD: Duchenne Muscular Dystrophy

Table-2. Patients' outcomes with oocyte and trigger details

Trigger Agent	Outcome	COC	MII	PO	ETD	ETF
Ovitrelle	Live Birth-Twin	12	10	10	5	1
Ovitrelle	Live Birth-Twin	8	7	7	5	2
Ovitrelle	Live Birth-Twin	3	3	3	5	1
Ovitrelle	Live Birth-Three	3	3	3	4	2
Ovitrelle	Live Birth-Three	3	3	3	3	2
Ovitrelle	23w Two Baby Ex	5	4	3	5	1
Ovitrelle	Live Birth-One	8	8	6	5	2
Ovitrelle	Live Birth-Twin	9	7	6	5	2
Ovitrelle	Live Birth-Twin	9	8	8	5	1
Lucrin	Live Birth-One	14	13	12	5	2
Dual	22w Three Abort	5	4	4	5	2
Ovitrelle	11w Twin Missed	7	6	6	5	1
Lucrin	15w Abort-Three	10	8	8	5	2
Lucrin	Live Birth-One	15	12	11	5	2
Lucrin	Live Birth	11	9	8	5	1
Lucrin	Spontan Abort	11	8	8	5	2

Abbreviations. COC: Cumulus-Oocytes Complexes, MII: Metaphase II Oocytes, PO: Pronucleus Oocytes, ETD: Embryo Transfer Day, ETF: Frequency of Embryo Transfer