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Longitudinal cohort study of uterine artery Doppler in singleton pregnancies obtained by IVF/ICSI with fresh or frozen blastocyst transfers in relation to pregnancy outcomes

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What are the novel findings of this work?

IVF/ICSI pregnancies with frozen blastocyst transfer as compared to fresh blastocyst transfer present lower uterine pulsatility index from 7 to 37 weeks and greater fetal growth.

What are the clinical implications of this work?

1. IVF/ICSI pregnancies with FBT as compared to fresh present heterogeneous prenatal behaviour probably due to inherent group differences. 2. Pregnancies after fresh ET require increased fetal surveillance due to higher UtA-PI and risk of SGA. 3. Placental perfusion and maternal cardiovascular function need further investigation in IVF/ICSI pregnancies.

ABSTRACT

Background. Pregnancies obtained by frozen blastocyst transfer (FBT) present higher gestational age and birthweight as compared to those derived by fresh blastocyst transfer (fresh). The aim of this study was to evaluate uterine artery pulsatility index (UtA-PI) during pregnancies conceived by in vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) techniques using either fresh or cryopreserved blastocysts, in relation to pregnancy outcomes.

Methods. Prospective longitudinal study of singleton evolutive IVF/ICSI pregnancies performing serial ultrasound assessment at San Raffaele Hospital at 7-37 gestational weeks. The study groups were composed of women undergoing fresh or frozen blastocyst transfer (FBT). Miscarriages, abnormalities, twins and egg donations were excluded. Pregnant women underwent ultrasound assessments at 7-10, 11-14, 18-24, 25-32 and 33-37 weeks. Mean uterine artery PI (UtA-PI) was measured with Doppler study at each time point according to FMF criteria. Pregnancy outcomes were recorded. UtA-PI values were made Gaussian after Log10 transformation. Analysis of repeated measures with a multilevel mixed linear model (LMM) (fixed effect and random effect) was performed. The possible effect of other covariates on UtA-PI Doppler values including BMI, SGA and PE was also evaluated.

Results. 625 observations in 367 IVF/ICSI cycles were collected and analyzed (median 2.5; range 2-4; fresh = 164 ; FBT = 203). The FBT group resulted in an average 14% lower UtA-PI when compared with the fresh group. In pregnancies with small-for-gestational-age (SGA) fetuses, UtA-PI resulted 18% higher irrespectively of the group. The fresh group showed significantly reduced birthweight centile (Mean (95%CI): fresh 43.4 (39.1-46.8); FBT 50.0 (46.5-53.7); p=0.007) and an increased rate of SGA (*p-value*=0.008), as compared to FBT. No significant differences were found for gestational age at birth, preterm-birth, preeclampsia, gestational diabetes mellitus and fetal macrosomia.

Conclusions. UtA-PI and proportion of SGA are lower in pregnancies after frozen as compared to fresh blastocyst transfer.

INTRODUCTION

Background and rationale

Transfer of cryopreserved blastocysts (FBT) is now very common in Europe involving more than 50% of in vitro fertilization (IVF) cycles in several countries.[1] FBT presents clinical benefits, of which primarily the significant reduction of ovarian hyperstimulation syndrome (OHSS).[2]

Two meta-analyses including 47 studies described an increased risk of small for gestational age (SGA) babies and fetal growth restriction (FGR) cases by 40 to 65% in IVF/ICSI pregnancies from fresh cycles.[3,4] On the other hand, a meta-analysis of 11 studies and two large cohort studies concluded for a reduced risk of SGA and FGR by about 45% with an associated increased risk of large for gestational age (LGA) by 30 to 90% in IVF/ICSI pregnancies after FBT as compared to fresh.[5-7] No long-term weight and height differences were found above 5 years of age in a large meta-analysis of 20 studies investigating childhood growth of singletons conceived after IVF/ICSI.[8] The basis for a reduced fetal growth in IVF/ICSI pregnancies from fresh cycles and greater fetal growth in FBT remains to be determined; however, it is clear that fetal and placental development in these different settings needs further investigation.

Extensive research established the use of uterine arteries Doppler throughout all trimesters to assess placental circulation in the prediction of SGA[9-18]. Few studies on spontaneous pregnancies evaluated uterine artery pulsatility index (UtA-PI) during early first trimester[19-23], few others evaluated this measurement at 11-14 weeks or later in assisted reproductive technologies (ART) pregnancies as compared to spontaneous conceptions. [24-27]

Objective

The aim of this study was to evaluate and to compare UtA pulsatility index from the first to the third trimester in pregnancies obtained with the transfer of fresh or frozen-thawed blastocysts.

METHODS

Study design

Prospective monocentric cohort study of singleton pregnancies achieved after IVF/ICSI procedures with transfer of fresh or frozen-thawed blastocysts. The study was conducted and reported according to the STROBE guidelines.[28]

Setting

Recruitment of the study groups was carried out at Reproductive Science Unit of San Raffaele Hospital (Milan, Italy) from January 2016 to December 2018. Follow-up and data collection were carried out at San Raffaele Hospital Maternal and Fetal Medicine Unit up to April 2019. Data were entered into a dedicated database (ViewPoint 6, GE Healthcare GmbH, Germany) and retrieved at the end of the study for analysis. Data of pregnant patients undergoing IVF/ICSI with blastocyst cryopreservation by vitrification or fresh ET were collected.

Controlled ovarian stimulation (COS) was based on antagonist fixed protocol. R-FSH was started on day 3 of spontaneous menstrual cycle and trigger with hCG was scheduled when a minimum of one follicle of diameter > 16 mm was detected at US scan. In case of risk of OHSS, trigger was performed with A-GnRH and elective freeze-all strategy was performed. In our center, the protocol for FBT is based in hormone replacement therapy for all women.

Study Participants

Eligibility criteria were pregnancies following autologous fresh or frozen/thawed embryo transfer at blastocyst stage. Other assisted reproductive techniques such as egg donations, intrauterine insemination, cleavage-stage embryo transfers, gamete or zygote intrafallopian transfers were not considered eligible in this study. Patients with twin pregnancies, aneuploidies, significant pregestational diseases, major fetal defects and spontaneous abortions were all excluded. (Table 1)

Variables

The exposure in analysis is blastocyst cryopreservation with vitrification as compared to fresh embryo transfer of IVF/ICSI conceptions. Gestational age (GA) was calculated setting conception at day of oocyte retrieval for fresh blastocyst group and establishing a pseudo-last menstrual period 19 days before embryo-transfer for FBT group. Maternal age, body mass index (BMI), ethnicity, cigarette smoking during pregnancy, obstetric history including parity (parous/nulliparous if no previous pregnancies ≥ 24weeks' gestation) were recorded. Data about IVF/ICSI procedures were collected: number of oocytes retrieved, progesterone and estrogen levels, use of cryopreservation, ICSI and preimplantation genetic testing (PGT) techniques. Antiplatelet dose aspirin and other medication assumption were recorded.

The primary outcome of this study was to measure the mean pulsatility index of uterine arteries (UtA-PI).

The left and right UtPI were measured and average of the two was used for statistical analysis. UtPI was calculated as follows: $UtPI = \frac{\text{UtPSV}-\text{UtPDV}}{\text{Vm}}$; UtPSV= uterine artery peak systolic velocity; UtPDV= uterine peak diastolic velocity; Vm= time average velocity. Secondary outcomes were gestational age at birth, birthweight, fetal and maternal complications with particular regard to SGA rate, preeclampsia (PE) and large for gestational age rate.

Data sources and Doppler measurements

Repeated Doppler ultrasound assessments were performed at 7-10; 11-14; 18-23; 24-30 and 31-37 weeks. Pregnancy was confirmed by transvaginal ultrasound (TVUS) at 6-10 weeks with visualization and measurement of gestational sac, yolk sac, crown-rump length (CRL) and evaluation of embryonic heart activity. All ultrasound scans and measurements, including Doppler assessment, were performed by operators with extensive experience in obstetric ultrasound and certification of competence granted by The Fetal Medicine Foundation (FMF) in order to minimize inter-observer variability. Physicians performing US and Doppler measurements US were blinded to the use of embryo cryopreservation and were not those performing IVF procedures. Blinding was achieved concealing history of the patients to the sonographers performing Doppler and delegating clinical management to different clinicians not involved in the trial. In pregnancies at less than 7 weeks of gestation, CRL was measured as the greatest length of the embryo and an appointment was rescheduled at 7-10 weeks. Above 7 weeks of gestation, CRL was measured in a sagittal section of the embryo, avoiding inclusion of the yolk sac. Transvaginal Doppler assessment at 7-10 weeks was obtained with identification of cervical canal and internal os in the midsagittal section of the uterus, gentle tilting of the transducer from side to side with color flow mapping used to identify each UtA along the side of the cervix and uterus at the level of the internal ostium. Pulsed wave Doppler was applied for less than 60 seconds necessary to assess both arteries, with no direct embryonic insonation. A detailed transabdominal sonographic exam, including CRL and UtA Doppler measurements was carried out at 11 –14 gestational weeks. UtA Doppler study at this stage was carried out following the FMF and ISUOG guidelines, obtaining a sagittal section of the uterus, identifying the cervical canal and internal os, tilting gently the transducer from side to side and using color-flow mapping to identify each uterine artery along the side of the cervix and the uterus, at the level of the internal os. The sampling gate was set at 2 mm to cover the whole vessel, insonation angle was kept smaller than 30◦ and peak systolic velocity greater than 60 cm/s to ensure that the UtA, rather than the arcuate artery, was being examined. When three similar waveforms were obtained consecutively, the PI was measured by manual tracing of the waveform trace and the mean observed PI of the left and right arteries was calculated. [17, 28]

All examinations were performed using adequate ultrasound equipment (Voluson E8 or E10; General Electric, USA) equipped with multi-frequency endocavitary or convex transducers. Ultrasound mechanical index and soft-tissue thermal index were set at 1.0 and 0.5 throughout the examination process.

Bias

We evaluated possible sources of information and selection biases. In particular, we have taken into account Aspirin treatment, medication use, maternal age and ovarian reserve.

Sample size

Power analysis for a comparison of 2 independent means was performed using Power Analysis Sample Size (PASS) software (Kaysville, UT, USA). Power analysis was conducted before the enrollment started. We estimated that, a total of 165 cases per group (given the sample allocation ratio =1:1) and with an UtA-PI coefficient of variation of SD/mean= <= 0.35 (as calculated by published data), would be needed to detect an absolute decrease in the primary outcome measure (PI in FBT) of at least 10% between groups, with a type I error of 5% and a power of 80% **.**

Statistical analysis

A regression model was applied to establish the best curve fitting estimation for UtA-PI against gestational age. UtA-PI values were made Gaussian after Log10 transformation. Analysis of repeated measures with a multilevel mixed linear model (LMM) with fixed effects and random effects was performed. The mixed modeling procedure simultaneously estimates the fixed effect parameters for the observed data (i.e., group effects) and the variance of the random effects (i.e., between subject effects). Such model is ideal for datasets with repeated measurements in which some missing values may occur. The fixed effect component included up to quadratic terms of gestational age (GA) expressed in days and group (fresh vs. FBT). The random effect component includes the intercept as well as linear effects of GA (slope). Repeat measurements at different weeks of gestation in the same patient constituted level 1 and each individual of the series constituted level 2. Covariance parameters and -2 Log likelihood ratio reduction were used to evaluate the performance of the statistical analysis at each step (fixed effect alone, random intercept, random slope). The possible effect of other covariates or factors on UtA-PI Doppler values including BMI, SGA and PE was also evaluated. In order to evaluate the intercepts variance, prior to performing LMM, continuous variables were centered by subtracting the mean from each measured value (grand-mean centering). Multiples of the median (MoMs) for UtA PI were also produced to summarize the effect (Table 3). Z-scores and centiles birthweight were calculated from reference equation of published dataset on similar population. [29] All tests were two sided, and a $p<0.05$ was considered significant. SPSS software was used for the statistical analyses (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA).

Ethical approval

Local Institutional Review Board approval (protocol OSTE-PMA) was obtained prior to the start of this prospective study. All women provided informed consent signing a written form for participation in the study for their clinical data and anonymized records to be used for research purposes.

RESULTS

Study Participants

Eight-hundred and twenty-seven IVF/ICSI pregnancies following autologous fresh or frozen/thawed embryo transfer at blastocyst stage were assessed for eligibility in the study period and after exclusion of 460 cases, 367 cases were available for analysis (median observation per patient 2.5; range 2-5; number of patients after fresh IVF/ICSI = 164; number of patients after FBT = 203; Table 1).

Descriptive data

Table 2 shows patients and exposure characteristics in the two study groups. Each patient recruited in the study was followed-up until delivery with collection of all outcome data.

Outcome data and main results

Outcomes of the study groups are summarized in Table 3. Supplemental table 1 shows the results of LMM in Doppler observations (n=625). In both study groups, there was a quadratic decrease in log10 UtA-PI with GA. However, at paired days of pregnancies, lower UtA-PI values were observed for the FET group (Supplemental table 1; Figure 1). The FBT group resulted in an average 14% lower UtA-PI when compared with the fresh group. (Supplemental table 1; Figure 1). In pregnancies where there was a SGA diagnosis, UtA-PI resulted 18.5% higher irrespectively of the study group (Supplemental table 1).

In the random part of the model, the level 1 (repeat measurements at different weeks of gestation) was significant allowing us to detect a Doppler PI variation on the same subject over the time. The level 2 (random intercept model) was also significant, allowing us to differentiate the variation in outcome explained by individual-level factor. Analysis of the partitioned variances of the random effects in Table 3 showed that in the LMM, 35% of the total between-subject variance was due to total between-subject differences in the intercept. The other component of the level 2 i.e the slope, did not contribute to explain the variance. Random-intercept model was significant, allowing us to differentiate the variation in outcome explained by individual-level factor. Analysis of the partitioned variances of the random effects in Supplemental table 1 showed that in the LMM, 35% of the total between-subject variance was due to total between-subject differences in the intercept.

The slope did not contribute to explain the variance. The fresh group resulted in a wider dispersion of the random intercepts values around the mean probably related to a more inhomogeneous population than that observed in the FBT group (data not shown).

The outcome of the fresh group showed significantly reduced birthweight centile (Mean (95%CI): fresh 43.4 (39.1 to 46.8); FBT 50.1 (46.5 to 53.7); *p-value*=0.023) and an increased rate of SGA fetuses (p=0.006) when compared to FBT. No significant differences were found for gestational age at birth, preterm-birth, PE, gestational diabetes mellitus and fetal macrosomia.

DISCUSSION

Key results

IVF/ICSI pregnancies from FBT demonstrated significantly greater uterine perfusion and fetal growth as compared to those from fresh embryo transfer. In particular, UtA-PI is 14% lower and birthweight centile is 7% greater in IVF/ICSI pregnancies obtained with FBT as compared to those from fresh transfer. SGA babies were 3 folds more common among the fresh group as compared to FBT. No differences were found concerning secondary outcomes. Mixed modeling detected a random inter-subjects effects and the fresh group was found to be composed of a more inhomogeneous population.

Interpretation

IVF/ICSI pregnancies are associated with increased risk of maternal and fetal complications including anomalies, prematurity, abnormal placentation, hypertensive disease, impaired fetal growth with a globally worse perinatal outcome.[3-7, 31-32] Etiology of these abnormalities is yet to be elucidated and is probably multifactorial, including intrinsic differences in the patient's characteristics as well as procedure-related factors.[5,33-38] Supra-physiologic estradiol levels typical of IVF/ICSI procedures with fresh embryo transfers were described as predictors of SGA.[35-38] Additionally, endometrial preparation and absence of corpus luteum (CL) in frozen embryo transfer (FET) might predispose to adverse obstetric outcomes (hypertensive disorders, PE, postpartum hemorrhage, accreta, post-term birth and macrosomia).[39-44] Critical role of CL in favoring the physiological transformation of the maternal cardiovascular system in early gestation was shown, demonstrating increased PE risk and reduced aortic compliance with FET and absent CL.[45-46] Since UtA-PI is also related to maternal cardiovascular adaptation, apparent contradiction between our findings of reduced UtA-PI in a group known for higher risk of PE may be explained by increased maternal age and IVF technique which may be proxy markers for worse pre-pregnancy UtA-PI and cardiovascular function.[47-48] Additionally, longer pregnancy duration of FET [5,6] may be on the basis of their higher prevalence of PE, according to competing risk modelling of PE with delivery.[49]

Conversely, in FET as compared to fresh transfer there is lower risk of SGA, [40,41,50,51] and perinatal mortality [51]. However, all cited studies have a very high risk of selection bias, since the characteristics of the women undergoing fresh transfer are different from those undergoing FET. One study with low risk of bias compared sibling oocytes from donors and no significant difference was observed between fresh transfer or FET; indeed, the observed proportion of SGA in FET was even higher than in fresh transfer (12.5% vs. 9.4%). Unfortunately, this study did not evaluate the effect of ovarian stimulation on the reproductive outcomes.[52]

Increased pulsatility index of UtA Doppler is associated with abnormalities in trophoblast invasion and is predictive of PE, hypertensive disorders and fetal growth restriction [9-15;53-56]. In addition, previous studies found a significant inverse correlation between UtA-PI and blood flow volume in the uterine arteries, strengthening the concept of reduced placental blood perfusion in placental dysfunction. [57,58]

UtA-PI declines progressively throughout pregnancy from 11 weeks to term as an expression of reduced vascular resistances in the placenta.[59] Few small studies evaluating UtA Doppler before 11 weeks showed progressive reduction of resistances from very early stages of pregnancy. [19-23] A previous study assessed the added benefit of longitudinal evaluation of UtA Doppler, in relation to pregnancy outcome taking into account both UtA-PI and change with gestational age.[60]

Previous studies failed to find differences of UtA-PI values in IVF/ICSI pregnancies as compared to spontaneous conceptions but they considered the heterogeneous IVF group as a single category.[24,26] Studies including ART with controlled ovarian hyperstimulation (COS), found a trend towards an increase of UtA-PI, in particular in cases developing PE.[10,26] These findings are consistent with the observation of higher frequency of SGA and PTB likely due to abnormal placentation in IVF/ICSI pregnancies and fresh transfer.[61] First trimester UtA-PI and 3D placental volumes in natural conceptions and IVF from fresh or cryopreserved embryos failed to show differences of UtA-PI MoMs but found smaller placental volumes in IVF, particularly from fresh cycles. [27] Lower UtA-PI at 11-14 weeks has been previously described in ICSI cycles with egg-donation as compared to spontaneous conceptions and, interestingly, that group of egg donations included all cases of FET

[62].

A meta-analysis showed that children born after FET had a higher risk of being LGA, lower risk of being SGA and better neonatal outcomes such as higher birthweight and lower risk of perinatal mortality compared with children born after fresh cycles.[63] This was suggested to be the result of the more natural intrauterine environment associated with frozen cycle allowing physiological early placentation, whereas COS in fresh cycles may alter endometrial angiogenesis and implantation.[35] Conversely, FBT was shown as a cause of mildly increased fetal nuchal translucency and free-βhCG concentrations in euploid fetuses with normal obstetric outcome.[64] FBT is usually performed when there are multiple good quality embryos available more frequently occurring in younger patients with a better ovarian reserve. Thus, the lower uterine artery PI in FBT may be a consequence rather than the cause of higher birthweights. Based on the present findings, UtA-PI may be proposed as an additional tool to individualize follow-up scheduling when assessing fetal growth in IVF/ICSI pregnancies. Benefits of IVF/ICSI cycles with freeze-all strategy needs to be weighed against their risk of PE and LGA. [39-44]

Limitations and strengths

Limitations of this study are related to: 1. aspirin use in 10% of patients in both groups; 2. difficulty in adjusting for all potential confounders with potential risk of bias (e.g. time of cryopreservation, morbidities, maternal age, ovarian reserve); 3. inadequacy of sample size to address all secondary outcomes; 4. the observed difference between groups in UtA-PI is relatively small when compared to differences due to intra and inter-observer variabilities for UtA-PI measurements, which were respectively estimated 20-30% and 30-40%, within 95% CI bounds. [65] The observed variability in this study was similar: the mean difference of the measurements of each operator with the expected value of UtA-PI of 1.00 MoM showed inter-observer variability of 35-40% within the 95% CI. However, for some operator the mean difference was below 5% (supplemental table 2). Again, no difference among and between the UtA-PI distributions quoted for each operator was detected (supplemental table 3).

Strengths of this study are related to: 1. the longitudinal design with early pregnancy uterine Doppler studies for which there are no available data; 2. robust statistics with LMM detecting a between-subject effect 3. monocentric design (homogeneous IVF and ultrasound protocols); 4. methodological quality of ultrasound and Doppler measurements (certified operators); 5. homogeneous subsets of ART procedures.; 6. vast majority of nulliparous (studies failing to show maternal age differences between FBT and fresh included a large proportion of parous women, particularly for FET).[6,7]

Generalisability

Our results suggest a greater utero-placental perfusion from very early gestation up to early term associated with a better fetal growth in IVF/ICSI pregnancies achieved after FBT. Further studies are required to confirm and explore the extent and quality of differences in embryo-fetal growth, UtA Doppler PI and placental and cardiovascular function in pregnancies among different ART subgroups and exploring the impact of prenatal genetic testing and specific details of both patients and of IVF/ICSI procedures.

Other information

Declaration of interests

The authors have no conflict of interests to declare.

Congress presentations of the data

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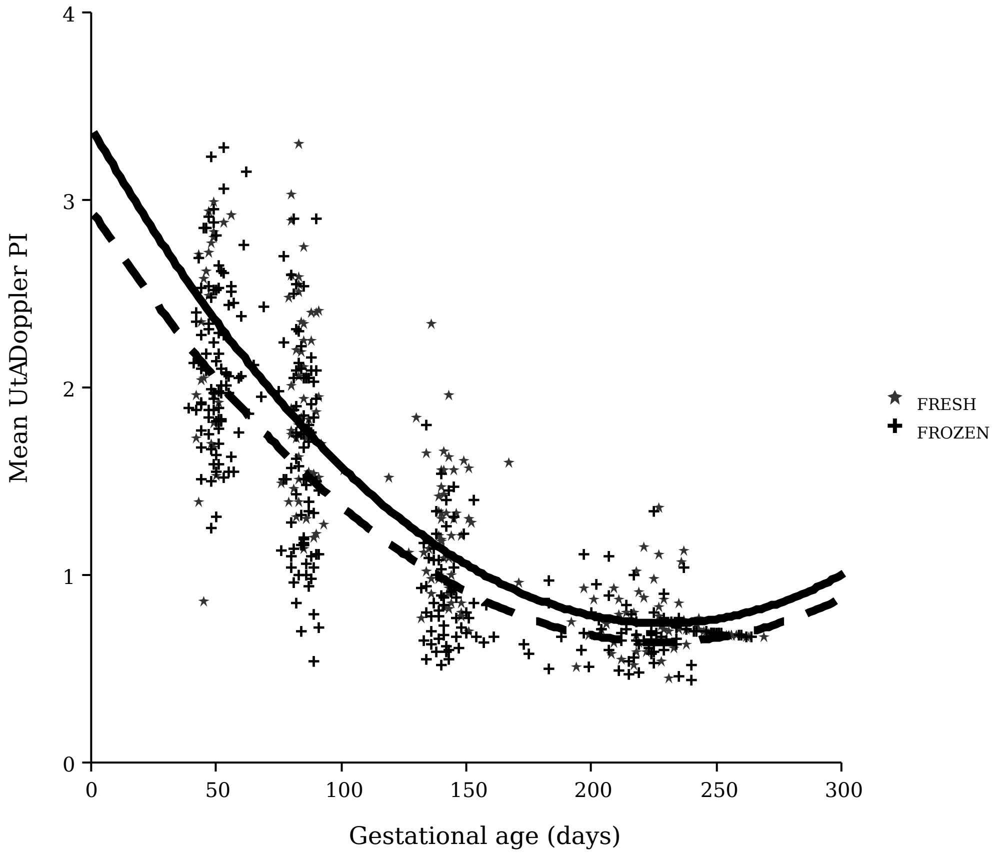
FIGURE LEGEND

Figure 1: Uterine artery Doppler mean pulsatility index (PI) in pregnancies obtained from fresh (solid line) and frozen (dotted line) embryos with a normal outcome.



Eligible

IVF/ICSI pregnancies following autologous fresh or frozen/thawed embryo transfer at blastocyst stage assessed for eligibility in the study period (January 2016 to December 2018; n=827)

Excluded (not meeting inclusion criteria; n=261):

- Significant pregestastional maternal diseases (n=116)
- Spontaneous abortions < 7 weeks or ectopic pregnancy (n=145)
- Declined consent (n=0)

Enrolled

Viable intrauterine IVF/ICSI pregnancies following autologous fresh or frozen/thawed embryo transfer at blastocyst stage confirmed by TVUS at 7-10 weeks enrolled in the study (n= 566)

Excluded after enrollment (n=199):

- Spontaneous abortions >7 weeks (n=33)
- Aneuploidies and major fetal defects (n=15)
- Twin pregnancies (n=151)

Investigated

Evolutive intrauterine singleton IVF/ICSI pregnancies following autologous fresh or frozen/thawed embryo transfer analyzed (n=367)

Table 1. Flow chart of the study design.

Table 2. Patients and exposure characteristics in the two study groups. For continuous variables mean and standard deviation, for categorical variables frequency and percentage shown.

Variable	Fresh (N=164) Mean ± SD or N (%)	Frozen (N=203) Mean ± SD or N (%)	p-value
Maternal age years	35.9 ± 3.9	34.9 ± 5.1	0.033*
BMI	22.38 ± 3.68	21.86 ± 3.63	0.175
Nulliparous	153 (93.3)	187 (92.1)	0.662
Cigarette smoker	22 (13.4)	27 (13.3)	0.978
IVF	18 (11.2)	37 (18.2)	0.055
ICSI	143 (88.8)	166 (81.8)	0.159
Estrogens peak at ovocyte pick-up	1651.6 ±727.1	2799.6 ± 1540.5	<0.001*
Progesterone peak at ovocyte pick-up	0.82 ± 0.43	1.25 ± 1.34	<0.001*
AMH pre-gestational (ng/mL)	2.28 ± 3.06	3.67 ± 3.00	<0.001*
FSH pre-gestational (IU/mL)	7.50 ± 2.87	7.24 ± 2.26	0.359
Male infertility	61 (37.2)	77 (37.9)	0.891
Ovulatory dysfunction	18 (11.0)	31 (15.2)	0.239
Endometriosis	13 (7.9)	14 (6.9)	0.715
Idiophatic sterility	43 (26.2)	44 (21.7)	0.314
Mixed sterility	8 (4.9)	7 (3.4)	0.470
Low ovarian reserve	14 (8.5)	10 (4.9)	0.165
Tubal factor	10 (6.1)	16 (7.9)	0.505
Other causes	5 (3.0)	21 (10.3)	0.007*
Metaphase II ovocytes	6.38 ± 3.5	11.32 ± 4.8	<0.001*
Available Embryos at D3	2.72 ± 1.3	4.34 ± 2.1	<0.001*
Blastocysts transferred	1.68 ± 0.6*	1.24 ± 0.5	<0.001*

Table legend: BMI: body mass index; IVF: in vitro fertilization; ICSI: intra-cytoplasmic sperm injection; AMH: anti-mullerian hormone; FSH: follicle-stimulating hormone; D3: day-three of embryonic life; *: statistical significance.

Table 3. Primary and secondary outcomes of the pregnancy in the study groups. For continuous variables mean and standard deviation, for categorical variables frequency and percentage shown.

Variable	Fresh (N=164)	Frozen (N=203)	p-value
Primary outcome			
MoM UtA-PI [Mean (SD)]	1.00 (0.29)	0.86 (0.28)	<0.001*
Secondary outcomes			
SGA [N (%)]	13 (7.9)*	4 (2.0)	0.008*
LGA [N (%)]	4 (2.4)	8 (4.4)	0.301
PE [N (%)]	8 (4.8)	3 (1.5)	0.065
GDM [N (%)]	12 (7.3)	17 (8.4)	0.698
GA at delivery in days [Median (IQR)]	272 (265-269)	274 (267-281)	0.370
PTB<37 wks [N (%)]	19 (11.7)	13 (6.4)	0.117
PTB<34 wks [N (%)]	5 (3.0)	7 (3.4)	0.427
Birtweight in grams (Mean ±SD)	3051 ± 575	3262 ± 542*	<0.001*
Birtweight centile (Mean ±SD)	43.44 ± 23.3	50.00 ± 23.1*	0.007*
Birtweight Z-score (Mean ±SD)	-0.22± 0.78	0.05 ± 0.88*	0.002*

Table Legend: MoM UtA-PI: Mean left and right uterine artery pulsatility index; SGA: small for gestational age (<10° centile); LGA: large for gestational age (>90° centile); PE: preeclampsia; GDM: gestational diabetes mellitus; GA: gestational age; PTB: preterm birth; *: statistical significance.