The incidence of monozygotic twinning in assisted reproductive technology: Analysis based on results from the 2010 Japanese ART national registry

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Abstract

Purpose To assess the incidence of monozygotic twinning (MZT) among cases undergoing assisted reproductive technology (ART) treatment.

Methods We performed a retrospective observational study and analyzed the data of patients who were registered in the national ART registry system of Japan from January to

December 2010; only the data of patients with single embryo transfer (ET) were included.

Results Of 30,405 pregnancies, 425 resulted in MZT following fresh and frozenthawed ET. The MZT incidence among women undergoing ART was 1.4 %. Multiple logistic regression analysis indicated that cases undergoing fresh and frozenthawed ET, blastocyst transfer had a significantly increased MZT rate ($P<0.01$). Assisted hatching (AH) and frozenthawed ET and maternal age did not significantly affect the MZT incidence. Of 8510 fresh ET pregnancies, 104 resulted in MZT. Multiple logistic regression analysis indicated that blastocyst transfer significantly increased the MZT rate in cases undergoing fresh ET. Ovarian stimulation, intracytoplasmic sperm injection, AH, and maternal age did not significantly affect the MZT incidence.

Conclusions Blastocyst transfer was associated with an increased MZT incidence. We have to be aware of the potential risk of MZT caused by blastocyst transfer. However, further studies are required to assess the correlation among specific AH types, embryo culture conditions, and MZT incidence.

Capsule MZT in ART.

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Keywords Monozygotic twinning · Assisted hatching · Blastocyst transfer · Frozen-thawed embryo transfer · National ART registry system

Introduction

Multiple births resulting from assisted reproductive technology (ART) can cause certain obstetric and perinatal problems. From January to December 2010, 1,087,148 newborns were born in Japan, and 21,282 (2.0 %) of these resulted from a multiple pregnancy (1). According to data from the National ART Registry System of the Japan Society of Obstetrics and

Gynecology (JSOG), the multiple pregnancy rate for ART treatment was 11.54 % in 2008. With an aim to reduce the incidence of multiple births, JSOG established the following recommendations in April 2008: (i) elective single embryo transfer (ET) should be performed for all patients aged <36 years who have no history of repetitive implantation failure; and (ii) the maximum number of transferred embryos should be restricted to 2, irrespective of the patient's age. Thus, the multiple pregnancy rate was reduced to 4.85 % in 2010; this may have primarily resulted from an increase in the use of single ET (2).

In general, most twin cases involve dizygotic twins that arise from multiple ET. Monozygotic twinning (MZT) is rarely observed naturally, and it only occurs in approximately 0.4 % of all births (3). In contrast, the incidence of MZT after ART is 0.9 % (4). However, the underlying reasons for the increase in the MZT rate following ART treatment remain unclear. Some studies have proposed ovarian hyperstimulation (5–7), zona pellucida manipulation (4, 8–10), ET stage (4, 11–13), and maternal age (14–16) to have an effect on the increased incidence of MZT.

As the occurrence of MZT is relatively rare, a large-scale study is required for analyzing the causes of MZT following ART. In the present study, the incidence of MZT, along with factors which might influence the MZT rate, were evaluated utilizing variables available in the national ART registry system of Japan in 2010. Specifically, ovarian stimulation, fertilization methods, fresh or frozen-thawed ET, ET stage, zona manipulation, and maternal age were evaluated.

Materials and methods

To examine the incidence of MZT, a retrospective observational study was performed. Since 2007, registered ART clinics are mandatorily required to report their cycle-based clinical data via a web-based registry system; since 2010, data on assisted hatching (AH) have also been added to the registry system. Of the 591 registered ART clinics, 587 (99.3 %) clinics reported their cycle-based clinical data. The data of patients in the national ART registry system of Japan from January to December 2010 were analyzed.

The definition of MZT was identified based on the detection of >1 fetal poles on ultrasonography in the cycle with single ET. The type of AH could not be analyzed because there were no detailed records for procedures of AH in the registry system. Moreover, because of the JSOG regulations against egg donation in Japan, data regarding egg donation were not officially included in the present study. In the present study, 242,161 cycles were evaluated. We excluded cycles with no embryo transfer and cases in which the following data were missing: type of ovarian stimulation, fertilization method, duration of embryo culture, and use of AH. To identify the possible factors affecting the incidence of MZT, a multiple logistic regression analysis was performed with the data from

30,405 pregnancies resulting from 105,455 single ET cycles. The adjusted variables included maternal age, fresh or frozen-thawed ET, duration of embryo culture (early cleavage stage ET or blastocyst transfer), and the use of AH. In addition, subgroup analysis was also performed for cases undergoing fresh ET. In the subgroup analysis, the adjusted variables included maternal age, ovarian stimulation protocols (no stimulation vs clomiphene citrate, human menopausal gonadotropin, or follicle stimulating hormone), fertilization methods, duration of embryo culture, and use of AH. Thus, the crude and adjusted odds ratios, 95 % confidence intervals (CI), and *P*-values of the likelihood-ratio test for each independent variable were calculated. All the analyses were performed using the IBM SPSS software version 21.0 for Windows (IBM Corp, NY, USA). A *P*-value of <0.05 was considered to indicate a significant difference in all analyses.

The present study was approved by the Registration and Research Subcommittee of the JSOG ethics committee, and all the data analyzed in the present study were provided by the JSOG.

Results

Table 1 shows the characteristics of infertility factors for ART treatments. The number of clinical pregnancies and the MZT rate in fresh and frozen-thawed ET are shown in Table 2. There were 425 cases of MZT in 30,405 pregnancies undergoing fresh and frozen-thawed ET. The incidence of MZT in pregnant women following ART was 1.4 %, and the incidence of MZT amongst all transfers was 0.40 %. There were 348 (0.6 %) cases of MZT in 59,692 blastocyst transfers, and 77 (0.2 %) cases of MZT in 45,763 early cleavage stage ETs.

The predicting factors for MZT in cases undergoing fresh and frozen-thawed ET are shown in Table 3. Blastocyst transfer significantly increased the incidence of MZT ($P<0.01$). AH, frozen-thawed ET, and maternal age were not associated with a significant increase in the incidence of MZT. The number needed to treat, which was defined as the number of people needed to treat with early cleavage stage ET instead of blastocyst transfer, to reduce MZT incidence by 1 was 241.

Table 1 Characteristics of infertility factors

	n.(%)
Male factor	33,100(31.4)
Tubal factor	19,532 (18.5)
Endometriosis	8,690(8.2)
Antisperm antibody	770(0.7 %)
Others	15,871(15.1 %)
Multiple answers allowed	43,765(41.5)

Table 2 Number of clinical pregnancies and MZT rate in each of variables with fresh and frozen-thawed ET

Category	Variable	MZT n.(%)	Total pregnancy n.
Fresh/frozen-thawed	fresh	117(1.2)	9,914
	frozen-thawed	308(1.5)	20,491
Duration of embryo culture	early cleavage stage	77(0.9)	8,675
	blastocyst stage	348(1.6)	21,730
AH	not performed	203(1.3)	15,902
	performed	222(1.5)	14,503

MZT monozygotic twinning; ET embryo transfer; AH assisted hatching

The number of clinical pregnancies and cases of MZT undergoing fresh ET are shown in Table 4. A total of 104 cases of MZT resulted from 8,510 pregnancies of fresh ET. The predicting factors for MZT in cases undergoing fresh ET are shown in Table 5. Blastocyst transfer significantly increased the incidence of MZT ($P<0.01$). Maternal age, ovarian stimulation, intracytoplasmic sperm injection (ICSI), and AH were not significantly associated with the incidence of MZT.

Discussion

To our knowledge, among retrospective observational studies on the relationship between MZT and ART, the present study included the largest sample size of MZT pregnancies. The incidence of MZT in women undergoing ART was 1.4 %. This was approximately 3.5 times higher than that in natural pregnancy (0.4 %) (3). The present study showed that the incidence of MZT among women treated by frozen-thawed ET, blastocyst transfer, and AH was 1.5, 1.6, and 1.5 %, respectively. The multiple logistic regression analyses indicated that blastocyst transfer significantly increased the incidence of MZT pregnancies. However, the incidence of MZT was not

associated with fertilization methods, AH, frozen-thawed ET, maternal age, and ovarian stimulation. Blastocyst transfer significantly increased the MZT rate, consistent with previous studies (4, 11). The MZT rate after blastocyst transfer was estimated to be 1.7 %, which is 4.25 times higher than that in natural pregnancy (MZT rate of 0.4 %) (4).

The increased MZT rate with blastocyst transfer may be explained by the following mechanisms. The extended exposure to culture media might induce hardening of the zona pellucida before blastocyst transfer. This might result in pinching and splitting of the inner cell mass at the time of hatching and the consequent development of 2 fetal plates (4). On the other hand, a recent study reported that improvements in culture systems significantly reduce the likelihood of MZT with blastocyst transfer (17). The present study showed that the incidence of MZT among women treated by single blastocyst transfer was 1.7 %. A previous study with single ET showed the incidence of MZT among women treated by blastocyst transfer was 1.2 % (18). Sills et al. reported that multiple ET might partially increase the MZT rate for patients undergoing in vitro fertilization (IVF) (19). With regard to the number of blastocysts transferred, several studies have reported the increasing incidence of MZT with multiple blastocyst transfer. The MZT rate per pregnancy among multiple blastocyst transfer has been reported as 3.9 % (13), 12.5 % (20), and 13.2 % (21). Comparing these previous studies and the present study, the MZT rate is apparently lower with single blastocyst transfer than multiple blastocyst transfer.

Theoretically, the artificial manipulation of the zona pellucida during the process of AH could lead to herniation and subsequent splitting of the inner cell mass, resulting in MZT (4). Previous studies have suggested that AH increases the incidence of MZT (9, 10). However, the present study showed that the likelihood of MZT when undergoing AH was 1.5 %, and AH was not associated with the incidence of MZT in the multiple logistic regression analysis. This is consistent with

Table 3 Predicting factor for MZT in each of variables with fresh and frozen-thawed ET

Category	Variable	cOR (95%CI)	aOR(95%CI) ^a	P ^a
Age(by 1 year)		0.98(0.96–1.00)	0.98(0.95–1.00)	0.05
Fresh/frozen-thawed	fresh	reference	reference	
	frozen-thawed	1.28(1.03–1.58)	0.95(0.74–1.22)	0.70
Duration of embryo culture	early cleavage stage	reference	Reference	
	blastocyst stage	1.82(1.42–2.33)	1.84(1.38–2.44)	<0.01
AH	not performed	reference	Reference	
	performed	1.20(0.99–1.46)	1.07(0.87–1.31)	0.54

When considering fresh or frozen-thawed, fresh was set as the standard and the odds ratio of frozen-thawed was measured. During embryo culture, early cleavage stage was set as the standard and the odds ratio of blastocyst stage was measured. When considering the AH, AH not performed was set as the standard and the odds ratio of AH performed was measured

MZT monozygotic twinning; ET embryo transfer; AH assisted hatching; cOR crude odds ratio; aOR adjusted odds ratio; CI confidence interval

^a Regression model adjusted for all variables listed in the table

Table 4 Number of clinical pregnancies and MZT rate in each category of fresh ET

Category	Variable	MZT n.(%)	Total pregnancy n.
Fresh ET (Total)		104(1.2)	8,510
Ovarian stimulation	no stimulation	14(1.7)	837
	performed	90(1.2)	7,673
Fertilization method	IVF	37(0.9)	3,898
	ICSI	67(1.5)	4,612
Duration of embryo culture	early cleavage stage	43(0.8)	5,433
	blastocyst stage	61(2.0)	3,077
AH	not performed	75(1.1)	6,906
	performed	29(1.8)	1,604

Ovarian stimulation include use of clomiphene citrate, human menopausal gonadotropin, follicle stimulating hormone, gonadotropin releasing hormone agonist and antagonist

MZT monozygotic twinning; ET embryo transfer; AH assisted hatching; IVF in vitro fertilization; ICSI intracytoplasmic sperm injection

the findings of previous studies (22, 23). The MZT rate when undergoing AH was estimated to be 0.7–0.8 % (4, 24). Therefore, this theory is still controversial, and further studies are needed to confirm the relationship between MZT and AH.

In the present study, ICSI, which involves an artificial breach of the zona pellucida, similar to AH, was not associated with significant changes in the MZT rate; this finding was consistent with that of a previous study (8). However, a previous meta-analysis indicated that the MZT rate when undergoing ICSI was 0.97 % (95 % CI, 0.5–1.6), and ICSI was associated with a 2.25 times higher likelihood of resulting in MZT compared to natural pregnancy (MZT rate, 0.4 %) (4).

In addition, multiple logistic regression analysis of the current data did not indicate any association between frozen-thawed ET and MZT, as reported in previous studies (14, 25). A recent meta-analysis reported that the MZT rate when undergoing frozen-thawed ET was 3.0 %. However, the total number of pregnancies in that analysis was not sufficient to ensure the estimation (4). Previous studies have suggested a possible association between MZT and frozen-thawed ET (26, 27). The increase in the MZT rate when undergoing frozen-thawed ET was hypothesized to be associated with zona pellucida hardening (28). However, the association between an increase in the MZT rate and frozen-thawed ET remains controversial.

In the present study, we did not identify an association with maternal age and MZT. A decreasing trend in the MZT rate ($P=0.05$) was noted with advancing maternal age. Knopman et al. reported that the risk for MZT pregnancy after IVF is highest when the maternal age of the oocyte source is < 35 years (29). In contrast, other studies reported that the rate of spontaneous MZT increased in women with advancing maternal age (> 35 years) (14–16).

Some studies have reported an association between ovarian stimulation and MZT (5–7). In the analysis of the present study, ovarian stimulation was not associated with significant changes in MZT. Although various stimulation protocols were performed, the association of MZT with specific stimulation protocols was not analyzed. Therefore, it is difficult to demonstrate an association between stimulation protocols and MZT.

This study had some limitations. First, sufficient data describing the method of AH performed could not be obtained from the registry system. AH was performed via different strategies among different clinics to achieve optimal outcomes.

Table 5 Predicting factor for MZT in each variable in fresh ET

Category	Variable	cOR (95%CI)	aOR(95%CI) ^a	p^a
Age(by 1 year)		0.99(0.95–1.04)	0.99(0.95–1.04)	0.78
Ovarian stimulation	no stimulation	reference	reference	
	performed	0.70(0.40–1.23)	0.61(0.34–1.09)	0.10
Fertilization method	IVF	reference	reference	
	ICSI	1.54(1.03–2.30)	1.43(0.95–2.16)	0.09
Duration of embryo culture	early cleavage stage	reference	reference	
	blastocyst stage	2.53(1.71–3.76)	2.47(1.65–3.69)	<0.01
AH	not performed	reference	Reference	
	performed	1.68(1.09–2.58)	1.31(0.84–2.06)	0.24

When considering ovarian stimulation, natural cycles was set as the standard and the odds ratio of ovarian stimulation performed was measured. When considering fertilization method, IVF was set as the standard and the odds ratio of ICSI was measured. During embryo culture, early cleavage stage was set as the standard and the odds ratio of blastocyst stage was measured. When considering the AH, AH not performed was set as the standard and the odds ratio of AH performed was measured

MZT monozygotic twinning; ET embryo transfer; AH assisted hatching; IVF in vitro fertilization; ICSI intracytoplasmic sperm injection; cOR crude odds ratio; aOR adjusted odds ratio; CI confidence interval

^a Regression model adjusted for all variables listed in the table

The size of the hole created in the zona pellucida might also differ between patients. Second, data regarding ovarian stimulation and fertilization methods were not obtained in cases undergoing frozen-thawed ET. Therefore, the ovarian stimulation and fertilization method could not be used as confounding factors in the multivariate analysis (Table 3). Third, this study found ET stage as a risk factor for MZT, but detailed information on the duration of embryo culture (e.g., day 2 or day 3 ET, day 5 or day 6 blastocyst transfer) was not available. Fourth, information on embryo quality, which may have an impact on MZT, was not available (14).

In the present study, MZT was significantly associated with blastocyst transfer. In MZT, there are monochorionic monoamniotic, monochorionic diamniotic, and dichorionic diamniotic twins. In general, monochorionic monoamniotic and monochorionic diamniotic twins greatly increase perinatal risk. Therefore, it is important to inform patients and obtain full consent when performing blastocyst transfer. Further studies are required to assess the association of MZT with embryo culture conditions and specific types of AH.

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Conflict of interest The authors declare that they have no conflict of interest.

References

- Ministry of Health, Labour and Welfare. Statistics information-white book. <http://www.mhlw.go.jp/> Accessed 20 December 2013.
- Japan Society of Obstetrics and Gynecology. ART Registry of Japan, 2008 in English. Available at: <http://plaza.umin.ac.jp/~jsog-art/> Accessed 6 April 2013.
- Bulmer MG. The biology of twinning in man. Oxford: Oxford University Press; 1970.
- Vitthala S, Gelbaya TA, Brison DR, Fitzgerald CT, Nardo LG. The risk of monozygotic twinning after assisted reproductive technology: a systematic review and meta-analysis. *Hum Reprod Update*. 2009;15:45–55.
- Schachter M, Raziel A, Friedler S, Strassburger D, Bem O, Ron-El R. Monozygotic twinning after assisted reproductive techniques: a phenomenon independent of micromanipulation. *Hum Reprod*. 2001;16:1264–9.
- Derom C, Vlietinck R, Derom R, Van den Berghe H, Thiery M. Increased monozygotic twinning rate after ovulation induction. *Lancet*. 1987;1:1236–8.
- Blickstein I, Keith LG. On the possible cause of monozygotic twinning: lessons from the 9-banded armadillo and from assisted reproduction. *Twin Res Hum Genet*. 2007;10:394–9.
- Saito H, Tsutsumi O, Noda Y, Ibuki Y, Hiroi M. Do assisted reproductive technologies have effects on the demography of monozygotic twinning? *Fertil Steril*. 2000;74:178–9.
- Hershlag A, Paine T, Cooper GW, Scholl GM, Rawlinson K, Kvapil G. Monozygotic twinning associated with mechanical assisted hatching. *Fertil Steril*. 1999;71:144–6.
- Schieve LA, Meikle SF, Peterson HB, Jeng G, Burnett NM, Wilcox LS. Does assisted hatching pose a risk for monozygotic twinning in pregnancies conceived through in vitro fertilization. *Fertil Steril*. 2000;74:288–94.
- Chang HJ, Lee JR, Jee BC, Suh CS, Kim SH. Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: a systematic review and meta-analysis. *Fertil Steril*. 2009;91:2381–90.
- Behr B, Fisch JD, Racowsky C, Miller K, Pool TB, Milki AA. Blastocyst-ET and monozygotic twinning. *J Assist Reprod Genet*. 2000;17:349–51.
- da Costa AA, Abdelmassih S, de Oliveira FG, Abdelmassih V, Abdelmassih R, Nagy ZP, et al. Monozygotic twins and transfer at the blastocyst stage after ICSI. *Hum Reprod*. 2001;16:333–6.
- Alikani M, Cekleniak NA, Walters E, Cohen J. Monozygotic twinning following assisted conception: an analysis of 81 consecutive cases. *Hum Reprod*. 2003;18:1937–43.
- Abusheikha N, Salha O, Sharma V, Brinsden P. Monozygotic twinning and IVF/ICSI treatment: a report of 11 cases and review of the literature. *Hum Reprod Update*. 2000;6:396–403.
- Steinman G. Mechanisms of twinning. II. Laterality and intracellular bonding in monozygotic twinning in in vitro fertilization. *J Reprod Med*. 2001;46:473–9.
- Moayeri SE, Behr B, Lathi RB, Westphal LM, Milki AA. Risk of monozygotic twinning with blastocyst transfer decreases over time: an 8-year experience. *Fertil Steril*. 2007;87:1028–32.
- Kawachiya S, Bodri D, Shimada N, Kato K, Takehara Y, Kato O. Blastocyst culture is associated with an elevated incidence of monozygotic twinning after single embryo transfer. *Fertil Steril*. 2011;95:2140–2.
- Sills ES, Moomjy M, Zaninovic N, Veeck LL, McGee M, Palermo GD, et al. Human zona pellucida micromanipulation and monozygotic twinning frequency after IVF. *Hum Reprod*. 2000;15:890–5.
- Tarlatzis B, Qublan H, Sanopoulou T, Zepiridis L, Brimbizis B, Bontis J. Increase in the monozygotic twinning rate after intracytoplasmic sperm injection and blastocyst stage embryo transfer. *Fertil Steril*. 2002;77:196–8.
- Jain JK, Boostanfar R, Slater CC, Francis MM, Paulson RJ. Monozygotic twins and triplets in association with blastocyst transfer. *J Assist Reprod Gen*. 2004;21:103–7.
- Frankfurter D, Trimarchi J, Hackett R, Meng L, Keefe D. Monozygotic pregnancies from transfers of zona-free blastocysts. *Fertil Steril*. 2004;82:483–5.
- Milki AA, Jun SH, Behr B, Giudice LC, Westphal LM. Incidence of monozygotic twinning with blastocyst transfer compared to cleavage-stage transfer. *Fertil Steril*. 2003;79:504–6.
- Carney SK, Das S, Blake D, Farquhar C, Seif MM, Nelson L. Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)). *Cochrane Database Syst Rev* 2012:CD001894.
- Blickstein I, Jones C, Keith LG. Zygotic-splitting rates after single-embryo transfers in in vitro fertilization. *N Engl J Med*. 2003;348:2366–7.
- Toledo MG. Is there increased monozygotic twinning after assisted reproductive technology? *Aust N Z J Obstet Gynaecol*. 2005;45:360–4.
- Faraj R, Evbuomwan I, Sturgiss S, Aird I. Monozygotic triplet pregnancy following egg donation and transfer of single frozen-thawed embryo. *Fertil Steril*. 2008;89(1260):e9–e12.
- Aston KI, Peterson CM, Carrell DT. Monozygotic twinning associated with assisted reproductive technologies: a review. *Reproduction*. 2008;136:377–86.
- Knopman J, Krey LC, Lee J, Fino ME, Novetsky AP, Noyes N. Monozygotic twinning: an eight-year experience at a large IVF center. *Fertil Steril*. 2010;94:502–10.