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Conjoined twins after frozen embryo transfer: a case report and literature review

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Summary

Objective: To present a case of conjoined twins (CTs) in a triplet pregnancy after frozen blastocyst transfer and a literature review. *Design:* Case report. *Setting:* Reproductive medicine center of a university teaching hospital. *Case Report:* A 24-year-old patient underwent in vitro fertilization (IVF) and received two frozen blastocyst transfers. Sequential examination by transvaginal ultrasonography and transvaginal multifetal pregnancy reduction were performed during early pregnancy. Ultrasound images of the fetus in the gestational sac were assessed. Two gestational sacs were found in the uterus at 30 days after the embryo transfer (ET). A germ with primitive heart beat was observed in each sac. At the seventh week of gestation, one heart was still observed in each sac. At the tenth week, a single fetus was observed in one sac, whereas thoracopagus CTs with one heartbeat were seen in the other sac. Selective fetal reduction of the CTs was performed at the 11th week. Unfortunately, abortion occurred at the 20th week because of premature rupture of membranes. *Conclusions:* The authors presented a case of CTs that occurred after the transfer of two frozen blastocysts, and suggested that factors such as ovulation induction, intracytoplasmic sperm injection (ICSI), preimplantation genetic diagnosis (PGD), culture time and culture condition, assisted hatching and blastocyst transfer, and maternal age might be responsible for the CTs. The importance of early transvaginal ultrasound in pregnancies resulting from assisted reproductive technologies (ARTs) is emphasized. Selective embryo reduction is an appropriate choice for the management of these pregnancies.

Key words: Conjoined twins; Assisted reproduction; Selective fetal reduction; Frozen embryo; Triplet pregnancy.

Introduction

With the widespread use of assisted reproduction technologies (ARTs) throughout the world, multiple pregnancies have become a common complication of ART in the past 20 years. Recently, the number of embryo transfers (ETs) per cycle have been strictly controlled as the risks of maternal, fetal, and neonatal associated with ARTs is increasingly recognized. Multifetal pregnancies are mostly multizygotic, but recent reports have shown that the incidence of monozygotic multifetal pregnancies increases after ARTs. The incidence of monochorionic pregnancies associated with ARTs is 3.2%, about eight times higher than that in natural pregnancies (0.4%) [1, 2]. So far, the mechanism for monozygotic twins (MZT) has not been clarified. Many factors may be related to MZT, such as maternal age [3], ovarian stimulation [4, 5], manipulation of the zona pellucida [6, 7], and blastocyst transfer [6,8].

Conjoined twins (CTs) are a rare phenomenon occurring approximately once in every 200,000 to 100,000 pregnancies [9], which result from an abnormal process during the development of MZT and make up about 1% of MZT [10].

Here the authors present a case of CTs in a triplet pregnancy conceived after two frozen-thawed blastocyst transferred with laser assisted hatching and a literature review of CTs associated ART.

Case Report

A 24-year-old woman with two years of primary infertility due to polycystic ovary syndrome underwent IVF treatment. Her husband's sperm analysis was normal. Briefly, she underwent six cycles of ovulation induction with clomiphene and human menopausal gonadotropin before IVF. Because of irregular menstrual cycle, she had previously taken oral contraceptives for pituitary down-regulation prior to the IVF treatment. She underwent the standard long protocol, pituitary down regulation was achieved by 1.2 mg GnRH agonist administered at the day equivalent to the midluteal phase of the previous cycle. Fourteen days after pituitary down-regulation, ovulation induction was performed on day 5 of the menstrual cycle by daily injection of recombinant follicle stimulating hormone. At 22 days of ovulation induction (a total dose of Gn: 17,75I U) when the maximal diameter of more than two oocytes reached 18 mm, oocyte maturation was triggered by 10,000 IU of hCG. Transvaginal oocyte retrieval was performed under sonographic guidance at 36 hours after hCG administration. A total of 18 oocytes were harvested and incubated in fertilization medium. A total of 13 oocytes were fertilized and incubated in cleavage medium with 10% human serum albumin. A total of seven blastocysts were obtained. Two high-quality embryos were obtained at three days after oocyte retrieval,

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and all other embryos were cultured in sequential media. All the embryos were frozen in order to prevent ovarian hyperstimulation syndrome (OHSS). After three months, the patient received hormone replacement therapy to prepare the endometrium due to her irregular menstrual cycle. Two frozen-thawed blastocysts with laser assisted hatching were transferred to the uterus on day 22 of the menstrual cycle. Luteal support was given by daily administration of 40 mg of progesterone injection and 20 mg of dydrogesterone tablets. Urine hCG test was positive and serum hCG level was 905.0 mIU/ml at 14 days after ET. A single fetal heartbeat was identified in each of the two gestational sacs in the uterus as revealed by transvaginal unltrasound at 30 days after ET. Transabdominal ultrasound revealed one fetal with a crown-rump length (CRL) of 49 mm in one sac, and inseparable fetal bodies with CRL of 46 mm in the other sac at 60 days after ET (about 11 weeks of gestational). The fetuses had two arms, two legs, one trunk, but two heads. The CTs in the triplet pregnancy were subsequently confirmed by two-dimensional scanning, and the family chose selective reduction of the CTs. Selective fetal reduction was performed by a transvaginal approach at 12 weeks of gestation. A 17-gauge needle was directed into the heart of the CTs, and 1.0 ml of 10% potassium chloride was injected till the heartbeat disappeared. Both the termination of the heartbeat of the CTs and the normal heartbeat of the single fetal in the other sac was detected by sequential scanning at one and seven days after the operation. Unfortunately, abortion occurred because of premature rupture of membranes at 20 weeks of gestation.

Discussion

To date, ARTs have successfully assisted countless couples to conceive. However, multiple pregnancies have always been a common and serious complication of these technologies in the past 20 years. The number of ETs is strictly controlled in ARTs in order to reduce the incidence of multiple pregnancies. The method has been quite effective in reducing the incidence of dizygotic twins, but is inefficient for the prevention of MZT. A increasing rate of ART-associated MZT has been frequently reported since the first reported case of MZT after IVF in 1984 [11]. To date, the exact mechanisms behind the formation of MZT remain unclarified. A number of factors are suspected to contribute to the increasing rate of MZT including ovulation induction, manipulation of the zona pellucida (e.g. Intracytoplasmic sperm injection (ICSI), preimplantation genetic diagnosis (PGD), and assisted hatching), blastocyst transfer, and maternal age. Herein the authors present a literature review on these factors and cases that might be associated with them.

Ovulation induction

Many studies have shown that ovulation induction may increase the incidence of MZT by hardening of the zona pellucida [1, 12] or a delayed implantation [13] induced by ovulatory hormones. Blickstein and Keith [14] believed that ovarian stimulation increased the number of available splitting-prone oocytes, and thereby caused the high incidence of such oocytes to develop into monozygotic twins.

ICSI

Studies have shown a significant higher incidence of MZT in ICSI cycles than that in conventional IVF cycles [15, 16]. It is believed that ICSI introduces an artificial gap in the zona pellucida that may cause splitting of the inner cell mass (ICM) to form identical twins. However, a contrary result was also reported in a study by Luke *et al.* in which the incidence of MZT decreased with in ICSI cycles compared with conventional IVF cycles [17].

PGD

Haimov-Kochman *et al.* [18] suggested that repeated manipulation of the zona pellucida, as well as extended embryo culture during ICSI-PGD treatments, might result in MZT and even triplet pregnancies. In contrast, Verpoest *et al.* [19] claimed that the embryo biopsy procedure of PGD did not induce higher incidence of MZT compared with ICSI cycles. However, the incidence of MZT related to PGD and ICSI in their study was 1.5% and 2.1%, respectively, which was significantly higher than that in natural pregnancies.

Assisted hatching

It has previously been suggested that assisted hatching caused the occurrence of MZT by splitting of the inner cells, particularly when the space was too small to allow the embryo to hatch properly [17]. Contrarily, no significant difference in the incidence of MZT was observed between the assisted hatching and the control groups in recent studies [20, 21].

Blastocyst transfer

Numerous studies have shown a higher incidence of MZT associated with blastocyst transfer [16, 18, 21, 22]. For example, in the latest ten-year retrospective study conducted by Takaski *et al.*, the incidence of MZT in women undergoing blastocyst transfer was 1.7%, which was 4.25 times higher than that in natural pregnancy (a MZT rate of 0.4 %) [21]. Although the exact mechanisms underlining the inductive effect of blastocyst transfer on MZT are not clear, it was suspected that the extended exposure to culture media might cause hardening of the zona pellucida before blastocyst transfer, which might result in pinching and splitting of the ICM during hatching, and consequently the development of two fetal plates [16]. However, some studies had opposite findings. For instance, Moayeri *et al.* [23] demonstrated that improvements in the culture systems significantly decreased the incidence of MZT induced by blastocyst transfer. Wu *et al.* discovered that the incidence of MZT after blastocyst transfer (1.4%) was not significantly different from that transferred at the cleavage stage (1.3%) [24].

Culture duration and culture condition

Cassuto *et al.* [25] suggested that prolonged in vitro cul-

Table 1. — *A summary of reported cases of conjoined twins after ART.*

Case no	Age	Gravity	Parity	Day of ET	Treat-ment	Type of IVF cycle	Assisted hatching transferred	No. of embryos	No. of GS	No. of fetuses	Gestational age at diagnosis	Selective termination	No. of new fetuses	Result	Author
1	27	ND	0	ND	IVF	Fresh	-	2	2	3	10w	+	1	NND	Boulot *et al.* [36]
2	35	ND	2	D3	IVF	Fresh	+	4	2	3	12w	+	1	Ongoing in third trimester	Skupski *et al.* [37]
3	28	1	0	ND	ICSI	Fresh	-	3	2	3	8w4d	+	1	NND (37w)	Goldberg *et al.* [38]
4	30	0	0	D3	ICSI	FET	+	2	2	3	10w	-	1	Abortion (12w)	Sugawra *et al.* [39]
5	30	2	0	ND	ICSI	Fresh	-	2	1	2	28w	-	1	CS (30w, nneonatal death)	Fujimori *et al.* [40]
6	36	0	0	D3 and D5	IVF	Fresh	-	3	1	2	10w	-	0	Termination (11w)	Shimizu *et al.* [41]
7	37	2	1	ND	ICSI	FET	-	4	3	4	11w3d	+	1	CS(38w)	Maymon *et al.* [42]
8	20	ND	ND	ND	IVF	Fresh	ND	2	2	3	10w	+	0	Spontaneous abortion (21w)	Charles *et al.* [43]
9	38	1	0	D3	ICSI	Fresh	+	3	3	4	11w3d	+	2	CS (38w)	Allegra *et al.* [44]
10	22	1	0	-	OI	-	NA	NA	NA	4	11w	+	2	CS (36w)	Mendilcioglu *et al.* [5]
11	34	2	1	D5	ICSI	Fresh	ND	2	2	3	8w	-	1	NVD (11w)	Hirata *et al.* [45]
12	30	1	0	D2	ICSI	Fresh	ND	1	1	2	9w	-	0	Termination (11w)	Poret *et al.* [46]
13	32	0	0	D3	ICSI	Fresh	ND	3	1	2	10w	-	0	Termination	Mercan *et al.*[47]
14	33	3	0	ND	IVF	FET	ND	1	1	2	8w	-	0	Missed Abortion (9w)	Varma *et al.* [48]
15	32	0	0	-	OI	-	NA	-	2	3	13w1d	+	1	NND (40w)	Shepherd *et al.* [49]
16	24	0	0	D5	IVF	FET	+	2	2	3	11w	+	1	Spontaneous abortion (20w)	This report

ture might induce the hardening of the zona pellucida and the formation of MZT. In addition, some studies have shown that in vitro culture conditions, such as high glucose concentration in culture medium, are responsible for the increased incidence of MZT. The induction effects of embryo culture conditions on MZT have also been confirmed in numerous animal experiments [18, 26-28]. Splitting of the ICM of humans takes place at four to eight days after fertilization and around the time of implantation, when culture conditions may affect the embryonic division. Increased apoptosis in certain culture conditions, such as high levels of free radicals, might cause disruption and splitting of the ICM [29]. It was also suggested that lower concentrations of calcium in the culture medium weakened intercellular junctions in the ICM, and thereby induced the division of the embryo [13].

Maternal age

Previous studies have suggested that age-related changes in the zona pellucida may induce MZT [30-34]. However, a recent study by Dennis *et al.* [24] revealed a higher incidence of MZT in women under age 35 compared with those above age 35. The result was consistent with the finding in a study by Knopman *et al.*, in which the incidence of IVF-associated MZT was higher in young acolyte age due to higher developmental potential in young oocytes compared with older women [35].

CTs conceived through ARTs are monozygotic twins whose bodies are joined in uterus as a result of abnormal process during the development of MZT. CTs are extremely rare accounting for approximately 1% of MZT, but their incidence has recently increased. The most common types of CTs are thoraco-omphalopagus (28%), thoracopagus (18.5%), and omphalopagus (10%) [10]. The ratio of girl to boy CTs was 3:1 [35]. The mechanism of the formation of CTs is not yet clarified. Currently, the most generally accepted is fission theory, in which there is an incomplete splitting of the embryonic axis. In contrast, the fusion theory explains an opposite mechanism in which the union of two initially distinct embryos occurs during early embryonic period.

To date, there are 16 cases of ART-associated CTs including this case report. These cases are summarized in Table 1 [36-49]. Out of the 16 cases of CTs, ten occurred after fresh embryos transfer, four after frozen embryos transfer, and two after ovulation induction (OI) by clomiphene. Patients were subject to zona manipulation by either ICSI or assisted hatching in most cases. Specifically, there are eight cases with ICSI, four cases with assisted hatching, and two cases with both ICSI and assisted hatching. Only two cases took place after blastocyst transfer, one case with both cleavage-stage, and blastocyst-stage ET. The low rate of CTs induced by blastocyst transfer is inconsistent with the previous finding that blastocyst transfer increases the incidence of MZT [16, 18, 21, 22,]. In this report, the authors presented a case of CTs after assisted hatching, which is the fourth case of CTs associated with frozen ET in the literature. This case is the authors' first case of CTs out of about 15,000 IVF/ICSI cycles in total. Factors including ovulation induction, in vitro culture condition, culture duration, blastocyst-stage transfer, and assisted hatching might have induced the CTs in the present case. However, further research is needed to elucidate the exact mechanism behind the formation of CTs in this report. In addition, studies may be performed to investigate if the super-physiological status of estrogen can promote the splitting of the ICM and thereby cause the CTs.

Ultrasound is the most non-invasive, accurate, and convenient method for the diagnosis of CTs. The ultrasound criteria for CTs in the second and third trimesters have been well-established. Ultrasonographic identification of any of the classical signs including lack of separating membrane, inability to separate fetal bodies, and two or more vessels in cord may suggest the diagnosis of CTs [50, 51].

Early diagnosis of CTs allows better counseling of the parents regarding management options, including continuation of the pregnancy, termination of the whole pregnancy, and selective termination of the CTs. Three- and four-dimensional ultrasound substantially improve the accuracy of prenatal assessment. The importance of vaginal ultrasound in early diagnosis of CTs was emphasized in a case reported by Mercan *et al.* [47] in which fetal anomaly was detected by ultrasonographic diagnosis at as early as six weeks and four days of pregnancy. In all previous cases of CTs in the literature, all patients were examined by ultrasound in the first trimester of pregnancy. In the present case, the patient was diagnosed at 11 weeks of pregnancy by ultrasound, and it was discovered that the CTs had one trunk, two arms and two legs, but two heads. The family chose selective reduction of the CTs after thorough consulting with OB/GYN Specialists. Selective fetal reduction has been an appropriate choice for the management of such CTs, which is part of a high-order multifetal pregnancy, despite that the outcome of this particular case was not satisfactory.

In summary, although CTs are extremely rare in IVF/ICSI pregnancies, ultrasound examination is necessary for early diagnosis and management of such extremely risky pregnancies. The authors presented a case of CTs that occurred after the transfer of two frozen blastocysts, and suggested that factors such as ovulation induction, manipulation of the pellucid zone, culture duration and culture condition, blastocyst transfer, and maternal age might be responsible for the CTs. Selective embryo reduction is an appropriate choice for the management of these pregnancies.

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