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Original Article

Monozygotic twinning after *in vitro* fertilization/intracytoplasmic sperm injection treatment is not related to advanced maternal age, intracytoplasmic sperm injection, assisted hatching, or blastocyst transfer

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ABSTRACT

Objective: To evaluate the effect of assisted reproductive techniques on the incidence of monozygotic twins (MZT) and the associated pregnancy outcomes.
Materials and methods: This was a retrospective study of all *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles with MZT pregnancies in our center from January 2001 to December 2011. The diagnosis of MZT pregnancies with their respective placental configurations was based on the results of ultrasonographic examinations performed during either the first or second trimester. The treatment characteristics and outcomes of each IVF cycle were recorded and stored in a computer database.
Results: A total of 17 cycles with MZT pregnancies were identified, resulting in an overall incidence of MZT of 1.3%. The incidence of MZT for women aged <35 years and ≥35 years were 1.5% and 0.8%, respectively ($p = 0.319$). The incidence was not significantly different between ICSI and non-ICSI cycles (1.4% vs. 1.0%; $p = 0.620$). In addition, the incidence was not increased in the assisted hatching (AH) group compared to those without AH (0.9% vs. 2.1%; $p = 0.103$). Finally, cycles with embryo transfer at the blastocyst stage had an MZT incidence that was not significantly different from those transferred at the cleavage stage (1.4% vs. 1.3%, respectively; $p = 1.000$). The incidence of each type of chorionicity, dichorionic–diamniotic, monochorionic–diamniotic, and monochorionic–monoamniotic, was 33.3%, 46.7%, and 20.0%, respectively. A total of 11 of 39 (28%) monozygotic babies and 16 of 19 (84%) coexisting heterozygotic babies were born alive.
Conclusion: Until definite conclusions are drawn from larger trials, patients receiving IVF should not be overly concerned about the increase in MZT risk when proceeding to various assisted reproductive procedures (i.e., ICSI, AH, and blastocyst transfer). However, there is some evidence that the incidence of monochorionic–monoamniotic twins may be significantly increased after IVF/ICSI cycles. Patients should be informed about the possible obstetric complications regarding this rare type of MZT.

Introduction

Monozygotic twinning (MZT) occurs rarely in natural conception, with an estimated overall incidence of 0.4–0.45% [1,2]. Unlike dizygotic twinning, in which the incidence varies among different races, monozygotic twinning is evenly distributed in all populations around the world [3]. The use of assisted reproductive techniques (ART) has been suggested to be associated with an increased occurrence of MZT, which is approximately 1.5% [1,4,5]. However, the actual ART-related incidence is obscured by multiple embryo transfer and early embryonic demise. Several previously proposed factors contributing to the increased incidence of MZT in ART include ovarian stimulation, intracytoplasmic sperm injection (ICSI), assisted hatching (AH), and embryo culture. However, the exact mechanism that leads to the association between ART and MZT is still unclear and needs further investigation.

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MZT occurs when a single zygote divides into two separate embryos. If the cleavage of the zygote occurs later than the 4th day after fertilization, monochorionic twins (with or without the sharing of placenta), monoamniotic twins, or even conjoined twins may develop. MZT is associated with several significant obstetric complications, including preterm labor, intrauterine growth restriction, and fetal death. Twin–twin transfusion syndrome (TTTS) occurs in approximately 9–15% of monochorionic twins [6,7], and the neurologic sequelae of the surviving twins generate further concerns. With the recent trend of the increasing use of ART, preconception counseling about the possibility of monozygotic twins becomes crucial prior to an ART protocol. Furthermore, early detection of the zygosity by transvaginal sonography in the first or early second trimester should be routinely performed in clinical practice.

In this retrospective study, we report all MZT gestations from *in vitro* fertilization (IVF) cycles performed between 2000 and 2011 in our fertility center. The available laboratory data, treatment protocols, and pregnancy outcomes were reviewed accordingly. The objective was to identify any association between the ART procedures and the incidence of MZT pregnancy.

Materials and methods

This study consists of all IVF/ICSI cycles with embryo transfer performed in our center from January 2001 to December 2011. Patients were assigned to either the long protocol (downregulation protocol) using a GnRH agonist (Lupron; Takeda, Osaka, Japan) or the GnRH antagonist (Cetrotide; Serono, Geneva, Switzerland) protocol according to their fertility evaluations and/or previous treatment responses. Both protocols combined the use of recombinant gonadotropin injections (Gonal-F; Industria Farmaceutica, Serono, Italy; or Puregon; N.V. Oganon, Oss, The Netherlands) initiating on Day 2–3 of the stimulation cycle. The dosage of recombinant gonadotropin was adjusted according to serum estradiol (E2) levels and follicular growth monitored by transvaginal ultrasonography. Final oocyte maturation was triggered by 10,000 IU of urinary human chorionic gonadotropin (Pregnyl; MSD, Haarlem, The Netherlands) when there were two or more follicles larger than 1.7 cm. Oocyte retrieval was performed 36 hours after human chorionic gonadotropin administration under the guidance of transvaginal ultrasonography. Progesterone supplementation began on the day of oocyte retrieval and continued until a fetal heartbeat was confirmed.

After serial washing, each oocyte recovered was maintained at 37°C in a separate drop of fertilization medium (Cook IVF, Brisbane, QL, Australia) equilibrated with 6% CO_2 in air. Insemination was carried out either through the conventional IVF procedure or ICSI based on the results of semen analysis. The zygotes were examined for the presence of pronuclei and polar bodies at 18–21 hours after insemination. Zygotes with two pronuclei were cultured at 37°C in a separate drop of cleavage culture medium equilibrated with 6% CO_2 in air until Day 3. Embryos were assessed daily on developmental rate and morphologic appearances to determine the length of culture. If embryos were cultured beyond Day 3, blastocyst culture medium (Cook IVF) was used. The best quality embryos were selected for transfer. These embryos were selectively treated with AH on the day of embryo transfer. We used laser quarter thinning of the *zona pellucida* (ZP), as described previously [8,9], as our method of AH.

Routine transvaginal ultrasonographic examinations were arranged for the 6th gestational week or 7th gestational week for patients with a positive urinary pregnancy test result. The following diagnostic criteria were used to determine monozygotic pregnancies: the number of fetal poles exceeded the number of embryos transferred or more than one fetal pole was identified in a single gestational sac. Follow-up obstetric ultrasonographic examinations were arranged at 10–14 weeks of gestation to confirm fetal viability, chorionicity, and fetal sex. Chorionicity was determined by the presence of either a "T-sign" (which signifies monochorionic twins) at the intertwin membrane-placental junction or a "λ-sign" (which suggests dichorionic twins) [10], whereas fetal sex was identified by checking for the sagittal sign, as described previously [11,12]. All ultrasonographic examinations were performed by well-experienced ultrasonographic technicians, and all images were reviewed by gynecologists to confirm the diagnosis. The treatment characteristics and outcomes of each IVF cycle were recorded and stored in a computer database. Chi-square tests were used to analyze the effect of each proposed risk factor on the incidence of MZT. A p value <0.05 was considered statistically significant.

Results

The total number of IVF cycles from January 2001 to December 2011 with a positive pregnancy result was 1338, in which 17 pairs of MZT were identified, resulting in an overall incidence of MZT of 1.3% in this study. All 17 pairs of MZT resulted from the transfer of fresh embryos. Only one pair of MZT resulted from the use of donated oocytes, and the rest of the MZT pairs resulted from embryo transfer of autologous oocytes. We did not analyze these two groups separately because the number of MZT pairs resulting from donated oocytes was small. The summary of patient characteristics, treatment protocols, and chorionicities of the 17 MZT pregnancies is shown in Table 1, and the proposed MZT risk factors with their respective incidences of MZT gestation are summarized in Table 2.

The mean maternal age of MZT pregnancies was 32.1 years. To analyze the effects of maternal age on the incidence of MZT pregnancy, we compared IVF cycles performed in women aged <35 years with women ≥35 years. The results showed that the incidence of MZT pregnancy was higher in cycles performed in women aged <35 years, although the difference was not statistically significant (1.5% vs. 0.8%, respectively; $p = 0.319$).

The effect of ICSI on the incidence of MZT pregnancy was analyzed. There were 853 fresh IVF cycles with positive pregnancy results in which ICSI was performed. Twelve ICSI cycles (1.4%) developed MZT gestations. For fresh IVF cycles without ICSI, the incidence of MZT was 1.0%. However, the difference was not statistically significant ($p = 0.620$). We also examined the effect of AH on the incidence of MZT pregnancy, but the results showed no increase in MZT incidence in the AH group compared to those without AH (0.9% vs. 2.1%).

The influence of embryo culture length on the incidence of MZT pregnancy was also reviewed. The incidence of MZT pregnancy in the Day 5 group was 1.4% (2 out of 151), which is not significantly different from the 1.3% (15 out of 1191) incidence in the cleavage stage group.

The chorionicity of MZT pregnancies was determined via transvaginal ultrasonographic examinations performed in the first and/or second trimester (Figure 1). Two MZT gestations had undetermined chorionicities due to early fetal demise. Ten (67%) of the MZT pairs with known chorionicities were monochorionic, whereas five (33%) were dichorionic. Of the monochorionic MZT pregnancies, seven (70%) were diamniotic and three (30%) were monoamniotic.

The obstetric outcomes of all 17 cases with MZT pregnancies are summarized in Table 3. Fourteen cases resulted in at least one live birth, including those with coexisting heterozygotic infant (s). In six of these cycles, at least one monozygotic baby was born alive. A coexisting viable heterozygotic infant was delivered at the same time in two cases. Only one pair of monochorionic–monoamniotic