

Factors associated with embryo splitting and clinical outcome of monozygotic twins in pregnancies after IVF and ICSI

A. MacKenna^{1,*}, J.E. Schwarze¹, J. Crosby¹, and F. Zegers-Hochschild^{1,2}

¹Unit of Reproductive Medicine, Clinica Las Condes, Lo Fontecilla 441, 7591046, Santiago, Chile ²Program of Ethics and Public Policies in Human Reproduction, Universidad Diego Portales, Ejercito 250, 8370056, Santiago, Chile.

*Correspondence. E-mail: amackenn@clinalascondes.cl <https://orcid.org/0000-0002-7810-2803>

Submitted on November 8, 2019; resubmitted on March 18, 2020; editorial decision on March 24, 2020

STUDY QUESTION: What factors are associated with monozygotic twins (MZT) after autologous IVF/ICSI with fresh and frozen/thawed single embryo transfer (SET) and what is the outcome of MZT?

SUMMARY ANSWER: Factors associated with increased MZT were blastocyst transfer and elective single embryo transfer (eSET), with MZT showing a lower gestational age at birth and neonatal weight but higher perinatal mortality only after fresh transfer.

WHAT IS KNOWN ALREADY: ART is associated with an increased incidence of MZT, which carries higher perinatal mortality. However, risk factors associated with MZT are still controversial.

STUDY DESIGN, SIZE, DURATION: A population-based retrospective analysis of data extracted from ART cycles reported to the Latin American Registry of ART between January 2012 and December 2016 was used in order to study the frequency and outcome of MZT after SET.

PARTICIPANTS/MATERIAL, SETTING, METHODS: In total, 2925 clinical pregnancies obtained after autologous IVF/ICSI with fresh SET were used to study biomedical factors possibly associated with MZT, such as maternal age, type of insemination, use of assisted hatching, stage of embryo development at transfer, elective or non-elective SET and preimplantation genetic testing. Another group of 3085 clinical pregnancies obtained after SET of frozen-thawed embryo transfer (FET) was also used to study the possible association between embryo freezing and MZT. Only pregnancies with complete follow-up until birth were included in this analysis. The diagnosis of MZT was established by transvaginal ultrasound performed at 6–8 weeks of amenorrhea. The rate of MZT for each potential risk factor was obtained and a multivariable logistic regression was performed in order to account for the above-mentioned factors. Pregnancies were followed until birth and the early neonatal period in order to assess the rate of miscarriage and stillbirths, gestational age at birth, neonatal weight and early neonatal mortality.

MAIN RESULTS AND ROLE OF CHANCE: There were 76 MZT out of 2925 clinical pregnancies with fresh SET (2.6%) and 69 MZT out of 3085 clinical pregnancies after FET (2.2%) (odds ratio (OR) = 0.85, 95% CI 0.61–1.19). A statistically significant increase in MZT rate was observed with blastocyst compared with cleavage stage ET (3.4 versus 2.0%, respectively; OR = 1.70, 95% CI 1.05–2.76). When confounding variables were considered, eSET was also significantly associated with an increase in the odds of MZT (OR = 1.74, 95% CI 1.04–2.92). Overall perinatal mortality was higher in MZT compared with singletons, but this rise was only significant after fresh ET.

LIMITATIONS, REASONS FOR CAUTION: Limitations of the current study result from the fact that MZT were diagnosed with ultrasound performed at 6–8 weeks of amenorrhea; therefore, spontaneous embryo reductions taking place earlier were missed.

WIDER IMPLICATIONS OF THE FINDINGS: Reproductive health providers must inform their patients that blastocyst transfer and eSET of fresh embryos are associated with a statistically significant increase in the odds of MZT and that perinatal mortality after fresh ET is significantly higher in MZT than in singletons.

STUDY FUNDING/COMPETING INTEREST(S): The Latin American Registry of ART receives direct funding from Ferring Pharmaceuticals, but no specific funding was received to undertake this study. None of the authors declare conflict of interest.

Key words: IVF/ICSI outcome / ART safety / multiple pregnancy / miscarriage / perinatal mortality

WHAT DOES THIS MEAN FOR PATIENTS?

We looked at the parts of the *in vitro* fertilization process that might influence whether a pregnancy after the transfer of a single embryo results in identical (monozygotic) twins. Based on our findings, we think that couples should be advised that transferring a single embryo, especially if it is at the blastocyst stage (Day 5 or 7 of embryo development), is not a guarantee for having only one baby. Furthermore, a pregnancy of identical twins after fresh embryo transfer is linked with an increased chance of premature delivery and a baby dying during late pregnancy, during childbirth or after birth.

Introduction

In humans, twins can result either from the ovulation and fertilization of two oocytes with the outcome of dizygotic twins (DZT) or from the splitting of a single embryo at some stage of its early development, resulting in monozygotic twins (MZT).

It is widely recognized that ART is associated not only with an increased incidence of DZT but also an increased risk of MZT (Aston et al., 2008). A population study suggests that the risk of MZT is ~60% higher among offspring of ART than after spontaneous conception (Parazzini et al., 2016).

Recent systematic reviews and meta-analyses, including articles from 1995 to 2018, have shown an increased incidence of MZT in women undergoing ART when compared with spontaneous conception (Hviid et al., 2018; Busnelli et al., 2019). Actually, MZT accounts for 0.4% of spontaneous pregnancies (Derom et al., 1987; Corsello and Piro, 2010; Luke et al., 2014) and up to 4.9% of pregnancies after ART (Hviid et al., 2018; Busnelli et al., 2019). This increased risk of MZT after ART has raised considerable clinical concern because MZT not only increases maternal and fetal complications associated with multiple gestation by itself but also increases the rates of premature delivery, fetal growth discordance and perinatal mortality when compared with DZT (Dickinson, 2005; Djaafri et al., 2017). Indeed, Hack et al. (2008) reported a perinatal mortality of 5% in DZT and 11.6% in MZT.

Although the ultimate cause of the increased risk of MZT after ART is unknown, many factors inherent to women undergoing fertility treatments and ART itself have been postulated as possible causes such as maternal age, type of insemination, embryo developmental stage at embryo transfer (ET), use of assisted hatching (AH), embryo biopsy for preimplantation genetic testing (PGT) and frozen-thawed embryo transfer (FET) (Knopman et al., 2014; Ikemoto et al., 2018; Busnelli et al., 2019).

The objective of this study was to assess possible factors associated with MZT in a large cohort of women undergoing single embryo transfer (SET) after autologous IVF and ICSI and to evaluate the gestational and perinatal outcomes of MZT.

Materials and Methods

Data was obtained from the Latin American Registry of ART (RLA), which collects data on ~80% of treatment cycles performed in the region and which are published annually by RBMO (Zegers-Hochschild et al., 2019). Follow-up of each case was possible since RLA has been a cycle-based multinational registry since 2012. All centers reporting to the registry have been certified by an accreditation team consisting of a biologist and clinician from a different country. There are strict regulations, including personnel, equipment and facilities,

quality controls and minimum standards of success that need to be accomplished before the data from a specific center is included in the registry.

Data was obtained from 2925 clinical pregnancies taking place after autologous IVF/ICSI and fresh SET, performed between January 2012 and December 2016. Also, in order to study the effect of FET on MZT, 3085 clinical pregnancies obtained after SET/FET in the same period of time were examined. Data regarding pregnancies generated after SET of embryos resulting from vitrified/warmed oocytes was available since 2015 but, because there were only a few cases, was not included in this study (60 clinical pregnancies). Only pregnancies having an ultrasound at 6–8 weeks of amenorrhea and followed up until birth and the early neonatal period were included in this study. MZT were diagnosed when two gestational sacs and/or two embryos with fetal heartbeats were observed.

The terminology used corresponds to definitions adopted by the International Glossary of Infertility and Fertility Care (Zegers-Hochschild et al., 2017).

Factors possibly associated with MZT

The following biomedical data with possible associations with MZT were studied: maternal age in completed years, type of insemination (IVF or ICSI), AH, stage of embryo development at the time of ET (cleavage stage embryo or blastocyst), elective SET (eSET), PGT and FET.

Clinical outcome

Pregnancies were followed until birth and the early neonatal period in order to assess the rate of miscarriage and stillbirths, gestational age at birth, neonatal weight and early neonatal mortality.

Statistical analysis

Statistical analyses were completed using Stata software, version 14.0 for Mac® (Statcorp, TX, USA). Descriptive statistics were calculated as counts and percentages or mean and SD. The baseline sample characteristics were compared between the MZT and singleton groups using Fisher's exact tests. MZT rates were obtained for each factor and odds ratio (OR) with 95% CI calculated. Furthermore, multivariable logistic regression was performed to account for the previously mentioned risk factors studied. A *P* value was considered significant if <0.05.

Ethical approval

As part of the accreditation process, all participating institutions agreed to have their data shared with and published by RLA. On the other hand, as part of the institutional consent forms used by REDLARA, all

Table I Comparison of monozygotic twin rates between fresh and frozen-thawed single embryo transfer.

	Singletons	MZT	MZT rate	OR (95% CI)	P value ^(a)
Fresh embryo transfer	2849	76	2.6	0.85	0.361
Frozen-thawed embryo transfer	3016	69	2.2	(0.61–1.19)	

MZT: monozygotic twins; OR: odds ratio

^(a) Fisher's exact tests**Table II Comparison of MZT rates between different age-categories with fresh single embryo transfer.**

Age (years)	Singletons (2849)	MZT (76)	MZT rate ^(a) (%)
≤34	1236	31	2.4
35–36	507	18	3.4
37–38	510	12	2.3
39–40	369	10	2.6
≥41	227	5	2.2

^(a) Fisher's exact tests. $P = 0.989$

patients are informed and consent obtained to have their unindividualized data used for clinical studies. Therefore, no other consent form was requested for the scientific analysis of the data collected for this study.

Results

There were 76 MZT out of 2925 clinical pregnancies in fresh SET (2.6%) and 69 MZT out of 3085 clinical pregnancies in FET (2.2%) (OR = 0.85, 95% CI 0.61–1.19) (Table I). There was one further case of MZT among 69 clinical pregnancies resulting from SET of embryos obtained from vitrified/warmed oocytes, not included in Table I.

A comparison of MZT rates by age category is shown in Table II. The 76 MZT after fresh ET were distributed in all age categories from ≤34 to ≥41 years old, with a similar proportion at every age interval ($P = 0.989$).

Table III shows the association between possible risk factors and the occurrence of MZT after fresh SET. A statistically significant increase in MZT rate was observed with blastocyst compared with cleavage stage ET (3.4 versus 2.0%, respectively; OR = 1.70, 95% CI 1.05–2.76). When confounding variables were considered, performing a multivariable logistic regression of the factors included in this study, eSET was also significantly associated with an increase in the odds of MZT (OR = 1.74, 95% CI 1.04–2.92) (Table IV). In cases of MZT after eSET, the proportion of blastocyst ET was 76.5% compared with 39% in MZT after the transfer of only one blastocyst because there were no more embryos available for transfer (oSET). Furthermore, all blastocysts transferred in cases of MZT after eSET were on Day 5, compared with 20% of Day 6 blastocysts in MZT after oSET. Moreover, there were 23/34 (67.6%) cases of MZT after eSET with three or more embryos cryopreserved.

Table V shows the outcome of clinical pregnancies in singletons and MZT after fresh and FET.

Gestational age at birth and neonatal weight were significantly reduced in MZT compared with singletons in fresh and FET. Very low birth weight (<1500 grams) was also significantly higher in MZT than singletons. There were no neonatal deaths in MZT after FET compared with seven cases after fresh transfers and perinatal mortality was significantly increased in MZT after fresh ET but not after FET.

Discussion

The results of this study provide an overall similar proportion of MZT in 2925 clinical pregnancies after IVF/ICSI with fresh SET (2.6%) and 3085 clinical pregnancies after SET/FET (2.2%). This is much higher than the 0.4% rate observed in spontaneous pregnancies (Derom *et al.*, 1987; Corsello and Piro, 2010; Luke *et al.*, 2014) and confirms previous data reported in both recent systematic reviews and a meta-analysis (Hviid *et al.*, 2018; Busnelli *et al.*, 2019), which showed an increased risk of MZT after ART from fresh ET and FET when compared with MZT rate in spontaneous pregnancies.

Hviid *et al.* (2018) reported a higher rate of MZT following blastocyst transfer when compared with cleavage stage ET. Furthermore, Busnelli *et al.* (2019) not only reported an association between extended culture with blastocyst transfer and MZT but also showed an increase in MZT in women younger than 35 years old and a statistically significant association between IVF and AH with MZT, that we could not demonstrate. However, most of the studies included in these meta-analyses did not report the number of embryos transferred and MZT was assumed to exist when the total number of embryo poles identified by ultrasound exceeded the number of embryos transferred, which is less accurate than the assessment of MZT when only one embryo is transferred. Moreover, data from both meta-analyses did not allow appropriate control by different variables influencing the splitting of a single embryo, which ends in confounding results.

Table III MZT rates for each risk factor after fresh single embryo transfer and univariable logistic regression analysis.

Risk factors	Singletons (2849)	MZT (76)	MZT rate (%)	OR (95% CI)	P value
ICSI	2428	64	2.6	0.92 (0.50–1.90)	0.806
IVF	421	12	2.8		
With assisted hatching	499	11	2.2	0.80 (0.37–1.54)	0.490
Without assisted hatching	2350	65	2.7		
Blastocyst transfer	1201	42	3.4	1.70 (1.05–2.76)	0.022
Cleavage-stage embryo transfer	1648	34	2.0		
Elective embryo transfer	1008	34	3.3	1.48 (0.91–2.40)	0.092
Non-elective embryo transfer	1841	42	2.3		
With PGT	215	6	2.7	1.05 (0.37–2.44)	0.909
Without PGT	2634	70	2.6		

PGT: preimplantation genetic testing

Table IV Multivariable logistic regression analysis of variables likely to be associated with MZT after fresh single embryo transfer.

Risk factors	OR (95% CI)	P value
Age		
≤34 years	Reference	
35–36 years	1.05 (0.54–2.01)	0.878
37–38 years	0.84 (0.46–1.53)	0.581
39–40 years	0.73 (0.37–1.49)	0.404
≥41 years	0.25 (0.05–1.08)	0.064
IVF	1.45 (0.97–2.17)	0.067
Without assisted hatching	1.05 (0.72–1.55)	0.767
Blastocyst embryo transfer	2.17 (1.04–2.92)	0.002
Elective single embryo transfer	1.74 (1.04–2.92)	0.033
PGT	0.97 (0.31–2.01)	0.632

On the other hand, controversial results have been reported in studies assessing the incidence of MTZ in only SET cycles. While several authors (Luke et al., 2014; Nakasuji et al., 2014; Osianlis et al., 2014; Kanter et al., 2015; Mateizel et al., 2016; Ikemoto et al., 2018) concluded that blastocyst transfer is associated with an increased risk of MZT, Papanikolaou et al. (2010) not find such an association.

Our study showed a significant increase in MZT after the transfer of blastocysts compared with cleavage-stage embryos (3.4 versus 2.0%, respectively; OR = 1.70, 95% CI 1.05–2.76). Furthermore, after correcting for possible confounding variables, performing a multivariable logistic regression of the factors considered in this study for fresh ET, not only blastocyst transfer but also eSET were predictors that were statistically significantly associated with an increase in the odds of MZT. Conversely, we could not demonstrate a relation between maternal age, type of insemination (IVF/ICSI), AH and PGT with MZT when fresh ET was performed.

The ultimate cause of MZT is still unknown, but definitely, it seems to be associated with prolonged *in vitro* culture (Liu et al., 2018). Luke et al. also suggested that MZT might be related to the transfer of high-quality embryos, which are more often transferred in eSET because a better selection of the best embryo from a cohort occurs and cryopreservation of surplus embryos is performed (Luke et al., 2014). They hypothesized that these embryos could be more sensitive to changes in temperature and pH during culture, which might result in higher rates of MZT after blastocyst transfer (Luke et al., 2014). The question arises as to which are the underlying conditions, inherent to eSET, which may increase the rate of MZT. Two factors are worth mentioning from our study: first, all blastocysts transferred in eSET were at Day 5 while 20% of transfers in oSET were at Day 6 or 7; second, in 23/34 eSET (67.6%), there were three or more blastocysts available to freeze. One may assume that women having MZT are overall more fertile or reproductively efficient, but if there are more embryos to choose from at the time of transfer, it would be interesting to elucidate if the degree of expansion or other morphologic characteristics may influence embryo splitting. Unfortunately, the RLA does not register embryo morphology at the time of transfer since this has never been standardized, but it is perhaps something worth looking at within institutions where the criteria for embryo quality assessment are standardized.

Most publications regarding MZT in ART have focused mainly on determining its incidence and etiology, but few studies have addressed clinical outcomes of MZT after ART. Cohort studies assessing clinical outcome of twin pregnancies conceived by ART have shown an increased risk of premature delivery, lower birth weight, major neonatal morbidity and neonatal death in MZT versus DZT (Ghalili et al., 2013; Simões et al., 2015; Hack et al., 2018). Our study demonstrated significantly reduced gestational age at birth and neonatal weight in MZT compared with singletons, which has been also reported by other authors (Vela et al., 2011; Mascarenhas et al., 2014), but no differences in stillbirths between singletons and MZT. Perinatal mortality was higher in MZT compared with the birth of singletons; however, this was only significant in births generated after fresh SET, which is probably due to less extremely preterm births and very low birth weights, with no neonatal deaths in MZT after FET. Actually, the overall outcome was poorer after fresh ET than FET. Concerning this issue, although

Table V Clinical outcome of singletons and MZT from fresh and frozen-thawed single embryo transfer.

Clinical outcome	Fresh embryo transfer			Frozen-thawed embryo transfer		
	Singletons (2849)	MZT (76)	P value ^(e)	Singletons (3016)	MZT (69)	P value ^(e)
Miscarriage N (%)	347 (12.2)	16 (21.1)	0.032	321 (10.5)	8 (11.6)	0.697
Induced abortion N (%)	8 (0.3)	0	-	16 (0.5)	0	-
Stillbirth N (% ^(a))	32 (13.0)	2 (16.9) ^(d)	0.667	13 (4.9)	2 (16.7) ^(d)	0.134
Live born deliveries N	2462	59	-	2666	60	-
Newborns N	2462	118	-	2666	120	-
Gestational age at birth (weeks ± SD)	37.8 ± 1.9	35.1 ± 3.1	<0.0001	37.9 ± 2.0	34.9 ± 2.7	<0.0001
Extremely preterm birth <28 weeks N (%)	17 (0.7)	4 (3.4)	0.013	16 (0.6)	2 (1.7)	0.180
Birth weight (grams ± SD)	3091 ± 518	2243 ± 580	<0.0001	3200 ± 538	2305 ± 471	<0.0001
Very low birth weight <1500 g N (%)	14 (0.6)	8 (6.8)	<0.0001	14 (0.5)	4 (3.3)	0.006
Neonatal death N (% ^(b))	7 (2.9)	7 (59.3)	<0.0001	7 (2.6)	0	-
Perinatal mortality N (% ^(c))	39 (15.8)	9 (76.3)	0.003	20(7.5)	2(16.7)	0.244

^(a)Expressed per 1000 newborns

^(b)Expressed per 1000 newborns

^(c)Expressed per 1000 newborns

^(d)Stillbirth of both fetuses in one pregnancy

^(e)Fisher's exact tests

published studies have some limitations because they are observational and not randomized, available evidence suggests improved perinatal outcomes after FET (Bhattacharya, 2016; Maheshwari et al., 2018). Perhaps, this could be interpreted as the consequence of an ET in a more physiological environment which, together with the inherent risks of MZT, could also explain the 2-fold higher miscarriage rate we observed in MZT after fresh ET than FET.

The main strengths of this study are the large number of cases of SET included in it, the thoroughness of the registry software and the rigor in follow-up by centers reporting to RLA, which allows adjustment for the most relevant confounding variables and to obtain reliable clinical outcomes. A potential limitation of the current study is that data obtained from RLA is based on early ultrasound with fetal heart beats at 6–8 weeks of amenorrhoea and cannot determine either whether one embryo loss occurred before this ultrasound or MZT chorioamnicity, which is relevant for pregnancy outcome and prognosis (Dickinson, 2005).

Based on our findings, we believe that patients undergoing IVF/ICSI should be educated that SET, especially of blastocysts, is not a guarantee for delivering singletons and that MZT after fresh ET is associated with a significantly higher risk of miscarriage, prematurity and perinatal death.

However, patients should not be discouraged to undergo eSET at blastocyst stage because enhanced embryo selection, higher pregnancy rates, a lower number of transferred embryos and lower multiple pregnancy rates have been reported when extended culture and blastocyst instead of cleavage-stage ET is performed (Glujovsky et al., 2016). Moreover, good prognosis patients should be encouraged to undergo blastocyst eSET because it has been demonstrated that this practice reduces multiple pregnancies without compromising cumulative live birth rates (Pandian et al., 2013).

We believe there are three options for future development: first, as suggested by Sutherland et al. (2019), include the assessment of inner cell mass (ICM) splitting prior to ET in current blastocyst embryo grading, in order to avoid transferring these embryos in favor of blastocysts with intact ICM and reduce the risk of MZT; second, identify dynamic markers capable of recognizing cleaving embryos that will reach blastocyst stage, in order to transfer them at an early stage of development and therefore reduce MZT rates; third, opt for FET as the preferred mode of treatment in SET in order to reduce perinatal mortality associated with MZT. Of course, the latter option could be undertaken after proper randomized trials with sufficient follow-up have been conducted and shown no adverse effect.

Acknowledgements

We thank Carolina Musri, administrator of the RLA, and all centers reporting to the registry for their efforts, which allow this research.

Authors' roles

All authors were involved in the design of the study, interpretation of data and revision of the final manuscript.

Funding

No specific funding was received to undertake this study.

Conflict of interest

None of the authors declare a conflict of interest.

References

- Aston KI, Peterson CM, Carrell DT. Monozygotic twinning associated with assisted reproductive technologies: a review. *Reproduction* 2008;**136**:377–386.
- Bhattacharya S. Maternal and perinatal outcomes after fresh versus frozen embryo transfer—what is the risk-benefit ratio? *Fertil Steril* 2016;**106**:241–243.
- Busnelli A, Dallagiovanna C, Reschini M, Paffoni A, Fedele L, Somigliana E. Risk factors for monozygotic twinning after in vitro fertilization: a systematic review and meta-analysis. *Fertil Steril* 2019;**111**:302–317.
- Corsello G, Piro E. The world of twins: an update. *J Matern Neonatal Med* 2010;**23**:59–62.
- Derom C, Vlietinck R, Derom R, Vanden BH, Thiery M. Increased monozygotic twinning rate after ovulation induction. *Lancet* 1987;**8544**:1236–1238.
- Dickinson JE. Monoamniotic twin pregnancy: a review of contemporary practice. *Aust New Zeal J Obstet Gynaecol* 2005;**45**:474–478.
- Djaafri F, Stirnemann J, Mediouni I, Colmant C, Ville Y. Twin–twin transfusion syndrome – what we have learned from clinical trials. *Semin Fetal Neonatal Med* 2017;**22**:367–375.
- Ghalili A, McLennan A, Pedersen L, Kesby G, Hyett J. Outcomes of monochorionic diamniotic twin pregnancies: a comparison of assisted and spontaneous conceptions. *Aust New Zeal J Obstet Gynaecol* 2013;**53**:437–442.
- Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev* 2016;**6**:CD002118.
- Hack KEA, Derks JB, Elias SG, Franx A, Roos EJ, Voerman SK, Bode CL, Koopman-Esseboom C, Visser GHA. Increased perinatal mortality and morbidity in monochorionic versus dichorionic twin pregnancies: clinical implications of a large Dutch cohort study. *BJOG An Int J Obstet Gynaecol* 2008;**115**:58–67.
- Hack KEA, Vereycken MEMS, Torrance HL, Koopman-Esseboom C, Derks JB. Perinatal outcome of monochorionic and dichorionic twins after spontaneous and assisted conception: a retrospective cohort study. *Acta Obstet Gynecol Scand* 2018;**97**:717–726.
- Hviid KVR, Malchau SS, Pinborg A, Nielsen HS. Determinants of monozygotic twinning in ART: a systematic review and a meta-analysis. *Hum Reprod Update* 2018;**24**:468–483.
- Ikemoto Y, Kuroda K, Ochiai A, Yamashita S, Ikuma S, Nojiri S, Itakura A, Takeda S. Prevalence and risk factors of zygotic splitting after 937 848 single embryo transfer cycles. *Hum Reprod* 2018;**33**:1984–1991.
- Kanter JR, Boulet SL, Kawwass JF, Jamieson DJ, Kissin DM. Trends and correlates of monozygotic twinning after single embryo transfer. *Obstet Gynecol* 2015;**125**:111–117.
- Knopman JM, Krey LC, Oh C, Lee J, McCaffrey C, Noyes N. What makes them split? Identifying risk factors that lead to monozygotic twins after in vitro fertilization. *Fertil Steril* 2014;**102**:82–89.
- Liu H, Liu J, Chen S, Kang X, Du H, Li L. Elevated incidence of monozygotic twinning is associated with extended embryo culture, but not with zona manipulation or freeze-thaw procedure. *Fertil Steril* 2018;**109**:1044–1050.
- Luke B, Brown MB, Wantman E, Stern JE. Factors associated with monozygosity in assisted reproductive technology pregnancies and the risk of recurrence using linked cycles. *Fertil Steril* 2014;**101**:683–689.
- Maheshwari A, Pandey S, Raja EA, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? *Hum Reprod Update* 2018;**24**:35–58.
- Mascarenhas M, Muthukumar K, Aleyamma T, Chandy A, Kamath M, Mangalaraj A. Obstetric outcomes of monochorionic pregnancies conceived following assisted reproductive technology: a retrospective study. *J Hum Reprod Sci* 2014;**7**:119.
- Mateizel I, Santos-Ribeiro S, Done E, Van Landuyt L, Van De Velde H, Tournaye H, Verheyen G. Do ARTs affect the incidence of monozygotic twinning? *Hum Reprod* 2016;**31**:2435–2441.
- Nakasuji T, Saito H, Araki R, Nakaza A, Nakashima A, Kuwahara A, Ishihara O, Irahara M, Kubota T, Yoshimura Y et al. The incidence of monozygotic twinning in assisted reproductive technology: analysis based on results from the 2010 Japanese ART national registry. *J Assist Reprod Genet* 2014;**31**:803–807.
- Oslanlis T, Rombauts L, Gabbe M, Motteram C, Vollenhoven B. Incidence and zygosity of twin births following transfers using a single fresh or frozen embryo. *Hum Reprod* 2014;**29**:1438–1443.
- Pandian Z, Marjoribanks J, Ozturk O, Serour G, Bhattacharya S. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection. *Cochrane Database Syst Rev* 2013;**7**:CD003416.
- Papanikolaou EG, Ph D, Fatemi H, Ph D, Venetis C. Monozygotic twinning is not increased after single blastocyst transfer compared with single cleavage-stage embryo transfer. *Fertil Steril* 2010;**93**:592–597.
- Parazzini F, Cipriani S, Bianchi S, Bulfoni C, Bortolus R, Somigliana E. Risk of monozygotic twins after assisted reproduction: a population-based approach. *Twin Res Hum Genet* 2016;**19**:72–76.
- Simões T, Queirós A, Marujo AT, Valdoleiros S, Silva P, Blickstein I. Outcome of monochorionic twins conceived by assisted reproduction. *Fertil Steril* 2015;**104**:629–632.
- Sutherland K, Leitch J, Lyall H, Woodward BJ. Time-lapse imaging of inner cell mass splitting with monochorionic triamniotic triplets after elective single embryo transfer: a case report. *Reprod Biomed Online* 2019;**38**:491–496.
- Vela G, Luna M, Barritt J, Sandler B, Copperman AB. Monozygotic pregnancies conceived by in vitro fertilization: understanding their prognosis. *Fertil Steril* 2011;**95**:606–610.
- Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, Rienzi L, Sunde A, Schmidt L, Cooke ID et al. The international glossary on infertility and fertility care, 2017. *Hum Reprod* 2017;**32**:1786–1801.
- Zegers-Hochschild F, Schwarze JE, Crosby JA, Musri C, Urbina MT, Latin American Network of Assisted Reproduction (RED-LARA). Assisted reproductive techniques in Latin America: the Latin American Registry 2016. *Reprod Biomed Online* 2019;**39**:452–460.