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Greater estimated fetal weight and birth weight in IVF/ICSI pregnancy after frozen–thawed *vs* fresh blastocyst transfer: prospective cohort study with novel unified modeling methodology

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KEYWORDS: fetal growth; *in-vitro* fertilization; intracytoplasmic sperm injection; large-for-gestational age; LGA; SGA; small-for-gestational age; thawed blastocyst transfer; ultrasound

CONTRIBUTION

What are the novel findings of this work?
Unified modeling showed non-divergent estimated fetal weight (EFW) and birth-weight (BW) growth trajectories across gestational age in pregnancies conceived after frozen–thawed blastocyst transfer (BT) and those after fresh BT. Mean EFW *Z*-scores were highest in the midtrimester and decreased with advancing gestation, becoming negative after 32 weeks in the fresh and after 35 weeks in the frozen–thawed BT group. Concordance between predicted EFW and observed BW centiles was greater for small-for-gestational age (SGA) in the fresh and for large-for-gestational age (LGA) in the frozen–thawed BT group.

What are the clinical implications of this work?
The proportion of SGA fetuses increases rapidly across gestation in pregnancies from fresh BT and smoothly in those from frozen–thawed BT, whereas the rate of LGA fetuses decreases rapidly with progressing gestation in pregnancies from frozen–thawed BT and smoothly in those from fresh BT. The higher rate of SGA in pregnancies conceived after fresh BT and of LGA in those after frozen–thawed BT is due to the higher midtrimester EFW observed in the frozen–thawed BT group. The performance of prediction models for SGA and LGA may be improved if the mode of conception, unified prenatal/postnatal curves and the greater efficiency in prenatal prediction of SGA in fresh BT and of LGA in frozen–thawed BT pregnancies are taken into account.

ABSTRACT

Objective *To compare, using a unified approach, standardized estimated fetal weight (EFW) trajectories from the second trimester to birth and birth-weight (BW) measurements in* in-vitro *fertilization/intracytoplasmic sperm injection (IVF/ICSI) pregnancies obtained after frozen–thawed* vs *fresh blastocyst transfer (BT).*

Methods *This was a secondary analysis of a prospective longitudinal cohort study performed at the Fetal Medicine and Obstetric Departments of San Raffaele Hospital in Milan, Italy, from January 2016 to December 2020. Eligible for inclusion were singleton viable gestations conceived by autologous IVF/ICSI conception after fresh or frozen–thawed BT that underwent standard fetal biometry assessment at 19–41 weeks and had BW measurements available. All ultrasound assessments were performed by operators blinded to the employment of cryopreservation. Patients with twin gestation, significant pregestational disease, miscarriage, major fetal abnormalities and use of other types of medically assisted reproduction techniques were excluded. EFW and BW Z-scores and their trajectories were analyzed using general linear models (GLM) and*

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logistic regression with a unified modeling methodology based on the Fetal Medicine Foundation fetal and neonatal population weight charts, adjusting for major confounders. Differences between prenatal EFW and postnatal BW centiles in the two groups were assessed and compared using contingency tables, χ^2 *test and conversion of prenatal to postnatal centiles.*

Results *A total of 631 IVF/ICSI pregnancies were considered, comprising 263 conceived following fresh BT and 368 after frozen–thawed BT. A total of 1795 EFW observations were available (*n = *715 in fresh BT group and* n = *1080 in frozen–thawed BT group; median of three observations per patient). EFW and BW* $< 10^{th}$ *centile were significantly more frequent in the fresh than in the frozen–thawed BT group (*$P = 0.003$ *and* $P < 0.001$*, respectively). EFW and BW* $> 90^{th}$ *centile were significantly more frequent in the frozen–thawed* vs *fresh BT group (*$P = 0.034$ *and* $P = 0.002$*, respectively). GLM showed significantly decreasing EFW Z-scores with advancing gestational age (GA) in both groups. The effect of GA was assumed to be equal in the two study groups, as no significant interaction effect was found. Smoothed mean EFW Z-scores from 19 weeks of gestation to term and smoothed mean BW Z-scores were both significantly higher in the frozen–thawed compared with the fresh BT group (EFW Z-score,* 0.70 ± 1.29 vs 0.28 ± 1.43*;* $P < 0.001$*; BW Z-score,* 0.04 ± 1.08 vs -0.31 ± 1.28*;* $P < 0.001$*). Mean smoothed EFW Z-score values in the frozen–thawed* vs *fresh BT groups were* 1.01 ± 0.12 vs 0.60 ± 0.08 *at 19–27 weeks,* 0.36 ± 0.07 vs -0.06 ± 0.04 *at 28–35 weeks and* -0.66 ± 0.01 vs -0.88 ± 0.02 *at 36–41 weeks. Mean smoothed BW Z-score values in the frozen–thawed* vs *fresh BT groups were* -0.80 ± 0.14 vs -1.20 ± 0.10 *at 28–35 weeks and* 0.22 ± 0.16 vs -0.24 ± 0.14 *at 36–41 weeks. Assessment of EFW and BW concordance showed a significantly greater rate of postnatal confirmation of prenatally predicted small-for-gestational age (SGA)* $< 10^{th}$ *centile in the fresh compared with the frozen–thawed BT group (*$P < 0.001$*), whereas the rate of postnatal confirmation of large-for-gestational age (LGA)* $> 90^{th}$ *centile was significantly higher in the frozen–thawed* vs *the fresh BT group (*$P < 0.001$*). Logistic regression analysis showed that the smoothed rate of EFW* $< 3^{rd}$ *centile was about 6-fold higher in the fresh* vs *frozen–thawed BT group (*$P < 0.001$*), whereas the smoothed rates of EFW* 90^{th}–97^{th} *centile and* $> 97^{th}$ *centile were nearly double in the frozen–thawed compared with the fresh BT group (*$P < 0.05$ *and* $P < 0.001$*, respectively).*

Conclusions *Robust novel unified prenatal–postnatal modeling in IVF/ICSI pregnancies after frozen–thawed or fresh BT from 19 weeks of gestation to birth showed non-divergent growth trajectories, with higher EFW and BW Z-scores in the frozen–thawed* vs *fresh BT group. The mean EFW Z-scores in both IVF/ICSI groups were greater than those expected for natural conceptions, being highest in the midtrimester and decreasing with advancing gestation in both groups, becoming negative after 32 weeks in the fresh and after 35 weeks in the frozen–thawed BT group. Mean BW Z-scores were negative in both groups, with lower values in preterm fetuses, and increased with advancing gestation, becoming positive at term in the frozen–thawed BT group. IVF/ICSI conceptions from frozen–thawed as compared to fresh BT presented increased rate of LGA and reduced rate of SGA both prenatally and postnatally.*

INTRODUCTION

Evidence from two meta-analyses that included a total of 47 studies on assisted reproductive technologies (ART) and fetal growth demonstrated an increased rate of low (LBW) or very low (VLBW) birth weight or fetal growth restriction (FGR) and small-for-gestational-age (SGA) newborns derived from fresh embryo transfers during *in-vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles as compared to natural conception (mean relative risk for SGA, FGR, LBW and VLBW was 1.4, 1.5, 1.6 and 2.6, respectively)[1,2]. Meta-analyses and large population-based cohort studies have established a reduced rate of SGA and LBW and an increased risk of large-for-gestational age (LGA) and macrosomia at birth in pregnancies from thawed blastocyst transfers (BT) as compared to those from fresh BT (mean relative risk for SGA, LBW, LGA and macrosomia: 0.5, 0.7, 1.5 and 1.7, respectively)[3–6]. Overall, singleton IVF/ICSI pregnancies after thawed *vs* fresh transfer showed significantly higher birth weight (BW). These findings from observational studies are consistent with those from major randomized controlled trials or registry studies on the effectiveness and outcome of embryo cryopreservation[7–10]. No significant differences were found in the long-term weight and height of children from IVF/ICSI pregnancies in comparison to those of children born after spontaneous conception, supporting the concept of different intrauterine growth patterns in these pregnancies[11].

Recent evidence also showed that pregnancies obtained by IVF/ICSI techniques with thawed BT, as compared to those with fresh BT, present greater crown–rump length growth and uterine perfusion[12–14]. However, differences in fetal growth patterns, growth trajectories or BW between spontaneous conceptions and ART with fresh *vs* thawed cycles present several unanswered questions, including etiology, onset timing and extent of the known deviations from normal. Moreover, different methodologies and reference curves for the calculation of estimated fetal weight (EFW) or BW standardized coefficients hamper the reliability of a unified generalized model assessing growth trajectories from the prenatal to the neonatal period.

The aim of this study was to assess differences in standardized EFW longitudinal measurements from the second trimester to birth and BW observations in IVF/ICSI pregnancies obtained after frozen–thawed *vs* fresh BT, using a novel unified methodology based on the Fetal Medicine Foundation (FMF) fetal and neonatal population weight charts of Nicolaides *et al.*[15].

METHODS

Study design

This was a secondary analysis of an ongoing prospective single-center longitudinal cohort study of singleton pregnancies achieved after IVF/ICSI procedures with transfer of fresh or thawed blastocysts[13]. The study was conducted and reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines[16].

Setting

Recruitment was carried out at the Fetal Medicine and Obstetrics Departments of San Raffaele Hospital, Milan, Italy. Patients included were seen from January 2016 to December 2020. Follow-up and data collection were carried out at the Fetal Medicine Unit of San Raffaele Hospital up to April 2021 (extended by 24 months as compared to the primary study). Data were stored prospectively in a dedicated database (ViewPoint 6; GE Healthcare GmbH, Germany) and retrieved subsequently for analysis. The data collected were relative to patients undergoing fresh or thawed BT, after embryo cryopreservation by vitrification in IVF/ICSI conceptions.

Indications for cryopreservation, methodology of controlled ovarian stimulation and IVF protocols including clinical or laboratory procedures and policies, were standardized at our center and described in detail in recent publications of our group, on different, yet related, research questions analyzed on the smaller primary cohort[13,14].

Study participants

Eligibility criteria were autologous IVF/ICSI conceptions following fresh or thawed BT. Other ART, such as oocyte donation, intrauterine insemination, cleavage-stage embryo transfer, gamete or zygote intrafallopian transfer, were not eligible for inclusion. Patients with twin pregnancy, aneuploidy, significant pregestational disease, major fetal defect or fetal demise were also excluded. The STROBE flowchart of the study design is presented in Figure S1. Of the 1525 patients recruited to the present study, 827 had also been recruited to the primary cohort study[13]. Of these, 367 patients were included in the analysis after exclusions. A total of 698 additional patients were considered eligible for inclusion in the present research due to prospective collection of new cases, retrospective recovery of pregnancy outcomes and availability of fetal biometric measurements. Of these, 434 participants were excluded before or after enrolment, for specific reasons, leaving 264 new patients (thawed BT, $n = 165$; fresh BT, $n = 99$) for analysis. Therefore, a total of 631 patients were included in the present analysis (Figure S1).

Variables and outcomes

The exposures analyzed were blastocyst cryopreservation with vitrification as compared to fresh BT in autologous IVF/ICSI conceptions. Gestational age (GA) was calculated by considering as conception the day of oocyte retrieval for fresh cycles and establishing a pseudo-last menstrual period 19 days before BT for the frozen–thawed BT group, as described before by our group[13,14]. Patient data of interest collected were maternal age, body mass index (BMI), cigarette smoking during pregnancy and obstetric history including parity (parous or nulliparous if no previous pregnancy ≥ 24 weeks' gestation). The following data on IVF/ICSI procedures were collected: number of oocytes retrieved, progesterone and estrogen levels, use of cryopreservation and IVF *vs* ICSI. Medications used were also recorded. Pregnancy outcomes collected included GA at birth, BW, and fetal and maternal complications with particular regard to rates of pre-eclampsia, SGA and LGA.

The main outcomes of this study were EFW and BW in the two study groups, expressed as *Z*-scores using a unified modeling methodology based on the FMF fetal and neonatal population weight charts[15].

Data sources and biometry measurements

All cases underwent first-trimester ultrasound at 11–14 weeks of gestation for confirmation of fetal viability and screening for major chromosomal or structural defects. For the purposes of this study, it was mandatory for pregnancies to undergo a first-trimester scan at 11–14 weeks and at least one subsequent ultrasound scan for standard fetal biometry and for pregnancy outcome to be reported. Cases with significant maternal chronic disease, prenatally detected structural defect, non-viable fetus, multiple pregnancy and patients with inadequate or absent recording of measurements or outcome were excluded (Figure S1).

All operators performing the ultrasound examinations were blinded to the employment of embryonic cryopreservation and were not involved in the IVF procedures or in clinical management apart from the ultrasound scan. Blinding was achieved by concealing patient history from all sonographers involved in the trial.

Standard fetal biometry was performed at 19–27, 28–35 and/or at 36–41 weeks of gestation, including biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL). EFW was calculated using Hadlock's formula[17]. In our center, ultrasound measurements of fetal biometry are performed according to the methodology of the International Society of Ultrasound in Obstetrics and Gynecology[18]. For all measurements, clear images with sufficient magnification (region of interest occupying more than half of the total image) and correct depiction of landmarks were obtained to allow precise caliper placement. HC and AC were obtained using the ellipse measurement tool, by placing the calipers on the outer edges of the soft tissue circumference. BPD and HC were measured at the symmetric transthalamic axial view of the skull, including the cavum septi pellucidi and excluding the cerebellum. AC was measured in the symmetrical axial plane, including the stomach bubble and portal sinus, excluding kidneys. FL represented the diaphysis length and was measured at $< 45°$ from the horizontal, when both bony edges were clearly visible[18,19].

In order to minimize interobserver variability, all scans and measurements were performed by operators with extensive experience in obstetric ultrasound. Measurements were performed transabdominally in all cases using high-end ultrasound machines equipped with multifrequency convex transducers (Voluson E8 or E10; GE Healthcare, Zipf, Austria). Ultrasound mechanical index and soft tissue thermal index were always set at 1.0 and 0.5, respectively. According to the American Institute of Ultrasound in Medicine/American College of Radiology/American College of Obstetricians and Gynecologists/Society for Maternal–Fetal Medicine/Society of Radiologists in Medicine (AIUM-ACR-ACOG-SMFM-SRU) guidelines, a thermal index for soft tissue was used at or before 10 weeks' gestation, while a thermal index for bone was used after 10 weeks[20].

Bias

We evaluated possible sources of information and selection biases. In particular, we took into account medication use and performance bias.

Statistical analysis

In order to achieve comparable standardized coefficients for EFW and BW, a fractional polynomial regression model was applied to establish the best curve-fitting estimation of median EFW or BW with SD (Appendix S1), based on the FMF fetal and neonatal population weight charts[15]. The polynomial equations produced were used for calculating expected values and *Z*-scores of both EFW and BW values of the study participants by a unified modeling methodology, based upon observed values of EFW from the Hadlock equation[17] and neonatal BW.

We also used general linear models (GLM) to explore the *Z*-score trajectories of EFW during pregnancy and postnatally with BW and to detect possible divergent patterns between the two groups (fresh and thawed BT), with correction for major confounders, if necessary.

In order to calculate the smoothed rates of EFW and BW according to preseleted categories ($< 3^{rd}$, 3^{rd}–10^{th}, 10^{th}–90^{th}, 90^{th}–97^{th} and $> 97^{th}$ centile) in each group (frozen–thawed BT and fresh BT), we ran two separate logistic regressions for EFW and BW, with GA as independent predictor.

Rate of concordance between prenatal EFW and postnatal BW centiles was assessed and compared using contingency tables, χ^2 test and conversion of prenatal to postnatal centiles. All tests were two-sided and a *P*-value < 0.05 was considered significant. SPSS Statistics for Windows software was used for the statistical analysis (version 25.0; IBM Corp., Armonk, NY, USA).

Ethical approval

The study followed the World Medical Association Declaration of Helsinki–Ethical Principles for Medical Research Involving Human Subjects. Local institutional review board approval was obtained before commencing the study (Protocol OSTE-PMA). Data were entered into a database in which patient data were stored anonymized to be used for research purposes. All women provided written informed consent to participate in the study.

RESULTS

Descriptive data

A total of 631 IVF/ICSI pregnancies were included for analysis, comprising 263 conceived following fresh BT and 368 following frozen–thawed BT. A total of 1795 EFW observations were available: 715 in the fresh BT group and 1080 in the frozen–thawed BT group (median number of observations per patient was three in each group). Table 1 summarizes maternal and pregnancy characteristics in the study groups. No significant differences were observed between the two study groups with regards to maternal age, BMI, parity, cigarette smoking, maternal serum concentration of pregestational follicle-stimulating hormone, mean GA at fetal biometry assessment, GA at birth, and incidence of preterm birth < 37 or < 34 weeks, pre-eclampsia and gestational diabetes mellitus. In the fresh BT group, compared with the frozen–thawed BT group, we observed significantly lower maternal serum concentration of pregestational antimüllerian hormone, number of metaphase-II oocytes and number of available embryos on day 3. A significantly higher number of blastocysts was transferred in the fresh as compared to the frozen–thawed BT group. In comparison to the fresh BT group, the frozen–thawed BT group had greater mean EFW and BW *Z*-scores, increased rates of EFW and BW $> 90^{th}$ centile and reduced rates of EFW and BW $< 10^{th}$ centile (Table 1).

GLM of EFW *Z*-score trajectories

The GLM output of EFW *Z*-scores is presented in Table S1. The EFW *Z*-scores are a function of both GA and study group (fresh or frozen–thawed BT). Under the assumption of this model, the mean EFW *Z*-score from 19 to 41 weeks' gestation was significantly higher in pregnancies conceived after frozen–thawed BT compared to those after fresh BT (EFW *Z*-score, 0.70 ± 1.29 *vs* 0.28 ± 1.43; $P < 0.001$). Figure 1 shows the fitted EFW *Z*-scores for both groups plotted against GA. Decreasing *Z*-scores with advancing GA were observed for both study

Table 1 Maternal and pregnancy characteristics of 631 singleton pregnancies conceived following *in-vitro* fertilization or intracytoplasmic sperm injection, according to whether fresh or frozen–thawed blastocyst transfer (BT) was performed

Variable	*Fresh BT* (n = 263)	*Frozen–thawed BT* (n = 368)	P*
Maternal age (years)	35.6 ± 4.4	35.2 ± 4.2	0.206
Body mass index (kg/m^2)	22.2 ± 3.7	21.9 ± 3.5	0.164
Nulliparous	244 (92.8)	342 (92.9)	0.938
Cigarette smoker	31 (11.8)	46 (12.5)	0.787
Pregestational FSH (IU/mL)	7.8 ± 2.5	7.7 ± 2.5	0.621
Pregestational AMH (ng/mL)	2.25 ± 3.05	3.77 ± 3.33	< 0.001
Metaphase-II oocytes (*n* per patient)	1.69 ± 4.75	6.60 ± 3.60	< 0.001
Available embryos at day 3† (*n* per patient)	2.95 ± 1.70	4.60 ± 2.15	< 0.001
Blastocysts transferred (*n* per patient)	1.55 ± 0.60	1.28 ± 0.58	< 0.001
Pre-eclampsia	12 (4.6)	19 (5.2)	0.082
Gestational diabetes mellitus	21 (8.0)	33 (9.0)	0.663
GA at fetal biometry (weeks)	25.9 ± 5.8	25.9 ± 5.7	1.000
Preterm birth < 34 weeks	9 (3.4)	13 (3.5)	0.941
Preterm birth < 37 weeks	34 (12.9)	32 (8.7)	0.087
GA at birth (weeks)	39 ± 1.33	39 ± 1.42	1.000
EFW < 10th centile‡	23 (8.7)	12 (3.3)	0.003
EFW > 90th centile‡	49 (18.6)	95 (25.8)	0.034
EFW *Z*-score	0.28 ± 1.43	0.70 ± 1.29	< 0.001
BW < 10th centile	45 (17.1)	31 (8.4)	< 0.001
BW > 90th centile	11 (4.2)	40 (10.9)	0.002
BW *Z*-score	−0.31 ± 1.28	0.04 ± 1.08	< 0.001

Data are given as mean ± SD or *n* (%). *Student *t*-test or χ^2 test, as appropriate. †Day 3 of embryonic life. ‡At any fetal biometry observation in the second or third trimester. AMH, antimüllerian hormone; BW, birth weight; EFW, estimated fetal weight; FSH, follicle-stimulating hormone; GA, gestational age.

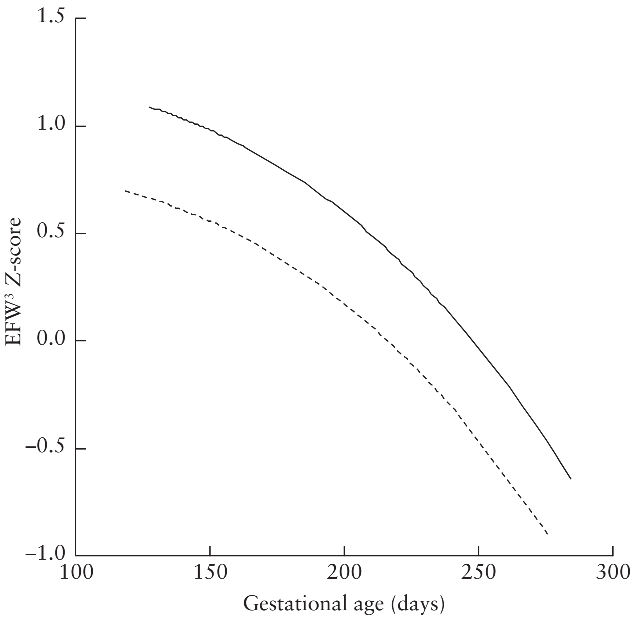

Figure 1 Estimated fetal weight (EFW) *Z*-scores according to gestational age in pregnancies conceived after fresh (------) or frozen–thawed (——) blastocyst transfer.

groups. Smoothed mean *Z*-score values were positive up to 32 and 35 weeks in the fresh and frozen–thawed BT groups, respectively, and became negative afterwards. Since no significant interaction effect (GA × group) was detected, the effect of GA was assumed to be equal in the two groups. When considering the data according to GA category, mean smoothed EFW *Z*-score values in the frozen–thawed *vs* fresh BT group were, respectively, 1.01 ± 0.12 *vs* 0.60 ± 0.08 at 19–27 weeks, 0.36 ± 0.07 *vs* −0.06 ± 0.04 at 28–35 weeks and −0.66 ± 0.01 *vs* −0.88 ± 0.02 at 36–41 weeks.

GLM of BW *Z*-score trajectories

The GLM output of BW *Z*-scores is presented in Table S1. Smoothed mean BW *Z*-scores were negative for preterm births and close to zero at term (Figure S2). Under the assumption of this model, the mean BW *Z*-score was significantly higher in pregnancies conceived after frozen–thawed *vs* after fresh BT, (BW *Z*-scores, 0.04 ± 1.08 *vs* −0.31 ± 1.28; *P* < 0.001). Figure S2 shows the fitted BW *Z*-scores for both groups plotted against GA. For both study groups, BW *Z*-scores increased with advancing GA at birth with a peak at about 270 days and subsequent trend inversion. Smoothed mean BW *Z*-score values were negative in preterm neonates and became positive at term in pregnancies from frozen–thawed BT, but not in those from fresh BT. Mean smoothed BW *Z*-score values in the frozen–thawed *vs* fresh BT groups, according to GA category, were −0.80 ± 0.14 *vs* −1.20 ± 0.10 at 28–35 weeks and 0.22 ± 0.16 *vs* −0.24 ± 0.14 at 36–41 weeks.

Comparison of EFW and BW

Table 2 shows the concordance between EFW at the last ultrasound scan performed at ≥ 28 weeks' gestation and BW measurements. Prenatal prediction of SGA was better in pregnancies from fresh *vs* frozen–thawed BT (58.3% *vs* 0%), in other words, after prenatal diagnosis of SGA it was more likely to have a postnatal diagnosis of SGA in fresh than in frozen–thawed BT pregnancies. Prenatal prediction of LGA was better in pregnancies from frozen–thawed *vs* fresh BT (81.1% *vs* 12.5%), meaning that a prenatal diagnosis of LGA was more likely to be confirmed postnatally in the frozen–thawed than in the fresh BT group. Prenatal prediction of SGA was better than prediction of LGA in pregnancies from fresh BT (concordance of 58.3% *vs* 12.5%), while prenatal prediction of LGA was better than prediction of SGA in pregnancies from frozen–thawed BT (concordance of 81.1% *vs* 0%).

Logistic regression analysis of prenatal and postnatal SGA and LGA rates

Table 3 shows the smoothed rates of EFW according to predefined centile categories (< 3rd, 3rd–10th, 10th–90th, 90th–97th and > 97th centiles) at three GA windows from 19 to 41 weeks' gestation. The output of the multinomial logistic regression analysis, using as a reference appropriate-for-gestational-age fetuses (EFW between the 10th and 90th centiles), was tested for between-group differences at each GA window. In pregnancies conceived after frozen–thawed BT, in comparison to those after fresh BT, the rate of EFW < 3rd centile was significantly lower at any stage of pregnancy. Overall, the frozen–thawed BT group presented rather stable, lower proportions of abnormally small fetuses, both in relation to the reference group and, to a greater extent, when compared to the fresh BT group. The fresh BT group showed a progressively increased rate of EFW < 3rd centile from the expected with advancing GA (Figure 2a,b). At all GA windows, the rates of EFW > 97th centile and 90th–97th centile were (in all but one case) about double in the frozen–thawed BT group as compared to the fresh BT group, with a progressive decrease from 19 to 41 weeks (Figure 2c,d).

Table S2 shows the smoothed rates of BW according to predefined centile categories (< 3rd, 3rd–10th, 10th–90th, 90th–97th and > 97th centiles) at 28–35 and 36–41 weeks of gestation. Table S3 shows the conversion of the prenatal centiles of EFW into neonatal centiles of BW according to the FMF fetal and neonatal model[15].

Table 2 Percentage concordance between estimated fetal weight (EFW) at the last ultrasound scan, in cases in which this was performed at 28–41 weeks ($n = 293$), and birth weight (BW), in pregnancies conceived after fresh or frozen–thawed blastocyst transfer (BT), and difference in concordance between the two groups

	BW		
EFW	*< 10th centile*	*10th–90th centile*	*> 90th centile*
Fresh BT			
< 10th centile ($n = 12$)	58.3*	41.7	0
10th–90th centile ($n = 95$)	12.6	85.3*	2.1
> 90th centile ($n = 32$)	15.6	71.9	12.5*
Frozen–thawed BT			
< 10th centile ($n = 6$)	0*	83.3	16.7
10th–90th centile ($n = 111$)	0	91.9*	8.1
> 90th centile ($n = 37$)	0	18.9	81.1*
Difference in concordance			
< 10th centile	58.3*†	−41.6†	−16.7†
10th–90th centile	12.6†	−6.6*‡	−6.0‡
> 90th centile	15.6†	53.0†	−68.6*†

Values are percent. Prenatal cases were used as denominator to calculate percentages. In all cases, prenatal and postnatal evaluations of each fetus were available. *Rate of concordance between prenatal and postnatal weight categories. The overall concordance between EFW and BW was greater in frozen–thawed *vs* fresh BT groups (85.7% (132/154) *vs* 66.2% (92/139); $P < 0.001$). Difference in percentage concordance between fresh and frozen–thawed BT groups for each weight category was assessed using χ^2 test: †$P < 0.001$; ‡$P < 0.05$.

DISCUSSION

Summary of results

First, at 19–32 weeks' gestation for fresh BT and at 19–35 weeks for frozen–thawed BT, IVF/ICSI conceptions presented greater mean EFW *Z*-scores as compared to those expected for natural conceptions, with the EFW *Z*-scores being higher in the frozen–thawed compared with the fresh BT group. After 32 weeks for fresh BT and 35 weeks for frozen–thawed BT, the mean EFW *Z*-scores became negative. The extent of differences in mean EFW *Z*-scores between the frozen–thawed and fresh BT groups remained constant from 19 weeks to birth.

Second, pregnancies conceived after frozen–thawed BT, compared to those after fresh BT, presented a lower rate of SGA fetuses/neonates and a higher rate of LGA fetuses/neonates. At midgestation, both groups presented a higher proportion of LGA fetuses than that expected

Table 3 Output of multinomial logistic regression analysis showing smoothed rates of estimated fetal weight (EFW) centiles at three gestational-age windows in pregnancies conceived after fresh or frozen–thawed blastocyst transfer (BT)

	EFW									
	< 3rd centile		*3rd–10th centile*		*10th–90th centile**		*90th–97th centile*		*> 97th centile*	
Gestational age	*Frozen BT*	*Fresh BT*	*Frozen BT*	*Fresh BT*	*Frozen BT*	*Fresh BT*	*Frozen BT*	*Fresh BT*	*Frozen BT*	*Fresh BT*
19–27 weeks	0.92†	2.53†	1.22	2.30	59.79	70.46	19.38‡	10.91‡	18.69†	13.80†
28–35 weeks	1.83†	8.83†	5.01	4.90	67.69	73.91	12.75‡	5.81‡	12.72†	6.54†
36–41 weeks	3.10†	24.66†	17.44†	8.37†	65.00	62.01	7.12‡	2.48‡	7.34†	2.48†

EFW was based on ultrasound biometry and Hadlock's equation[17]. *Reference group in each gestational-age category was that with EFW in the normal range (10th–90th centile). Differences between frozen–thawed and fresh BT groups were assessed using χ^2 test: †$P < 0.001$; ‡$P < 0.05$.

for natural conceptions (higher in the frozen–thawed than in the fresh BT group) with decreasing rates of LGA with advancing gestation and approaching normal values at term for BW. On the other hand, the rate of SGA at midgestation was similar to that expected for natural conceptions and increased with advancing gestation (more so in the fresh than in the frozen–thawed BT group).

Third, in both BT groups, the overall proportion of normal growth (10^{th}–90^{th} centiles) was lower prenatally than that expected for natural conceptions; however, it increased with advancing gestation approaching normal values for term neonates. Fourth, the overall prenatal prediction of BW category was good for SGA and poor for LGA in the fresh BT group and *vice versa* in the frozen–thawed BT group. Finally, the mean BW *Z*-scores were negative in preterm neonates (due to the effect of placental dysfunction occurring in spontaneous or iatrogenic preterm delivery) and increased with advancing GA, becoming positive at term in the frozen–thawed and nearly positive in the fresh BT group.

Interpretation of findings

The coronavirus disease 2019 (COVID-19) pandemic favored the use of cryopreservation in ART, representing both an opportunity to postpone pregnancy (avoiding higher risks of COVID-19 during gestation[21]), while concomitantly counteracting detrimental aging effects[22]. This study shows that the higher BW observed in IVF/ICSI pregnancies after frozen–thawed BT in comparison to fresh BT may be initiated and explained by increased EFW already evident in the second trimester and persisting in the third trimester up to birth[23,24].

Recent evidence from our group showed reduced uterine artery pulsatility index at 7–37 weeks' gestation and a greater crown–rump length measurement at

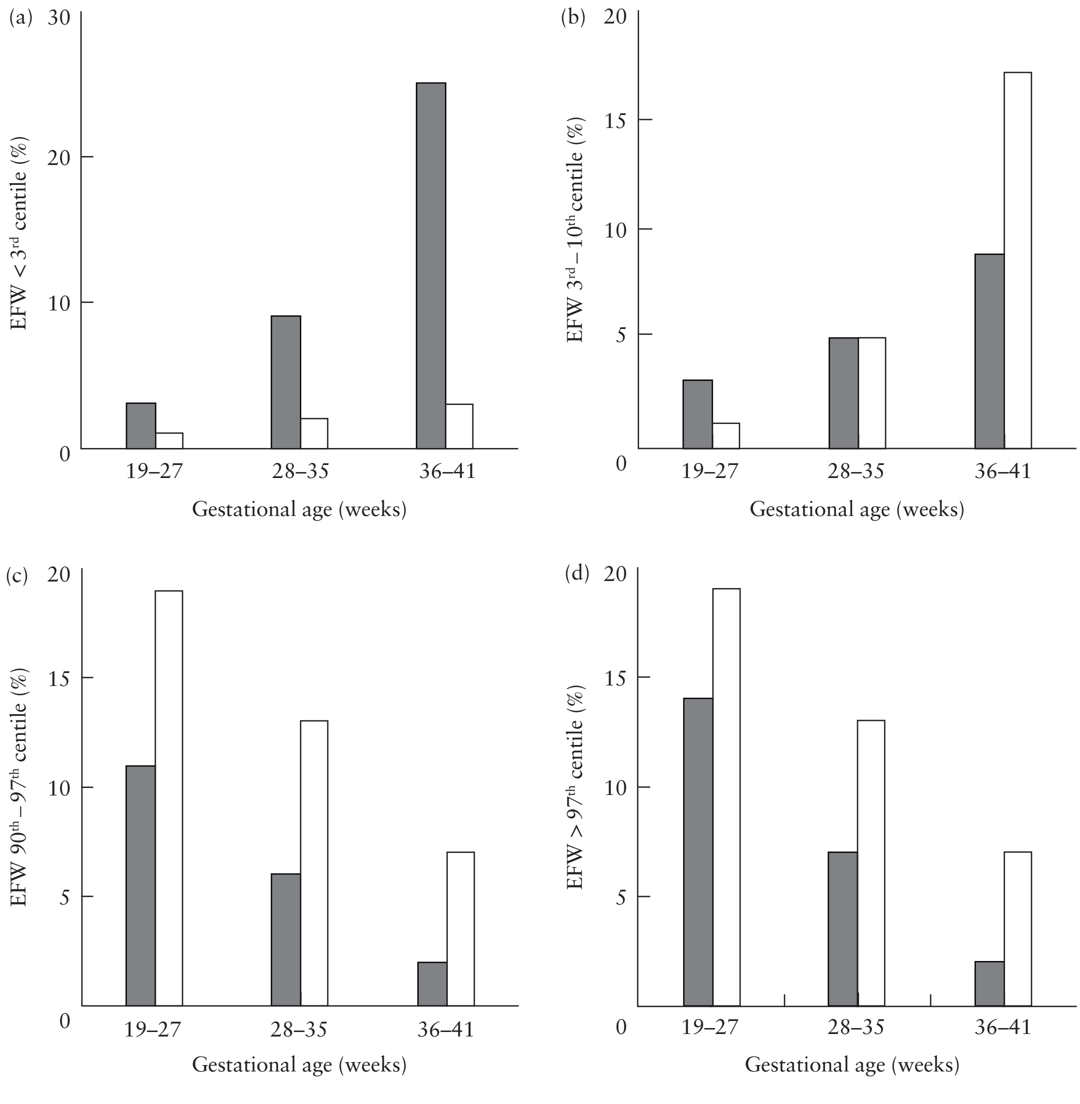

Figure 2 Proportions of estimated fetal weight (EFW) < 3^{rd} centile (a), 3^{rd}–10^{th} centile (b), 90^{th}–97^{th} centile (c) and > 97^{th} centile (d) in pregnancies conceived following fresh (■) or frozen–thawed (□) blastocyst transfer at 19–27 weeks, 28–35 weeks and 36–41 weeks of gestation.

7–14 weeks in frozen–thawed as compared to fresh BT pregnancies[13,14]. Greater embryo/fetal growth and greater placental perfusion in the first trimester are consistent with events favoring early fetal and placental development in frozen–thawed BT as compared to fresh BT. BW differences have been shown before to be related to variations in growth trajectories emerging as early as the first trimester and continuing with greater EFW in the midtrimester[25]. Previous studies also showed the impact of both maternal cardiovascular profile and embryo quality on embryo/fetal growth, which we believe would be promising research areas[26,27].

The choice of using the Hadlock formula[17] for calculation of EFW in our model was supported by the results of a previous study[28], comparing 70 models of EFW extracted from 45 publications, in which it was deemed the most accurate model with the lowest Euclidean distance and highest proportion of absolute mean error $\leq 10\%$.

In agreement with our findings, recent studies on first- and second–third-trimester fetal growth in IVF/ICSI conceptions showed greater fetal growth following thawed procedures, with higher rate of LGA and lower rate of SGA[12,29–31]. Some authors have attributed the differences in embryonic growth trajectories of spontaneously conceived compared to IVF/ICSI pregnancies to less precise pregnancy dating and differences in endometrial receptivity[32]. However, on one hand these hypotheses were not conclusive and on the other hand previous studies on this topic did not resolve the issue of a holistic approach for assessing prenatal and postnatal growth, examining prenatal and neonatal data as a whole, as it was done in this research. This is important in order to present an overall complete interpretation of prenatal growth, in particular given the higher rates of prematurity in IVF/ICSI conceptions with potential effects on estimates of fetal growth[33–35].

The discussion on the rates of abnormal fetal/neonatal growth in ART is essential, given the increased risk of SGA described in IVF/ICSI conceptions and its association with stillbirth[36–38]. Finally, the association between BW and long-term outcomes strengthens this concept, including the recently described positive association with intelligence quotient in children born at term[39].

Strengths and limitations

A limitation of this study is the fact that it was not possible to consider additional confounders, such as diet, gestational weight gain and socioeconomic status, in our analyses. In addition, different SD described for EFW and BW in the Nicolaides *et al.*[15] paper may be a source of bias for comparison of standardized fetal or neonatal weights. Strengths of this study include its longitudinal design involving repeat prenatal and postnatal measurements, showing the novel finding of a variation in standardized measurements across GA, which opens the door to future research on this topic. Moreover, the unified modeling allowed robust statistical analysis and reliable depiction of growth trajectories from the prenatal to the neonatal period.

Implications

The methodology used in this study could be reproducible in other fetal/maternal conditions, as it minimizes diagnostic errors arising from use of different heterogeneous charts and limits variance due to inherent biological differences in fetal growth in the study groups. Moreover, clarifying the timing and extent of growth deviation from normal in frozen–thawed *vs* fresh BT pregnancies may provide insights to help explain their etiology. This knowledge is the basis for defining timing of prenatal assessment, which should be tailored according to risk factors and individual characteristics of each patient, including conception method and trend of growth observed across pregnancy. Theoretically, the smaller the fetus the earlier the third-trimester scan should be performed, and *vice versa*. Based on our findings, as a general rule, pregnancies conceived after fresh BT should undergo ultrasound at 26–28 weeks of gestation, whereas the assessment should be postponed to 34–37 weeks for pregnancies from frozen–thawed BT .

Conclusions and generalizability

The option of frozen–thawed BT *vs* fresh BT should be considered for each patient undergoing IVF/ICSI in relation to the specific risks and benefits with several possible variants potentially affecting the outcome[40–43]. Interestingly, unlike autologous cycles, fresh embryo transfer in oocyte donations was associated with better birth outcome when compared with transfer of frozen embryos[44]. This requires investigation in light of the lower uterine artery pulsatility index shown in oocyte donations[45].

The observed growth differences between babies from thawed and those from fresh BT are associated with mechanisms that occur in early pregnancy and persist in the second and third trimesters. Improved embryo/fetal growth following frozen–thawed BT *vs* fresh BT deserves further investigation and is likely due to multifactorial mechanisms related to patient and procedure characteristics, placental development, hormonal status, epigenetics, uterine anatomy or perfusion and cardiovascular function. Prediction models of SGA and LGA should take into account the mode of conception and use of blastocyst cryopreservation in order to improve their performances.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Appendix S1 Regression equations calculated from Nicolaides *et al.*[15]

Figure S1 STROBE flowchart of patient recruitment.

Figure S2 Birth-weight (BW) *Z*-scores, according to gestational age, in pregnancies conceived after fresh (------) or frozen–thawed (——) blastocyst transfer.

Table S1 General linear model outputs of estimated fetal weight (EFW) and birth-weight (BW) *Z*-scores in fresh *vs* frozen–thawed blastocyst transfers

Table S2 Output of multinomial logistic regression analysis showing smoothed rates of birth-weight (BW) measurements at two gestational-age windows in pregnancies conceived after fresh or frozen–thawed blastocyst transfer (BT), according to BW $< 3^{rd}$, $3^{rd}–10^{th}$, $10^{th}–90^{th}$, $90^{th}–97^{th}$ or $> 97^{th}$ centiles

Table S3 Conversion of prenatal centiles of estimated fetal weight (EFW) into neonatal centiles of birth weight (BW) according to the Fetal Medicine Foundation fetal and neonatal population weight charts[15]