Split happens: a case of consecutive monozygotic twin pregnancies following elective single-embryo transfer in a 40-year old woman using donor oocytes

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Introduction

Monozygotic twins derive from a single zygote that undergoes division at varying stages of development, the timing of which ultimately determines placental sharing. Morula stage (days 1–4) division results in dichorionic-diamniotic twins that cannot be differentiated from dizygotic twins in utero. Blastocyst stage (days 4–8) division results in monochorionic-diamniotic twins. Division at the 8–12-day stage results in monochorionic-monoamniotic twins, and division after day 12 leads to conjoined twins [1, 2]. Monozygotic twinning is seen at a rate of 0.4% in natural conceptions, though that rate is significantly higher, ranging from 1.57–5.6%, when blastocysts are transferred using assisted reproductive technologies (ART) [3–5]. Although consensus has not been reached in the literature, it is thought that factors such as intracytoplasmic sperm injection (ICSI), blastocyst transfer, assisted hatching (AH), and individual embryologist technique may play a role in the increased frequency of monozygotic twinning in ART [2, 6]. It is widely agreed upon, however, that monozygotic twins carry a greater risk of adverse outcomes such as premature delivery, growth discordance, developmental abnormalities, and perinatal mortality when compared to singleton or dizygotic pregnancies [5].

Case report

We present the case of a 40-year-old nulliparous woman with a 1-year history of infertility and a primary diagnosis of diminished ovarian reserve (AMH 0.16 ng/mL). The couple underwent three IVF cycles prior to presenting at our clinic, two of which resulted in conversion to IUI while the third resulted in cancellation due to poor response. The initial consultation included counseling for the possible necessity of oocyte donation but the patient preferred to first attempt using autologous oocytes. She subsequently had two canceled cycles before proceeding with an ovum donor. The donor selected was a 26-year-old with a prior successful donation cycle that resulted in a singleton pregnancy. She underwent a stimulation cycle using recombinant follicle stimulating hormone in an antagonist protocol, which yielded 21 oocytes, all inseminated using conventional methods. Day 3 development showed 11 8-cell embryos (grade A) and 1 4-cell embryo (grade D). Assisted hatching for all 8-cell embryos was performed on day 3 following quality grading.

Four day 5 and five day 6 blastocysts were biopsied for preimplantation genetic screening for aneuploidy (PGT-A). Six of nine blastocysts were euploid using the Igenomix® next-generation sequencing platform. The patient underwent a single-embryo transfer (eSET) in a frozen embryo transfer cycle using transdermal estrogen administration and vaginal progesterone capsules for luteal support. The beta-HCG level was 458 mIU/mL 12 days after embryo transfer. Transvaginal ultrasound was done at 6.5 weeks gestation and demonstrated a twin intrauterine pregnancy (IUP) with two fetuses sharing a single gestational sac. Both embryos had positive fetal cardiac activity and appropriate growth. Repeat ultrasound done at 7 weeks and 4 days showed a loss of cardiac activity in both fetuses, and a dilation and curettage (D&C) was performed.

Four months following the initial embryo transfer, the patient underwent a second frozen embryo transfer cycle using a

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similar endometrial preparation with a single euploid embryo. Beta-HCG was 187 mIU/mL 10 days after embryo transfer. Transvaginal ultrasound was performed at 6.5 weeks gestation, and a twin IUP was confirmed with two fetal poles, both with cardiac activity within a single gestational sac. After the patient was counseled by a maternal fetal specialist, the couple elected to terminate the pregnancy, and a D&C was performed the following week.

A review of all cases of monozygotic twin pregnancies and twin pregnancies following elective single embryo transfer at our clinic is listed in Table 1.

Discussion

Though the mechanisms leading to monozygotic twinning are not well known, our review of the literature proposes several potential contributing factors for the increased risk of MZT in ART. A 7-year single-center retrospective study by Kawachiya et al. showed a MZT rate of 1.01% that was found to be associated with prolonged embryo culture to the blastocyst stage. Several factors for causes of embryo splitting were suggested, including maternal age, prolonged embryo culture, ovarian stimulation, and zona pellucida (ZP) manipulation [6]. Knopman et al. assessed the effect of oocyte source age (comparing autologous and donor oocytes from women < 35 years old) at the time of oocyte retrieval on the incidence of MZT pregnancies and found that age of the oocyte source could be a primary contributor to the incidence of monozygosity [7].

Results from a meta-analysis with data from over 40,000 cycles were in agreement that blastocyst culture may be a risk factor for MZT, and proposed mechanisms such as mechanical ZP breach by ICSI or AH prolonged exposure and manipulation of the ZP in culture and hardening of the ZP leading to herniation of the blastocyst [8, 9]. A supposition by Blickstein and Keith in a 2007 study indicated that some small number of oocytes may have an innate disposition towards splitting once fertilized and that ovarian stimulation increases the quantity of those oocytes, thereby increasing the likelihood of MZT upon fertilization [10]. Moayeri et al. suggested that extended time in culture, culture media composition, and embryologist experience level may also impact MZT rates [11]. Prolonged exposure to lower calcium levels in culture may also predispose blastocysts to greater rates of ICM division due to breakdown of intercellular bonds [12]. Additionally, exposure to sequential media with a higher glucose content could lead to the production of more free radicals which can induce apoptosis. Menezo and Sakkas suggested that increased levels of apoptosis could disrupt the ICM and potentially lead to splitting [13]. Lastly, it is possible that blastocysts undergo increased metabolic stress due to their prolonged exposure to culture media which lacks cytokines and other cellular factors that may mediate apoptosis [9, 11].

More recently, data from Franasiak et al. comparing patients undergoing blastocyst or cleavage-stage ET showed that MZT rates between the groups were similar when embryo cohort quality measures were controlled. Conclusions from the study suggest that

Table 1 Cases of monozygotic twin pregnancies in all ART cycles at our clinic between 2013 and 2018

Case	Age	Day of ET	IVF cycle	Treatment	PGT-A	AH	#ET	#GS	Chorion/amnion	Pregnancy Outcome
1	33	D4	Fresh	IVF	Yes	No	1	1	Mono/di	Demise ×2 at 21 wks
2	23 ^a	D3	Fresh	ICSI, OD	No	No	1	1	Di/di (reduced from triplets)	TAB at 21 wks
3	35	D3	Fresh	IVF	No	No	2	1	Mono/di	Live birth ×2 at 37 wks
4	21 ^a	D3	Fresh	IVF, OD	No	No	1	1	Di/di	Live birth ×2 at 36 wks
5 ^b	26 ^a	D5	FET	IVF, OD	Yes	Yes	1	1	Mono/di	SAB at 7wks
6 ^b	26 ^a	D5	FET	IVF, OD	Yes	Yes	1	1	Mono/di	TAB at 7 wks
7	34	D5	FET	IVF	Yes	Yes	1	1	Mono/di	SAB at 21 wks
8	35	D5	FET	IVF	Yes	Yes	1	1	Mono/di	Live birth ×2 at 37 wks
9	33	D3	FET	IVF	No	Yes	1	1	Mono/di	SAB at 7 wks
10	38	D5	FET	ICSI	Yes	Yes	2	1	Mono/di	SAB at 8 wks
11	23 ^a	D5	FET	IVF, OD	Yes	Yes	1	1	Mono/di	SAB at 16 wks
12	34	D5	FET	ICSI	Yes	Yes	1	1	Mono/di	TAB at 16 wks
13	35	D2	Fresh	IVF	No	No	3	3	Quadruplets with mono/di twins	Live birth ×4 via CS at 29 wks
14	32	D3	FET	IVF	No	Yes	1	1	Mono/di	Ongoing in second trimester

OD ovum donor, SAB spontaneous abortion, TAB therapeutic abortion, FET frozen embryo transfer

^a Indicates that ovum donor was used and age of donor at the time of egg retrieval

^b Cases 5 and 6 represent data from the patient discussed in this case report

increasing age was associated with decreased MZT rate, and a larger proportion of 6–8 cell embryos at the cleavage stage was associated with increased rates of MZT if a blastocyst transfer occurred [14].

Monozygotic twin pregnancies from both ART and natural conceptions are generally associated with higher incidences of premature delivery, growth discordance, developmental anomalies, and mortality rates [4, 15, 16]. MZT pregnancies conceived through ART are typically more complicated than naturally conceived MZT pregnancies and carry an increased incidence of being part of a high-order multiple pregnancy [15, 17]. Obstetric and perinatal risks associated with IVF pregnancies include lower birth weight, increased risk for preterm delivery, placental abruption, preeclampsia, and perinatal mortality [5, 18].

Though the risks of twin gestations are understood well in current literature, we have observed that patients tend not to be aware of these and depend instead on their physician to provide adequate counseling. SART has published ASRM guidelines for elective single-embryo transfer, multiple gestation risks, and impact of blastocyst culture on ART outcomes, but we believe these resources may be overseen by patients and that it is ultimately the duty of the treating physician to emphasize the risks of multiple gestations to patients [19–22]. We encourage all providers performing embryo transfers to counsel their patients regarding the real and observed risks of twin pregnancies, even in cases of eSET. This is especially important as PGT-A and single blastocyst transfer become more prevalent. Additionally, we recommend clinics to conduct internal reviews of MZT pregnancy rates to determine if their rates are similar to the published literature and if any pattern exists in these cases which may warrant investigation into the clinics laboratory protocols and techniques. A review of our own clinic's SART data ranging from December 2013 to February 2018 showed an overall monozygotic twin rate of 2.4% (14 MZT/591 clinical pregnancies, including autologous and donor oocyte cycles). Interestingly, among donor oocyte pregnancies, the MZT rate was 7% (5/69 clinical pregnancies) and among PGT-A pregnancies the MZT rate was 6% (8/135 clinical pregnancies). However, our overall rate is consistent with the current literature and within the parameters outlined in our embryo transfer consents. Using this data and an independent probability calculation, we determined that the likelihood of occurrence for the unlikely event described in this case report is 0.058%.

To our knowledge, this is the first reported case of consecutive monozygotic twin pregnancies in a patient undergoing IVF following two single-blastocyst embryo transfers. Potential risk factors for this patient include both intrinsic factors (young age of the oocyte donor, high quality cleavage stage embryos) and extrinsic factors (increased embryo manipulation from assisted hatching and trophectoderm biopsy). While these cases are rare, the increased complications and poor outcomes of pregnancies associated with monozygotic

twinning warrant continued investigation into the potential causes and risk factors of this outcome in assisted reproduction.

Compliance with ethical standards

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

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