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ARTICLE

Twin pregnancies and perinatal outcomes: a comparison between fresh and frozen embryo transfer: a two-centre study

BIOGRAPHY

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KEY MESSAGE

A retrospective cohort study of twin pregnancies resulting from fresh and frozen embryo transfers showed that the frozen group resulted in a significantly lower risk of LBW, VLBW and SGA infants, which are known risk factors for possible adverse perinatal outcome. Further studies are needed to confirm these preliminary results.

ABSTRACT

Research question: To assess the perinatal and obstetric outcomes of twin pregnancies resulting from IVF frozen embryo transfer (FET) in comparison with fresh embryo transfer.

Design: A retrospective cohort study of 773 twin pregnancies conceived via IVF treatment. Data were collected from the records of two outpatient fertility IVF clinics of cycles conducted between 2006 and 2016.

Results: A total of 773 pregnancies were evaluated: 614 (79.4%) following FET and 159 (20.6%) following fresh embryo transfer. The FET group had a significantly higher mean birthweight ($P = 0.002$), and lower rates of small for gestational age ($P = 0.003$), low ($P = 0.003$) and very low birthweight ($P = 0.006$) infants. Also, a significantly lower rate of spontaneous second trimester miscarriage compared with the fresh embryo transfer group was observed ($P = 0.001$). No significant difference was found between groups regarding gestational age at delivery, term birth (after 37 weeks of gestation), twin discordancy rate, fetal major malformation rate, and hospitalization duration.

Conclusion: In twin pregnancies, FET might have better perinatal outcomes compared with fresh embryo transfer in regards to birthweight and spontaneous second trimester miscarriages. Further research is needed to evaluate these results.

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INTRODUCTION

Multiple pregnancies are considered to be a complication of assisted reproductive technology (ART) treatment (Gerris, 2005). These pregnancies are associated with increased maternal and perinatal morbidity and mortality. In addition, multiple pregnancies impose higher costs on health services (Roque et al., 2015). These concerns have prompted the European Society of Human Reproduction and Embryology (ESHRE) and the Human Embryology and Fertilisation Authority (HFEA) to recommend the use of elective single embryo transfer as an effective way of reducing twin pregnancies associated with IVF (Bergh, 2005; los Santos et al., 2016). These recommendations have led to a change in practice and a reduction in multiple embryo transfers (El-Toukhy et al., 2006). However, these aforementioned recommendations were based on integrated data that included both fresh embryo transfers and frozen embryo transfers (FET).

FET has been increasingly used during the past few years and has become common practice in ART treatment (de Mouzon et al., 2010). FET has been shown to have several advantages including higher pregnancy rate and live birth rate in comparison with fresh cycles (Chen et al., 2016; Roque et al., 2013). One probable explanation can be the adverse effect of an artificial cycle on endometrial receptivity leading to implantation failure (Shapiro et al., 2011; Wang et al., 2017). Another benefit of FET is the possibility of delayed embryo transfer in a natural (non-stimulated) cycle, thus reducing the risk of ovarian hyperstimulation syndrome (Fatemi et al., 2014). Some IVF clinics are advocating these advantages and now use only FET, as a 'freeze-all' policy (Roque et al., 2015).

FET in singleton pregnancies was reported (Maheshwari et al., 2012; Zhao et al., 2016) to have reduced risk for low birthweight (LBW), small for gestational age (SGA) and preterm labour compared with fresh transfers. On the other hand, it was also reported (Maheshwari et al., 2016) to have increased risk for large for gestational age (LGA).

Although FET singleton pregnancies are less frequently associated with obstetric and perinatal complications, there is still inconclusive data regarding obstetric and perinatal outcomes in FET cycles compared with fresh embryo cycles in multiple pregnancies. To the best of our knowledge only three retrospective studies (Aflatoonian et al., 2016; Pereira et al., 2016; Shih et al., 2008) addressed this comparison in multiple pregnancies, yet none of these studies addressed second trimester miscarriage, different levels of prematurity, or complications specific to twin pregnancies, such as discordancy. Furthermore, two of these studies (Aflatoonian et al., 2016; Shih et al., 2008) were relatively small, including only 150–250 pregnancies each.

The current study aimed to evaluate the obstetric and perinatal outcomes of twin pregnancies conceived via IVF FET versus fresh embryo transfer; including spontaneous second trimester miscarriage, prematurity, birthweight discordancy, fetal major malformation rate and hospitalization duration during the pregnancy. In order to provide strong evidence based on a large sample size, data were collected from two medical centres.

MATERIALS AND METHODS

Study design

This was a two-centre retrospective cohort of all twin pregnancies resulting from IVF cycles between 2006 and 2016. Data were collected from the records of two outpatient fertility IVF clinics (Assuta Medical Centre, Rishon Letzion, Israel and Soroka University Medical Centre, Beer-Sheva, Israel).

Patients

All patients who conceived by IVF, had twin pregnancy and successfully completed the first trimester were included in the analysis. All the transferred embryos were cleaved embryos (day 3) due to our policy of single embryo transfer at blastocyst stage. Twin pregnancies resulting from egg donation were excluded, due to their older maternal age and their different medication protocol prior to the embryo transfer. Twin pregnancies that had undergone fetal reduction were excluded because this is an invasive procedure that may have implications for obstetric and perinatal outcome. Mono-chorionic twin pregnancies were also excluded, because these pregnancies are characterized by a lower median gestational age at delivery and have a different and unique set of obstetric complications.

Demographic characteristics of the women enlisted including age, gravity, parity, body mass index (BMI) and smoking were collected retrospectively from medical records. Ovarian stimulation protocol (long gonadotrophin-releasing hormone [GNRH] agonist or GNRH antagonist) and endometrial preparation protocol (spontaneous ovulation or hormonal replacement therapy) were analysed. Perinatal outcomes included gestational age at delivery, birthweight, twin discordancy (defined as >20% difference in intra-pair birthweight) and fetal major malformation rate. Fetal major malformation was defined as the incidence of defects of organs or body parts due to an intrinsically abnormal developmental process or chromosomal aberrations (either numerical abnormalities or structural). Preterm deliveries were defined as any live deliveries occurring earlier than 37 weeks of pregnancy. Late preterm delivery was defined as delivery between 34 and 36 weeks of pregnancy, while early preterm delivery was defined as delivery between 24 and 33 weeks of pregnancy (Spong et al., 2011). Neonatal weight below 2500 g at delivery, irrespective of gestational age, was defined as LBW and neonatal weight below 1500 g at delivery, irrespective of gestational age, was defined as very low birthweight (VLBW) (Spong et al., 2011). Neonatal weight below the 10th percentile of the weight appropriate to gestational age and fetal gender was defined as SGA. Dollberg twin birthweight standards were used (Dollberg et al., 2005), which best suit Israel's population, in order to determine the 10th percentile.

Obstetric outcomes included spontaneous second trimester miscarriage and hospitalization duration during the pregnancy for obstetric reasons. Spontaneous second trimester miscarriage was defined as spontaneous termination of pregnancy after completion of 14 weeks and before 24 weeks of pregnancy.

Ethical issues

The Institutional Review Board approved the study design in May 2017 and, as the protocol involved the retrospective review of patient charts, the need for individual consent from patients was

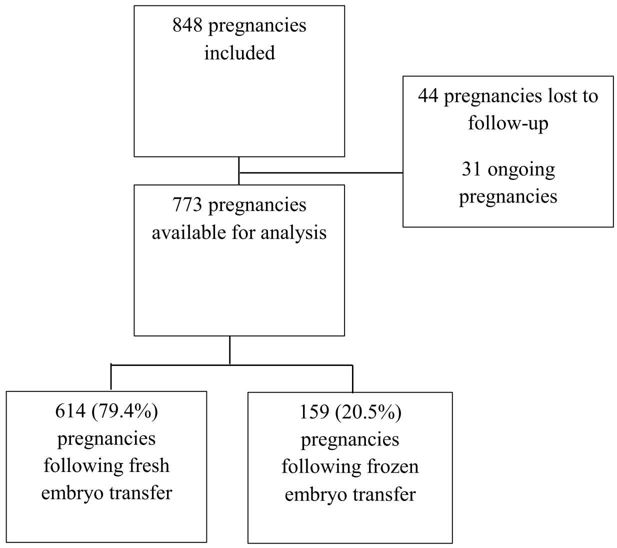

FIGURE 1 Flow diagram of cases included in the study.

waived. The approval reference number is 0019-17-ASMC.

Statistical analysis

Statistical analysis was performed using the SPSS® 20.0 package for windows (IBM Corp., USA). Categorical variables were analysed by chi-squared test or Fisher's exact test. Continuous variables were analysed by t-test. A *P*-value of <0.05 was considered to be statistically significant. All statistical tests were two-tailed.

Linear regression was used for continuous parameters and logistic regression for nominal ones.

Based on the assumption that the prevalence of births with LBW in twin pregnancy is 55% and that a reduction of 15% was expected for the FET group, it was calculated that enrolment of 170 participants in each group would provide a power of 80% to show a treatment effect, at two-sided alpha level of 5%.

RESULTS

A total of 848 IVF twin pregnancies were included in this study. Of these, 44 pregnancies (5.2%) were lost to follow-up and 31 pregnancies (3.7%) were still ongoing at the time of data analysis. A total of 773 pregnancies were evaluated: 614 (79.4%) following fresh embryo transfer and 159 (20.6%) following FET (FIGURE 1). There was no significant difference between the FET and fresh embryo transfer groups regarding the ovarian stimulation protocol; 64% and 69%, respectively, were treated with the antagonist protocol while 36% and 31%, respectively, were treated with the long agonist protocol. Endometrial preparation for FET was natural ovulation (58%) and hormone replacement therapy (42%).

The basic characteristics of the two groups are detailed in TABLE 1. Maternal age was lower in the FET group compared with the fresh embryo transfer group (30.19 ± 4.6 versus 31.37 ± 4.3 years, respectively, *P* = 0.003). Parity and gravity were significantly higher in the FET group compared with the fresh embryo transfer group (0.81 ± 0.8 versus 0.51 ± 0.7, *P* = 0.000 and 1.81 ± 1.6 versus 0.94 ± 1.2, *P* < 0.001, respectively). The proportion of women who were nulliparous was significantly lower in the FET group compared with the fresh embryo transfer group (37% versus 60%, *P* < 0.001). There were no significant differences between the FET and fresh embryo transfer groups regarding prevalence of smoking, BMI, fetal male gender (50% versus 45%, respectively) or Caesarean delivery (69% versus 61%, respectively).

Gestational age at birth and preterm delivery

No difference was found in gestational age at delivery for FET twins compared with fresh embryo transfer twins (35.89 ± 2.5 versus 35.99 ± 2.8).

No statistically significant difference was found in the prevalence of preterm, late preterm, early preterm and extreme early preterm (TABLE 2).

Birthweight

Average birthweight was significantly higher in the FET group compared with the fresh embryo transfer group (2382 ± 465 versus 2247 ± 486 g, *P* = 0.002; TABLE 2). The risk of having a LBW infant (defined as birthweight of <2500 g) was significantly lower in the FET group when compared with fresh embryo transfer (55% versus 64%, *P* = 0.003); calculated relative risk (RR) was 0.87.

TABLE 1 BASIC CHARACTERISTICS BY TREATMENT GROUP (FRESH AND FROZEN EMBRYO TRANSFER)

Characteristic	Fresh embryo transfer (*n* = 614)	Frozen embryo transfer (*n* = 159)	*P*-value
Maternal age (years ± SD)	31.37 ± 4.3	30.19 ± 4.6	0.003
Gravity (mean ± SD)	0.94 ± 1.2	1.81 ± 1.6	<0.001
Parity (mean ± SD)	0.51 ± 0.7	0.81 ± 0.8	<0.001
Nulliparous, *n* (%)	366 (60)	59 (37)	<0.001
Smoking (mean pack years ± SD)	0.91 ± 3.3	0.04 ± 0.2	NS
Pre-pregnancy BMI (kg/m^2 ± SD)	24.41 ± 5.0	25.38 ± 6.3	NS
Fetal male gender, *n* (%)	552/1228 (45)	159/318 (50)	NS
Delivery by CS, *n* (%)	375 (61)	110 (69)	NS

BMI = body mass index; CS = Caesarean section; NS = not statistically significant.

TABLE 2 OBSTETRIC AND PERINATAL OUTCOMES FOR TWIN PREGNANCIES FOLLOWING FRESH VERSUS FROZEN EMBRYO TRANSFER

Outcome	Fresh embryo transfer (*n* = 614)	Frozen embryo transfer (*n* = 159)	*P*-value
Gestational week at birth (mean ± SD)	35.99 ± 2.8	35.89 ± 2.5	NS
Term birth[a]	324/567 (57)	81/158 (51)	NS
Preterm birth[b]	243/567 (43)	77/158 (49)	NS
Late preterm[c]	157/243 (65)	49/77 (64)	NS
Early preterm[d]	86/243 (35)	28/77 (36)	NS
Extreme early preterm[e]	13/243 (5.3)	2/77 (2.6)	NS
Neonatal birthweight, g (mean ± SD)	2247 ± 486	2382 ± 465	**0.002**
LBW infants <2.5 kg	724/1130 (64)	172/314 (55)	**0.003**
VLBW infants <1.5 kg	109/1130 (9.6)	15/314 (4.8)	**0.006**
SGA infants <10%	180/1130 (15.9)	29/314 (9.2)	**0.003**
Discordancy	105/565 (18.6)	22/157 (14)	NS
Spontaneous second trimester miscarriages	47/614 (7.7)	1/159 (0.6)	**0.001**
Major malformation rate	30/614 (4.9)	8/159 (5)	NS
Hospitalization days during pregnancy (mean ± SD)	6.01 ± 13.4	5.20 ± 10.3	NS

Data are presented as *n* (%) unless otherwise stated.

LBW = low birthweight (<2500 g at delivery irrespective of gestational age); NS = not statistically significant; SGA = small for gestational age; VLBW = very low birthweight (<1500 g at delivery irrespective of gestational age).

[a] ≥37 weeks of pregnancy.

[b] <37 weeks of pregnancy.

[c] 34–36 weeks of pregnancy.

[d] <34 weeks of pregnancy.

[e] 24–28 weeks of pregnancy

The risk of having a VLBW baby (defined as birthweight of <1500 g) was also significantly lower in the FET group compared with the fresh embryo transfer group (4.8% versus 9.6%, $P = 0.006$); calculated RR was 0.50. The risk of having a SGA infant (defined as birthweight below the 10th percentile of the weight appropriate to gestational age) was also significantly lower in the FET group compared with the fresh embryo transfer group (9.2% versus 15.9%, $P = 0.003$); calculated RR was 0.58. Regarding the incidence of birthweight discordance (defined as intra-pair birthweight difference >20%), there was no significant difference between the groups (14% versus 18.6%).

Regression analysis did not show a significant effect of the group (FET/fresh embryo transfer) on birthweight.

Spontaneous second trimester miscarriages

The incidence of a second trimester miscarriage (defined as miscarriages occurring between 14 and 23 weeks of gestation) was significantly lower (0.6% versus 7.7%, $P = 0.001$) in pregnancies occurring as a result of FET when compared with those after fresh embryo transfer.

Logistic regression showed significant effect of the group (FET/fresh embryo transfer) on the rate of miscarriages ($P = 0.014$).

Fetal major malformations

There was no significant difference regarding fetal anomalies between the two groups (5% versus 4.9%).

Duration of hospitalization

No difference in duration of hospitalization was found between the two groups (mean 5.20 days versus 6.01 days). The most common indications for hospitalization in both the FET and fresh embryo transfer groups were premature contractions and shortening of the cervix (12% versus 9%, respectively), and placentation disorders such as bleeding, hypertension or pre-eclampsia (3% versus 1%, respectively).

Sub-analysis by parity

The proportion of women who were nulliparous in this study was significantly higher in the fresh embryo transfer group (60% versus 40%, $P < 0.001$). Nulliparity has been reported to be a risk factor for a poor outcome in twin pregnancies including late term miscarriages (Berkovitz et al., 2010).

It was verified in this cohort that nulliparity is indeed a risk factor as mentioned above; nulliparous in comparison to multiparous women in the study had a lower average gestational age at birth (35.7 versus 36.2, $P = 0.02$), a lower average birthweight (2202 versus 2360 g, $P < 0.001$) and higher second trimester miscarriage rate (8.7% versus 3.2%, $P < 0.001$).

In order to eliminate this possible confounder of parity, FET versus fresh embryo transfer were analysed and compared separately in the nulliparous women and multiparous women. Parity data were missing in eight women from the cohort, thus this analysis was made for the remaining 765 cases.

In this cross-sectional analysis it was found that in the nulliparous group, FET was related to a significantly lower incidence of second trimester miscarriages ($P = 0.011$). In the multiparous group, FET was found to be related to a significantly lower incidence of LBW and VLBW infants (TABLE 3). The

TABLE 3 OBSTETRIC AND PERINATAL OUTCOMES FOR TWIN PREGNANCIES FOLLOWING FRESH VERSUS FROZEN EMBRYO TRANSFER – NULLIPAROUS AND MULTIPAROUS WOMEN

Characteristic	Nulliparous women (425)			Multiparous women (340)		
	Fresh embryo transfer (*n* = 366)	Frozen embryo transfer (*n* = 59)	*P*-value	Fresh embryo transfer (*n* = 248)	Frozen embryo transfer (*n* = 92)	*P*-value
Gestational week at birth (mean ± SD)	35.81 ± 2.9	35.64 ± 2.7	0.690	36.25 ± 2.6	36.18 ± 2.3	NS
Term birth[a]	183/329 (55.6)	31/59 (52.5)	0.661	141/238 (59.2)	49/91 (53.8)	NS
Preterm birth[b]	146/329 (44.4)	28/59 (47.5)	0.661	97/238 (40.8)	42/91 (46.2)	NS
Late preterm[c]	85/146 (58.2)	14/28 (50)	0.533	72/97 (74.2)	32/42 (76.2)	NS
Early preterm[d]	61/146 (41.8)	14/28 (50)	0.533	25/97 (25.8)	10/42 (23.8)	NS
Extreme early preterm[e]	8/146 (5.5)	1/28 (3.6)	1.0	5/97 (5.2)	1/42 (2.4)	NS
Neonatal birthweight, g (mean ± SD)	2185 ± 489	2304 ± 527	0.095	2331 ± 471	2432 ± 430	NS
LBW infants <2.5 kg	445/654 (68)	71/116 (61)	0.149	279/476 (58.6)	90/182 (49.5)	0.034
VLBW infants <1.5 kg	76/654 (11.6)	10/116 (8.6)	0.344	33/476 (6.9)	5/182 (2.7)	0.04
SGA infants <10%	121/654 (18.5)	16/116 (13.8)	0.222	59/476 (12.4)	13/182 (7.14)	NS
Discordancy	69/327 (21)	10/59 (17)	0.502	36/238 (15)	10/91 (11)	NS
Second trimester miscarriages	37/366 (10.1)	0/59 (0)	0.011	10/248 (4.0)	1/92 (1.1)	NS
Major malformation rate	13/366 (3.6)	6/59 (10)	0.03	16/248 (6.5)	2/92 (2.2)	NS
Hospitalization days during pregnancy (mean ± SD)	7.73 ± 15.9	7.00 ± 9.3	0.634	3.66 ± 8.3	4.06 ± 10.8	NS

Data are presented as *n* (%) unless otherwise stated.

This analysis included 765 cases from our 773 cases cohort due to missing data regarding parity in eight cases.

LBW = low birthweight (<2500 g at delivery irrespective of gestational age); NS = not statistically significant; SGA = small for gestational age; VLBW = very low birthweight (<1500 g at delivery irrespective of gestational age).

[a] ≥37 weeks of pregnancy.

[b] <37 weeks of pregnancy.

[c] 34–36 weeks of pregnancy.

[d] <34 weeks of pregnancy.

[e] 24–28 weeks of pregnancy.

incidence of SGA infants appeared to be lower in this group; however, it did not reach significance ($P = 0.054$).

DISCUSSION

The major finding of the current study is that FET twin pregnancies resulted in a significantly higher average birthweight, lower risk of LBW, VLBW and SGA babies, and a significantly lower incidence of spontaneous second trimester abortion compared with the fresh embryo transfer group. No difference was found between groups regarding gestational age at birth, preterm birth incidence, twin discordancy and fetal major malformation rates.

With the wide use of FET, there were concerns about the possible negative effect of cryopreservation on the health of infants born using this method. However, in terms of obstetric and perinatal outcome, systematic reviews and meta-analysis of observational studies suggest that singleton pregnancies resulting from FET are associated with lower obstetric and perinatal morbidity in comparison with singleton pregnancies resulting from fresh embryo transfer (Maheshwari et al., 2016, 2012; Shapiro et al., 2014; Zhao et al., 2016). A possible explanation for this perinatal advantage is that the FET protocol is based on a more physiological level of hormones. Consequently, the physiological condition of the endometrium may have a positive influence not only on the endometrial receptivity and early implantation, but also on placentation, which affects both subsequent fetal growth and time of delivery (Shapiro et al., 2014). Another plausible biological mechanism is that high oestrogen and progesterone concentrations from controlled ovarian stimulation may affect genes involved in implantation and have a possible effect on fetal growth. Furthermore, high oestrogen levels during ovarian stimulation have been postulated to interfere with endometrial angiogenesis (Wang et al., 2017). Another hypothesis is that the freeze–thaw process in freeze-only cycles serves as a filter for embryos of poorer quality, which may not survive the thaw (Wang et al., 2017).

Although FET singleton pregnancies are less frequently associated with obstetric and perinatal complications, there are still only sparse and inconclusive data regarding obstetric and perinatal outcomes in FET cycles compared with fresh embryos cycles in multiple pregnancies. This study attempted to assess whether the aforementioned perinatal advantage of FET pregnancies applies to twin pregnancies as well. Three retrospective studies (Aflatoonian et al., 2016; Pereira et al., 2016; Shih et al., 2008) addressed this issue. Shih et al. (2008) showed that the combined birthweight for twins from FET was significantly higher than for those from fresh embryo transfer, while gestational age at birth and percentage of preterm births were found not to be significantly different. This study did not address different levels of prematurity, second trimester miscarriage or twin pregnancy complications such as discordancy. Furthermore, the basic characteristics of the enlisted women with twin pregnancy were not presented. Pereira et al. (2016) found that there was no difference between FET and fresh embryo transfer twins in rates of Caesarean deliveries, term deliveries, early and late preterm deliveries, neonatal weight at delivery, LBW or VLBW. Aflatoonian et al. (2016) found a higher average gestational age at birth and lower percentage of LBW in twins resulting from FET, yet no significant difference regarding prematurity percentage, average birthweight or SGA percentage was found. The two last-mentioned cohort studies (Aflatoonian et al., 2016; Pereira et al., 2016) were relatively small, including only 150–250 pregnancies each. This study focused on twin pregnancies only and was based on data with a larger sample size, which allowed a better evaluation of the differences between groups.

It is well established that twin pregnancies carry significant perinatal risks. Babies who are products of twin gestations have higher rates of LBW, VLBW, earlier gestational age at delivery, and higher rates of neonatal and infant death and cerebral palsy. This increased risk for morbidity and mortality of twin infants is predominantly attributed to higher rates of early preterm delivery and VLBW (Newman and Unal, 2011). For this reason, the focus of this study was to examine whether FET cycles have an advantage in twin pregnancies, in terms of birthweight and gestational age at birth.

No difference was found in average gestational age at birth or prematurity incidence between FET cycles compared with fresh ET cycles. The relatively large sample size allowed different severities of prematurity (late, early and extremely early) to be accounted for. The results were similar to findings by Shih et al. (2008) and by Pereira et al. (2016). In contrast, Aflatoonian et al. (2016) found higher gestational age at birth for FET twins compared with fresh embryo transfer (35.94 ± 1.78 versus 34.78 ± 2.91, $P = 0.002$), yet they found no difference regarding prematurity. Extensive research has been dedicated to understanding the pathophysiological pathways leading to preterm birth in twins, as well as to developing efficient screening tests and treatments aimed to prevent or to halt it. Among the main causes of premature birth in twins are over-distended uterus and preterm pre-labour rupture of membranes (Waldorf et al., 2015). A possible explanation for the unchanged gestational age at birth for twin pregnancies in the FET group is that FET has no influence on these aforementioned mechanisms causing preterm delivery in twin pregnancies.

A higher average birthweight and lower incidence of LBW, VLBW and SGA infants were found in the FET cycles compared with fresh embryo transfer cycles. The statistically significant difference in SGA incidence reflects a real difference in neonatal birthweight adjusted by the confounding factors of gestational age and neonatal gender. Results were similar to those of Shih et al. (2008) regarding birthweight and lower percentage of LBW, yet their study did not account for VLBW or SGA. Aflatoonian et al. (2016) also found a lower rate of LBW for FET, but no significant difference regarding SGA or birthweight. Pereira et al. (2016) found no differences at all between groups in terms of birthweight, LBW, VLBW or SGA. Yet this study was a relatively small cohort that included only 150 pregnancies.

A possible explanation for the higher average birthweight and lower incidence of LBW, VLBW and SGA infants in the FET group is, as mentioned above, the better process of placentation and endometrial angiogenesis in these cycles, due to the physiological level of hormones. This applies for twin pregnancies as well as singleton pregnancies. This study did not find any difference regarding intra-pair twin weight discordancy. This study is thought to be the first to evaluate this parameter in twin pregnancies following FET versus fresh embryo transfer.

A decision was made to address only second trimester miscarriages based on the assumption that different mechanisms exist for early and late pregnancy losses. While early abortions are mainly attributed to intrinsic abnormalities within the embryo, late (second trimester) miscarriages are mainly attributed to maternal or uterine factors. Another reason for the emphasis on late miscarriages is their associated morbidity and mortality, including their effect on future fertility ('Practice Bulletin No. 135: Second-Trimester Abortion,' 2013). A significantly lower rate of spontaneous second trimester miscarriages was found in the FET group compared with the fresh embryo transfer group (0.6% versus 7.7%, $P = 0.001$). This finding differs from previous published data. Aflatoonian et al. (2016) found no difference in pregnancy loss between pregnancies following FET versus fresh embryo transfer; however, their study was relatively small (including only 248 twin pregnancies, of which only 35 resulted from FET) and its miscarriage rate calculation included all pregnancies lost before 20 weeks of gestation, even biochemical pregnancies. Pereira et al. (2016) also found no difference in miscarriage (after a sonographic visualization of an intrauterine gestation); however, in their study miscarriage rate was analysed for singleton and twin pregnancies together. The relatively large cohort of twins-only in this study suggests high confidence of the findings.

Regarding fetal major malformation rate there was no significant difference between groups. This finding is consistent with current literature regarding fetal malformation rate in pregnancies resulting from FET (Belva et al., 2016, 2008; Berkovitz et al., 2010).

Duration of hospitalization reflects the risk for prematurity and fetal well-being as assessed by the medical treating team. No difference was found in duration of hospitalization between the two groups or in the distribution of hospitalization indications. This relates to the similar gestational age at birth between both groups.

As mentioned above, the proportion of women who were nulliparous in this study was significantly higher in the fresh embryo transfer group (60% versus 40%, $P < 0.001$). Given that nulliparity was reported to be a risk factor for a poor outcome in twin pregnancies (Berkovitz et al., 2010), FET versus fresh embryo transfer were analysed and compared separately in nulliparous women and multiparous women. This study is thought to be the first to evaluate perinatal outcomes following IVF twin pregnancies separately for nulliparous and multiparous women. This difference in parity may be explained by the fact that the frozen group had a previous successful IVF treatment with top-quality embryos that were suitable for freezing and future use.

Similar results to those mentioned above were found in the cross-sectional analysis, as detailed in TABLE 3. Among nulliparous women, FET showed an advantage over fresh embryo transfer in terms of significantly lower second trimester miscarriage rate. Among multiparous women, FET showed an advantage over fresh embryo transfer in terms of significantly lower rate of LBW and VLBW infants. SGA rate also appeared to be lower; however, statistical significance was borderline (P = 0.054). In this group, late miscarriages were almost four-fold more common in fresh embryo transfer in comparison to FET, although this difference did not reach statistical significance.

Another finding of the sub-analysis by parity was that among the nulliparous women the major malformation rate was higher in the FET group compared to the fresh embryo transfer group (P = 0.03). No such difference was found among multiparous women. This finding is thought to be incidental given the small cohort in this comparison.

This study also found a significant difference in age between the study groups; with a younger maternal age in the FET group compared with the fresh embryo transfer group (30.19 ± 4.6 versus 31.37 ± 4.3, P = 0.003). This difference is thought to reflect the better ovarian response of younger women and therefore the higher number of embryos suitable and available for the freezing process in these younger women. It was thought that maternal age could be a plausible confounder as previous reports show that among primiparous women the risk of delivering preterm twins decreased with increasing maternal age (*Branum and Schoendorf, 2005*). However, in this cohort the maternal age difference between groups was only 1.18 years, which is statistically significant, but most probably clinically insignificant. In addition, a regression analysis showed no significant effect of the maternal age, parity or gravity on prematurity.

The main strength of this study is that it is the first report to include a large sample size cohort of twin pregnancies to address the issue of obstetric and perinatal outcomes when comparing FET to fresh embryo transfer twin pregnancies, with emphasis on twin pregnancy common complications. The large sample size reflects data that summarize 10 years of the two-centre experience. The higher numbers provide greater precision.

This study has limitations inherent to any retrospective observational study analysis. Further research that will compare obstetric and perinatal outcomes in pregnancies following fresh and frozen embryo transfer in an adequately powered multicentre trial is warranted.

In conclusion, multiple pregnancies are considered to be a complication of ART treatment. This cohort shows that FET may have better obstetric and perinatal outcomes in twin pregnancies compared with fresh embryo transfer in regards to birthweight and second trimester miscarriages. Discussing this information with patients should be considered, as part of the decision process during fertility treatments. Although twin pregnancy can pose risks, each IVF cycle poses emotional stress and an economic burden. If further research, preferably a large multicentre cohort, confirms these findings, we believe that a more permissive policy regarding double embryo transfer in frozen cycles should be considered, accounting for their better 'twin performance'. Furthermore, in specific situations (i.e. severe male factor, elderly patients, diminished ovarian reserve, repeated IVF failure), when a double embryo transfer is planned, FET should be considered for its better outcome as described above.

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