Retrospective comparison of pregnancy outcomes of fresh and frozen-warmed single blastocyst transfer: a 5-year single-center experience

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Abstract

Purpose To assess whether live birth rates (LBR) and maternal/neonatal complications differed following single fresh and frozen-warmed blastocyst transfer.

Methods The present retrospective observational study analyzed 4,613 single embryo transfers (SET) (646 fresh and 3,967 frozen) from January 1, 2014, to December 31, 2018. Fresh embryo transfer at blastocyst stage was considered according to the age of the patient and her prognosis. In case of the risk of ovarian hyperstimulation syndrome, premature progesterone rise, non-optimal endometrial growth, or supernumerary embryos, cryopreservation with subsequent frozen embryo transfer (FET) was indicated.

Results No differences in LBR were recorded. Fresh embryo transfers yielded an increase both in neonatal complications OR 2.15 (95% CI 1.20–3.86, p 0.010), with a higher prevalence of singletons weighting below the 5th percentile (p 0.013) and of intrauterine growth retardation (p 0.015), as well as maternal complications, with a higher placenta previa occurrence OR 3.58 (95% CI 1.54–8.28, p 0.003), compared to FET.

Conclusion LBR appears not to be affected by the transfer procedure preferred. Fresh embryo transfer is associated with higher risk of neonatal complications (specifically a higher prevalence of singletons weighting below the 5th percentile and of intrauterine growth retardation) and placenta previa. Reflecting on the increased practice of ART procedures, it is imperative to understand whether a transfer procedure yields less complications than the other and if it is time to switch to a "freeze-all" procedure as standard practice.

Trial registration: Clinical Trial Registration Number: NCT04310761. Date of registration: March 17, 2020, retrospectively registered.

Keywords Pregnancy outcomes · Fresh ET · Frozen-warmed ET · Blastocyst · SET

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Introduction

Embryo cryopreservation has become a recognized tool in assisted reproductive technology (ART), performed to protect women's health, cryopreserve supernumerary embryos, and avoid further ovarian stimulation cycles if the fresh transfer is not successful [1]. The benefits of this procedure are many: it minimizes the risks of ovarian hyperstimulation syndrome (OHSS) [2, 3] and multiple pregnancy [4, 5] and maximizes cumulative live birth rate (CLBR) [6]. Additionally, cryopreservation allows to postpone embryo transfer when the endometrial preparation is suboptimal [7] or in case of premature progesterone rise. Moreover,

cryopreservation has also been extended to cycles with pre-implantation genetic diagnosis/screening [8, 9].

Therefore, cryopreserving embryos gives the couple the opportunity of performing further transfers undergoing only a single controlled ovarian stimulation (COS) and oocyte retrieval with good clinical outcomes while maximizing CLBR [10].

Previous studies that analyzed different pregnancy outcomes comparing fresh and frozen single blastocyst transfers have shown for the latter increased maternal risks of placenta accreta and pregnancy-induced hypertension [11], and lower risks of placenta previa and placental abruption [12]. With regard to neonatal complications, literature reports for frozen transfers an increase in the rate of large for gestational age (LGA) [13] and a lower risk of very preterm birth, small for gestational age (SGA), and perinatal mortality [12].

Despite the increased use of frozen embryo (ET) transfer procedure, literature still lacks concrete up-to-date evidence to whether one procedure is superior to another. As a matter of fact, the large-scale study by Ishihara et al. [11], which analyzes different single ET procedures from 2008 to 2010, concludes that with the continuous improvement in ART techniques, it is important to provide new and more recent data on the topic.

The present study purpose, therefore, was to assess whether live birth rate (LBR) and maternal/neonatal complications differed following single fresh and frozen-warmed blastocyst transfers. In addition, our study intended to analyze transfers performed only in a single center in order to reduce any bias given by different operators' dexterity or centers' equipment. Moreover, by analyzing only single blastocyst transfers, and thus reducing any bias from twin pregnancies, our study provides further relevant information and evidence on the present still open debate.

Materials and methods

Study design and population

A single-center retrospective study was conducted at a University affiliated, tertiary care referral ART center. The focus of the study were single fresh and warmed day-five blastocyst transfers between January 1, 2014, and December 31, 2018.

To minimize the selection bias, the following exclusion criteria were considered: oocyte or sperm donation cycles and embryos subjected to pre-implantation genetic testing (PGT). No age limitations were considered. Only single embryo transfers (SETs) were included in both arms to reduce the bias due to twin pregnancies. Maternal and neonatal outcomes in these pregnancies, over the same period of time, were compared, corrected for the common covariates.

Interventions

In fresh cycles, the internal protocol between 2014 and 2018 established the usage of SET in good prognosis patients [14]. SET was chosen according to the age of the patient (< 35 years of age) and favorable factors, such as first cycle of in vitro fertilization, previous live birth (either natural or following ART), evidence of morphological good quality embryos, and supernumerary good quality embryos to allow cryopreservation [14]. The male partner's infertility status was also considered, such as age, sperm quality, quantity, and source of spermatozoa. If the clinical presentation was feasible, after 5 days in culture, patients underwent fresh embryo transfer.

Cryopreservation with frozen embryo transfer (FET) was performed in women at risk of OHSS, premature progesterone rise, non-optimal endometrial growth, or in case of supernumerary embryos. Embryo transfer procedure relies on endometrial synchronization, and three main protocols were available: natural cycles (NC-FET), modified natural cycle (mNC-FET), and artificial replacement (AR-FET) [15].

ET procedure was carried out in a surgical theatre via transabdominal ultrasound scan (US) guidance and the use of a soft intrauterine catheter, following a pre-determined and standardized direct pre-load technique [16].

Patients' follow-up

Clinical pregnancy was defined according to the ASRM/ESHRE definition as a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy; in addition to intrauterine pregnancy, this definition includes clinically documented ectopic pregnancies. Delivery is defined as the complete expulsion or extraction of one or more fetuses, after at least 22 completed weeks of gestational age, irrespective of whether they are live births or stillbirths. Live birth rate was considered the complete expulsion or extraction from a woman of a product of fertilization, after 22 completed weeks of gestational age, which, after such separation, breathes or shows any other evidence of life, such as heartbeat, umbilical cord pulsation, or definite movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is attached, expressed per 100 ETs [17]. Twin delivery represents two live births [17].

Patient's follow-up is performed by dedicated personnel which includes physicians and clinical psychologists, supported by the fetal-maternal staff throughout the pregnancy, generally with a 30- to 90-day interval. This data

and those regarding pregnancy outcome, from delivery up to the end of the perinatal period (28 days after delivery), are reported in a dedicated section of the center's internal database. This reduces the possibility of bias in data recording, but also any potential clinical interference.

Main outcomes

The main outcome of this study was the live birth rate (LBR). Secondary outcomes were neonatal and maternal complications.

Investigated neonatal complications included stillbirth, neonatal death, intrauterine growth restriction (IUGR), prematurity, macrosomia, and fetal malformations. In addition, the birth weight percentile per gestational week was calculated [18].

Maternal complications were gestational diabetes; preeclampsia; hypertensive disorders; threatened preterm labor (i.e., progression of cervical dilation and ripening caused by regular uterine contractions occurring before 37 weeks of pregnancy, which may result in preterm birth) or miscarriage; oligohydramnios; placenta previa; placenta abruption; premature rupture of the membranes; endocrine, autoimmune, or infectious disorders; and the rate of urgent Cesarean (C-) sections.

The analysis was corrected for the following covariates: maternal age and paternal age at induction, maternal body mass index (BMI), smoking history, years of infertility, primary or secondary infertility, ovarian reserve expressed as follicular stimulating hormone (FSH), anti-Müllerian hormone (AMH), and antral follicular count (AFC), and indication to treatment.

Ethical approval

Internal Ethical Committee approved the study design on April 7, 2020, and the protocol was registered in ClinicalTrials.gov (ID NCT04310761) prior to any data extraction and statistical analysis. Patients who underwent ART cycles had consented in writing that their medical records could be used for research purposes if their anonymity was ensured and protected.

All the relevant data for the study were collected using an exclusive internal web-based database, as already described in a previous study [19].

Statistical methods

Results were expressed as mean ± SD (standard deviation) or median (interquartile range (IQR)) or percentage, as appropriate. All variables were analyzed by univariate logistic regression, and variables with a p value less than 0.2 were then submitted to multivariate logistic regression analysis, to identify factors associated to outcome. Results of the logistic regression analysis were expressed as odds ratio (OR) and 95% confidence interval (CI).

All analyses were made with Stata 15.0 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). Statistical significance was established at $p < 0.05$.

Results

Patient characteristics

During the 5-year period, a total of 4,613 single day-five blastocyst transfers were performed, among which 646 (14%) were fresh and 3,967 (86%) frozen transfers. As reported in Table 1, which displays the baseline characteristics of the embryo transfer population, significant differences existed between the two groups under investigation in maternal and paternal ages, infertility duration, ovarian reserve (expressed as AMH, AFC, FSH), and two specific indications to treatment: male and unexplained infertility. Mean female age at induction was 34.90 ± 3.97 for fresh and 35.97 ± 4.12 for frozen ($p < 0.001$) ETs. Regarding indication to treatment, male infertility accounted for 230 (35.6%) for fresh and 1,593 (40.2%) for frozen transfers (p 0.028), and unexplained infertility was recorded as 110 (17.0%) for fresh and 508 (12.8%) for frozen (p 0.003). Considering FET, the following transfer protocols were used: ART-ET 22.0%, NC-ET 15.2%, and mNC-ET 62.7%.

Live birth rate and other outcomes

The main outcome of the study is shown in Table 2: the live birth rate was 30.7%, 32.2% for fresh, and 30.5% for frozen ETs (p 0.382).

Out of 4,613 transfers, 2,047 (44.4%) clinical pregnancies were recorded. Biochemical pregnancies were not included in the analysis in accordance with the study protocol; these were 167.

Evolutive pregnancies were 1,653, of which 1,632 (86.8%) were single pregnancies, and 21(1.1%) twin pregnancies. Of these, 1,416 resulted in a live born neonate and 7 in stillbirth. The total number of abortions was 435 (23.1%); specific causes are shown in Table 2. Patients lost to follow-up were 4 (0.2%).

For both neonatal and maternal complications, complications were considered one if one or more complications occurred in a single neonate and woman, respectively.

Table 1 Baseline characteristics of embryo transfer population

Characteristics	All cycles	Fresh ET	Frozen ET	p
Embryo transfer (n)	4,613	646	3,967	
Female age at induction (years)	35.82 ± 4.11	34.90 ± 3.97	35.97 ± 4.12	< 0.001
Male age at induction (years)	39.07 ± 5.36	38.29 ± 5.37	39.20 ± 5.35	< 0.001
Infertility duration (years)	4.58 ± 2.59	4.08 ± 2.39	4.66 ± 2.61	< 0.001
BMI	22. 02 ± 3.15	22.03 ± 3.17	22.02 ± 3.14	0.744
Smoking (%)	921 (20.0%)	130 (20.1%)	791 (19.9%)	0.913
Primary infertility	2,585 (56.0%)	367 (56.8%)	2,218 (55.9%)	0.669
FSH	6.95 ± 2.50	7.37 ± 2.59	6.88 ± 2.48	< 0.001
Indication to treatment				
Male infertility	1,823 (39.5%)	230 (35.6%)	1,593 (40.2%)	0.028
Tubal	585 (12.7%)	93 (14.4%)	492 (12.4%)	0.158
Unexplained infertility	618 (13.4%)	110 (17.0%)	508 (12.8%)	0.003
Male and female factor	743 (16.1%)	109 (16.9%)	634 (16.0%)	0.568
Ovulatory	150 (3.3%)	13 (2.0%)	137 (3.5%)	0.055
Reduced ovarian reserve	346 (7.5%)	45 (7.0%)	301 (7.6%)	0.578
Multiple female factors	172 (3.7%)	22 (3.4%)	150 (3.8%)	0.640
Endometriosis	154 (3.3%)	19 (2.9%)	135 (3.4%)	0.544
Other	21 (0.5%)	4 (0.6%)	17 (0.4%)	0.504

Data are expressed as mean ± standard deviation or number (percentage)

BMI body mass index, *FSH* follicle stimulating hormone, *AMH* anti-Mullerian hormone, *AFC* antral follicular count

Table 2 Outcomes of the two different transfer procedures

Characteristics	All cycles	Fresh ET	Frozen ET	p
Live birth rate	30.7%	32.2%	30.5%	0.382
Clinical pregnancy (n)	2,047 (44.4%)	287 (44.4%)	1,760 (44.4%)	0.997
Biochemical pregnancies (% on total ET)	167 (3.6%)	18 (2.8%)	149 (3.8%)	0.221
Ectopic pregnancies	18 (1.0%)	4 (1.5%)	14 (0.9%)	0.335
Total abortions	435 (23.1%)	54 (20.1%)	381 (23.7%)	0.198
Therapeutics abortions	14 (0.7%)	2 (0.7%)	12 (0.7%)	1.000
Early miscarriage (< 12 weeks)	421 (22.4%)	52 (19.5%)	369 (22.9%)	0.193
Evolutive pregnancies (> 12 weeks)	1,653	242	1,411	0.101
Single pregnancies	1,632 (86.8%)	236 (87.7%)	1,396 (86.7%)	
Twin pregnancies	21 (1.1%)	6 (2.2%)	15 (0.9%)	
Live births	1422	210	1212	0.029
Singletons	1405 (98.8%)	204 (97.1%)	1201 (99.1%)	
Twins	17 (1.2%)	6 (2.9%)	11 (0.9%)	
Maternal complications (% on pregnancy)	515 (27.4%)	71 (26.4%)	444 (27.6%)	0.691
Neonatal complications (% on pregnancy)	59 (3.1%)	15 (5.6%)	44 (2.7%)	0.013
Lost to follow-up	4 (0.2%)	1 (0.4%)	3 (0.2%)	0.461

Data are expressed as mean ± standard deviation or number (percentage)

Neonatal complications

For singleton pregnancies with delivery (n = 1,399) the highest prevalence of complication (5.4%) was observed upon fresh ET, compared to 2.6% for frozen ET (p 0.041), as shown in Table 3.

The mean birth weight (grams) was 3,183 ± 520 and 3,282 ± 526 for fresh ET and frozen ET, respectively (p 0.001). In addition, the 95th and 5th birth weight percentile per gestational week was calculated following the work of Nicolaides et al. of 2018 [18]. Notably, fresh ET resulted in a higher prevalence of singletons weighting below the 5th percentile, 12.9% compared to 7.4% in the frozen ET

Table 3 Outcomes of singleton pregnancy

Characteristic	All cycles	Fresh ET	Frozen ET	*p* value
Singleton pregnancy (with delivery)	1,399	202	1,197	
Male/female ratio	709/690 = 1.03	93/109 = 0.85	616/581 = 1.06	0.154
Gestational week (± SD)	38.97 ± 1.92	38.95 ± 1.71	38.98 ± 1.96	0.426
Birth weight (g) (± SD)	3,268 ± 526	3,183 ± 520	3,282 ± 526	0.001
Premature delivery (< 37 weeks)	121 (8.7%)	17 (8.4%)	104 (8.7%)	0.899
Very premature delivery (< 32 weeks)	18 (1.23)	2 (1.0%)	16 (1.3%)	1.000
Weight at birth > 95th percentile	52 (3.7%)	7 (3.5%)	45 (3.8%)	1.000
Weight at birth < 5th percentile	115 (8.2%)	26 (12.9%)	89 (7.4%)	0.013
Macrosomia	6 (0.4%)	1 (0.5%)	5 (0.4%)	1.000
Maternal complications	501 (35.8%)	66 (32.7%)	435 (36.3%)	0.315
Neonatal complications	42 (3.0%)	11 (5.4%)	31 (2.6%)	0.041
Neonatal anomalies	31 (2.2%)	5 (2.5%)	26 (2.2%)	0.795

Data are expressed as mean ± standard deviation or number (percentage)

subgroup (*p* 0.013). The twin population (*n* = 17) was also analyzed, but no statistically relevant data were recorded concerning both neonatal and maternal complications in the two transfer groups. Investigating the single neonatal complications, a higher rate of intrauterine growth delay was reported by the fresh ET procedure subgroup: 3.3% versus 1.1% in FET (*p* 0.015).

Univariate and multivariate logistic regressions were performed to determine the relationship between the variables investigated and overall neonatal complications in fresh ET and frozen ET groups, as demonstrated by Table 4. Endometriosis and "other indication to treatment" were not included in the regression analysis since we had a recorded value of 0, and via the Fisher analysis, the *p* value was not statistically significant. In addition, only FSH was considered a variable for the assessment of female infertility as there is a strict correlation with the other two variables AMH and AFC.

The regression showed that the fresh ET procedure itself had a direct impact on the occurrence of the complications, with an OR of 2.15 (95% CI 1.20–3.86, *p* 0.010). *p*-values of less than 0.2 were then subjected to

Table 4 Univariate and multivariate logistic regressions in neonatal complications

Variable	Univariate		Multivariate	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Fresh cycle	2.15 (1.20–3.86)	0.010	2.19 (1.21–3.98)	0.010
Female age at induction (years)	0.96 (0.90–1.02)	0.159		
Male age at induction (years)	0.98 (0.93–1.03)	0.462		
Years of infertility	0.96 (0.86–1.07)	0.418		
BMI (kg/m²)	1.04 (0.96–1.12)	0.365		
Smoking	1.12 (0.60–2.08)	0.728		
FSH (mUI/mL)	1.07 (0.97–1.17)	0.159	-	
Indication to treatment				
Male	1.00 (0.60–1.67)	0.996		
Tubal	0.77 (0.33–1.80)	0.541		
Unexplained	1.88 (1.02–3.46)	0.042	-	
Male and female factor	0.55 (0.24–1.29)	0.170	-	
Multiple female factors	1.80 (0.64–5.11)	0.268		
Ovulatory	0.44 (0.06–3.24)	0.422		
Reduced ovarian reserve	1.56 (0.61–3.97)	0.355		
Oocytes retrieved	1.02 (0.97–1.06)	0.445		
Maternal complication	5.54 (3.31–9.26)	< 0.001	5.58 (3.33–9.34)	< 0.001

Results are presented as odds ratio (OR) and 95% confidence interval (CI). In multivariable analysis, the dash indicates that the given variable had entered the analysis and subsequently exited for lack of significance. *BMI* body mass index, *FSH* follicle stimulating hormone

multivariate logistic regression analysis, confirming this result.

Maternal complications

As for maternal complications, a total of 501 (35.8%) and 10 (58.8%) were recorded in the group of singleton pregnancies with delivery ($n=1,399$) and twin pregnancies with delivery ($n=17$), respectively. No significant differences were observed between the two transfer procedure subgroups.

When single complications were analyzed, a higher prevalence of placenta previa was reported following fresh ET: 4.3% compared to 1.2% in FET (OR 3.58, 95% CI 1.54–8.28, p 0.003).

The same regression analyses were performed for the maternal complications (Table 5). Of statistical significance were maternal BMI and FSH (OR 1.05, 95% CI 1.01–1.09, $p=0.010$ and OR 1.05, 95% CI 1.00–1.10, $p=0.035$, respectively). Of interest was primary infertility, which was very close to reaching statistical significance: p 0.052. Multivariate logistic regression analysis further showed the significant impact of BMI, FSH, and primary infertility on maternal complications (OR 1.06 (95% CI 1.02–1.10) $p=0.004$; OR 1.06 (95% CI 1.01–1.11) $p=0.019$; and OR 1.3 (95% CI 1.02–1.69) $p=0.033$, respectively).

Discussion

Our retrospective study shows that the LBR per total transfer was 30.7%, specifically 32.2% for the fresh ET procedure and 30.5% for frozen ET. Our study confirms the statement by Roy et al. that apparently there seems to be no statistical difference between the two transfer procedures (p 0.382) [20]. As for complications, fresh embryo transfers yielded an increase both in neonatal complications OR 2.15 (95% CI 1.20–3.86, p 0.010), with a higher prevalence of singletons weighting below the 5th percentile (p 0.013) and of intrauterine growth retardation (p 0.015), as well as maternal complications, with a higher placenta previa occurrence OR 3.58 (95% CI 1.54–8.28, p 0.003), compared to FET.

Research has suggested that singleton pregnancies after ARTs are associated with higher risks of obstetric and perinatal complications when compared with singleton pregnancies after spontaneous conception [21]. Understanding which procedure contributes to the greatest risk has now become mandatory. In fact, since the first successful live birth reported following frozen ET in 1984 [22], there has been an unsolved dilemma regarding which transfer procedure yields better neonatal and maternal outcomes, and if one has a higher pregnancy and LBR compared to another. As a matter of fact, the goal that an infertile couple seeks

Table 5 Univariate and multivariate logistic regressions in maternal complications

Variable	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Fresh cycle	1.03 (0.72–1.45)	0.889		
Female age at induction or vitrification (years)	0.99 (0.96–1.02)	0.658		
Male age (years)	0.99 (0.96–1.01)	0.322		
Years of infertility	0.99 (0.94–1.04)	0.642		
BMI (kg/m^2)	1.05 (1.01–1.09)	0.01	1.06 (1.02–1.10)	0.004
Smoking	0.94 (0.69–1.28)	0.693		
FSH (mUI/mL)	1.05 (1.00–1.10)	0.035	1.06 (1.01–1.11)	0.019
Primary infertility	1.28 (1.00–1.65)	0.052	1.32 (1.02–1.69)	0.033
Indication to treatment				
Male	0.99 (0.77–1.27)	0.932		
Tubal	1.09 (0.75–1.58)	0.656		
Unexplained	1.12 (0.79–1.59)	0.519		
Male and female factor	0.82 (0.58–1.16)	0.264		
Disovulatory	0.59 (0.26–1.32)	0.205		
Reduced ovarian reserve	1.43 (0.88–2.32)	0.149		
Multiple female factors	1.03 (0.55–1.95)	0.914		
Endometriosis	0.93 (0.46–1.88)	0.844		
Other (genetic)	1.70 (0.18–16.38)	0.648		
Retrieved oocytes	0.99 (0.97–1.02)	0.716		

Results are presented as odds ratio (OR) and 95% confidence interval (CI)

BMI body mass index, *FSH* follicle stimulating hormone

from ART procedures is a healthy, live born neonate. There have been multiple reports trying to solve this ongoing dilemma, without reaching a conclusive solution, with a handful of reports suggesting that warmed-thawed transfers yield an equivalent, or even higher pregnancy rate ([20, 23–25]. According to Aflatoonian et al., LBR resulted to be lower after FET [23]. Furthermore, it has been proved by Boomsma et al. that controlled ovarian stimulation (COS) causes direct and significant alterations on the endometrial milieu encountered by the embryo being transferred [26]. Thus, these deleterious effects on endometrial receptivity, and therefore on embryo implantation, during fresh-embryo transfer, could be avoided by exploiting natural cycle or artificial replacement cycles in frozen embryo transfer [27].

Considering that secondary outcomes of this study were the prevalence of both neonatal and maternal outcomes and that the factors that have an impact on the onset of obstetric complications in ART have been deeply investigated in the literature [28] and are still under debate, we decided to perform an analysis focusing on which, if any, individual complication had an association with the type of transfer procedure performed.

Our findings support the existing literature in that neonatal complications are more frequent in fresh ET than in frozen ET, with an OR of 2.15 (95% CI 1.20–3.86, p 0.01). Analyzing each single complication as a separate entity, this study revealed a higher prevalence of delay in intrauterine fetal growth in the fresh transfer procedure: 3.3% against 1.1% prevalence in frozen (p 0.019). Singletons born from the frozen ET had a statistically significant higher birthweight, on average 100 g heavier. But, despite this finding, only 6 newborns in singleton pregnancies appeared to be macrosomic (0.4% of all the live births). This could be explained by a higher prevalence of diabetes in the frozen population, i.e., 80 (6.6%), as compared to 8 (3.8%) in the fresh group. Nevertheless, this difference in weight does not appear to be of great clinical concern, considering also that only 3.7% of neonates had a birthweight above the 95th percentile.

In our study we performed a sub-analysis on the prevalence of neonatal complications in singleton and twin pregnancies. Despite only single embryo transfers were enrolled in this study, the overall twin delivery rate was 1.2%, 2.9% for fresh and 0.9% for FET. Despite only 17 twin pregnancies with delivery were recorded, a higher prevalence of complications occurred in this subgroup, 29.4% compared to only 3.0% in the singleton pregnancies. Indeed, of note is the potential impact of twin pregnancies on the total number of neonatal complications recorded. As a matter of fact, it is well known that multiple gestation pregnancies tend to have a more complicated clinical course than singleton pregnancies [29], and our results could have been influenced by their occurrence.

This finding could be explained by what Vega et al. reported [30] in a recent study. Vega et al. argue that there are three main causes responsible for twinning in SET:

1. Zygotic splitting, which it is known to have a 1.3% prevalence in SET [31]
2. Concomitant spontaneous conception
3. Errors at the time of transfer (both in clinical and laboratory procedures)

While it is not possible to calculate the rate of the latter, Vega stated that concomitant spontaneous conception could be accounted for especially in the unexplained infertility subpopulation. It is possible, in fact, that this subpopulation, with the help of ART procedures such as controlled ovarian stimulation, could be able to spontaneously conceive, after sexual intercourse, thus leading to two embryos implanting and to a dizygotic twin pregnancy. Our data seems to agree, in our investigation, that unexplained infertility was the reason of 618 indications to treatment, for all cycles, 110 (17.0%) in the fresh ET and 508 (12.8%) in the frozen ET population, with a significant difference of p 0.003. Another cause could be an elevated BMI, which could lead to the incomplete oocyte retrieval, leading to the possibility of being spontaneously fertilized. This last finding was not confirmed in our population. On this regard, in our dataset of born twins, the monochorionic pregnancies were 11, and the dichorionic ones were 6. A total of 16 couples of twins were the same sex, and only one couple had different sex. No test for zygosity was performed.

Maternal complications

Maternal complications were first analyzed as a whole, showing no significant differences. When analyzed one by one however, a higher prevalence of placenta previa was observed in the fresh ET (4.29%), compared to 1.24% of the FET group (p 0.003). Our finding is in accordance with the literature that reports frozen ET having a lower risk of placenta previa compared to fresh [12]. Furthermore, upon univariate and multivariate logistic regression analyses, our study demonstrates that BMI, FSH levels, and primary infertility directly affect the rate of maternal complications.

Saunders et al., in 1988, was the first to state that infertility per se is an independent risk factor for subsequent problems during pregnancy [32, 33]. The comprehensive review of the literature on this topic, published by Palomba et al., states that "the level of evidence seems to be sufficient to suggest a direct relationship between infertility, subfertility factors and many adverse obstetric and neonatal outcomes" [32]. Our investigation showed an OR of 1.32 (95% CI 1.02–1.69) upon multivariate logistic regression analysis, regarding primary infertility. Remarkably, neither

an increase in maternal nor paternal age seemed to have an effect on maternal complications, while it is well known that both have multiple adverse effects on pregnancy outcome and infertility [34, 35] even though maternal age seems to impact more spontaneous conception than ART-related pregnancies [36]. Another peculiar finding was the influence of high levels of FSH on the incidence of maternal complications, but since this was beyond the scope of the study, we decided not to investigate it further.

The primary strength of this study was the large number of single embryo transfers ($n = 4,613$) available for analysis and the length of the study period, i.e., 5 years. In addition, being the investigation focused solely on a single center, there are no confounding factors related to different procedures used by different centers nor on the method with which data were recorded and extrapolated. Furthermore, via the use of a dedicated phoning system, both embryo transfer and pregnancy follow-up could easily be guaranteed. Indeed, we only recorded a total of 4 patients lost to follow-up. Regarding possible study limitations, surely worth of mention is the retrospective design of the study. A further limitation of our study could be the design that did not consider a control arm of spontaneous conception and/or no history of infertility patients to compare neonatal and maternal complication rates with. It could have been important to perform such analysis on the very same population, but our university-affiliated tertiary care could not account on a physiological population, since it treats only infertile couples. Moreover, some data could have erroneously been recorded, despite the fact that data were retrieved from the center's exclusive internal web-based database.

Conclusions

In conclusion, this single-center study focusing on LBR and maternal and neonatal outcomes after elective day-five single ET showed that single FET had a lower risk of neonatal complications and lower prevalence of placenta previa and of IUGR compared to single fresh ET when analyzed together. Indeed, fresh transfer has a higher neonatal complication rate compared to the frozen procedure.

Our study would appear to agree with the literature reporting that FET seems to be a safer option than a fresh transfer, certainly regarding the fetal counterpart. We found no difference in the LBR, which can still be taken as a positive outcome considering the incoherent data in the literature regarding this parameter. Further research is imperative to safely decide whether a "freeze-all" strategy, independent of the patients' profile, should be implemented and whether it would be superior, in terms of live birth rate and outcomes, compared to a fresh transfer.

Declarations

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