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Obstetric and Perinatal Outcomes after Fresh versus Frozen Embryo Transfer

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Obstetric and Perinatal Outcomes after Fresh versus Frozen Embryo Transfer

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Estudante

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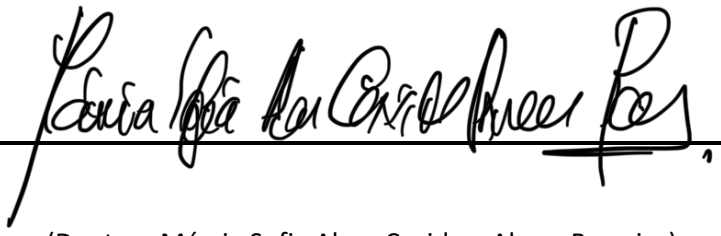

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Junho de 2026

Declaration of Scientific Integrity

I, Leonor Campelo Lopes do Rego a student in the Integrated Master's Program in Medicine at the Faculty of Medicine and Biomedical Sciences, University of Porto, hereby affirm that the present dissertation represents the result of my individual academic work and reflects my original research efforts, critical analysis, and interpretation.

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Dedicatória

Dedico este projeto à minha família, que, mesmo de forma silenciosa, me acompanhou e guiou ao longo de todas as etapas, transmitindo-me os valores que constituem o pilar de quem sou hoje. O seu exemplo foi um incentivo contínuo para ser e fazer melhor, particularmente nos momentos mais exigentes.

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Resumo

Introdução: A infertilidade afeta cerca de um em cada seis casais a nível mundial, pelo que o recurso às técnicas de procriação medicamente assistida (PMA) tem vindo a aumentar substancialmente. Embora os ciclos convencionais envolvam transferência de embriões a fresco, a estratégia de *freeze-all* e a transferência de embriões criopreservados (TEC) têm vindo a ser amplamente adotadas, suscitando debate sobre o seu impacto nos *outcomes* obstétricos e perinatais. A evidência disponível associa a TEC a um risco reduzido de síndrome de hiperestimulação ovárica (SHO), maior peso à nascença, maior proporção de recém-nascidos grandes para a idade gestacional e menor incidência de baixo peso ao nascimento. Ademais, tem sido reportado um maior risco de distúrbios hipertensivos, particularmente pré-eclâmpsia. Os achados relativos a outros *outcomes* permanecem heterogéneos.

Objetivo: Comparar os *outcomes* obstétricos e perinatais de gravidezes concebidas por PMA após transferência de embriões a fresco versus transferência de embriões criopreservados.

Métodos: Realizou-se um estudo de coorte retrospetivo no Centro de PMA do Centro Materno-Infantil do Norte (CMIN), incluindo 491 gravidezes concebidas por fertilização *in vitro* (IVF) ou injeção intracitoplasmática de espermatozoides (ICSI) entre janeiro de 2019 e dezembro de 2024. Os *outcomes* obstétricos e perinatais foram comparados entre TEC (n = 246) e transferências de embriões a fresco (n = 245). Os principais *outcomes* analisados incluíram o diagnóstico de distúrbios hipertensivos da gravidez, anomalias placentares, diabetes gestacional, colestase intra-hepática da gravidez, restrição de crescimento fetal, malformações congénitas, avaliação da idade gestacional ao nascimento, parto pré-termo, peso à nascença, incluindo a sua classificação em baixo e elevado peso, índices de Apgar e morte neonatal. Atendendo à diferença significativa na distribuição de gravidezes únicas e gemelares entre grupos, os desfechos perinatais foram analisados separadamente. Foram realizadas análises de regressão multivariável para ajustar potenciais confundidores.

Resultados: Os *outcomes* obstétricos foram comparáveis entre os dois grupos. Na análise multivariável, a TEC não foi um preditor independente de morbilidade obstétrica. O índice de massa corporal e a idade materna foram os principais determinantes das complicações hipertensivas e metabólicas, enquanto a transferência de dois embriões se associou a uma maior ocorrência de distúrbios hipertensivos (aOR = 2.377, IC 95% 1.104-5.114, p = 0.027). Nas gravidezes únicas, a TEC foi independentemente associada a maior peso ao nascer (3241 ± 542 g versus 3024 ± 541 g, B = +266.86 g, IC 95% 177.12-356.61, p < 0.001) e maior taxa de parto distócico (80.0% versus 68.1%, aOR = 1.65, IC 95% 1.029-2.653, p = 0.037). Contudo, o efeito protetor relativo ao baixo peso ao nascer foi atenuado após ajuste para o estadio embrionário, sugerindo que a maior prevalência de blastocistos nos ciclos de TEC contribui para este efeito. A idade gestacional foi significativamente inferior no grupo TEC (37.72 ± 1.85 versus 38.02 ± 1.73 semanas, p = 0.026), embora de magnitude clínica limitada, visto que os restantes *outcomes* perinatais, incluindo a proporção de parto pré-termo, índices de Apgar, restrição de crescimento fetal, malformações congénitas e morte neonatal, são comparáveis entre grupos. Nas gravidezes gemelares, a TEC foi associada a menor idade gestacional (34.00 ± 2.47 versus 34.55 ± 3.63 semanas, p = 0,024), sem diferenças significativas nos restantes *outcomes*.

Conclusões: A TEC não confere um aumento do risco obstétrico e está associada, de forma independente, a maior peso ao nascer e parto distócico nas gravidezes únicas. Estes resultados destacam a importância de individualizar a estratégia de transferência embrionária com base nas características do casal e contexto clínico, para otimização dos *outcomes* maternos e neonatais.

Palavras-chave: Procriação medicamente assistida; FIV; ICSI; Transferência de embriões frescos; Transferência de embriões criopreservados; *Outcomes* obstétricos; *Outcomes* perinatais; Peso ao nascer; Parto distócico; Gravidez gemelar.

Abstract

Background: Infertility affects around one in six couples worldwide, and the use of assisted reproductive technology (ART) continues to expand accordingly. While conventional ART cycles involve fresh embryo transfer (Fresh ET), the freeze-all strategy and frozen-thawed embryo transfer (FET) have gained widespread adoption, prompting debate regarding their comparative impact on obstetric and perinatal outcomes. Evidence consistently links FET with a decreased risk of ovarian hyperstimulation syndrome (OHSS), higher birth weight, increased rates of large for gestational age (LGA), and reduced low birth weight (LBW), while a higher risk of hypertensive disorders, particularly preeclampsia, has also been reported. Findings regarding other outcomes remain heterogeneous.

Objective: This study aims to compare obstetric and perinatal outcomes in pregnancies conceived through ART following fresh versus frozen-thawed embryo transfer.

Methods: A retrospective cohort study was performed at the Center of Assisted Medical Procreation of Centro Materno-Infantil do Norte (CMIN), including 491 pregnancies conceived through *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) between January 2019 and December 2024. Obstetric and perinatal outcomes were compared between FET (n = 246) and Fresh ET (n = 245). Main outcome measures included hypertensive disorders of pregnancy, placenta-related complications, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, fetal growth restriction, congenital anomalies, gestational age, preterm birth, birth weight including low and high birth weight, Apgar scores and neonatal death. Given the significant difference in the distribution of singleton and twin pregnancies between groups, perinatal outcomes were analyzed separately. Multivariable regression analyses were performed for selected outcomes to adjust for potential confounders.

Results: Obstetric outcomes were comparable between groups. In multivariable analysis, FET was not an independent predictor of obstetric morbidity. Body mass index and maternal age were the main determinants of hypertensive and metabolic complications, while double embryo transfer was independently associated with an increased risk of hypertensive disorders of pregnancy (aOR = 2.377, 95% CI 1.104-5.114, p = 0.027). In singleton pregnancies, FET was independently associated with higher birth weight (3241 ± 542 g versus 3024 ± 541 g, B = +266.86 g, 95% CI 177.12-356.61, p < 0.001) and a higher rate of dystocic delivery (80.0% versus 68.1%, aOR = 1.65, 95% CI 1.029-2.653, p = 0.037). The protective effect against low birth weight was weakened after adjustment for embryo stage, suggesting mediation by the higher prevalence of blastocyst-stage transfers in FET cycles. Gestational age was significantly lower in the FET group (37.72 ± 1.85 versus 38.02 ± 1.73 weeks, p = 0.026) but of limited clinical magnitude, with preterm birth, Apgar scores, fetal growth restriction, congenital anomalies, and neonatal death comparable between groups. In twin pregnancies, FET was associated with a shorter gestational age (34.00 ± 2.47 versus 34.55 ± 3.63 weeks, p = 0.024), although preterm birth rates and all other perinatal outcomes were similar.

Conclusions: FET does not confer a meaningful increase in obstetric risk compared with Fresh ET and is independently associated with higher birth weight and dystocic delivery in singleton pregnancies. These findings highlight the importance of individualizing embryo transfer strategy based on patient characteristics and clinical context to optimize both maternal and neonatal outcomes.

Keywords: Assisted reproductive technology; IVF; ICSI; Fresh embryo transfer; Frozen embryo transfer; Obstetric outcomes; Perinatal outcomes; Birth weight; Dystocic delivery; Twin pregnancy.

Abbreviations and Acronyms

aOR – Adjusted Odds Ratio

ART – Assisted Reproductive Technology

ASRM – American Society for Reproductive Medicine

BMI – Body Mass Index

CI – Confidence Intervals

CMIN – Centro Materno-Infantil do Norte

COS – Controlled Ovarian Stimulation

DET – Double Embryo Transfer

DOR – Diminished Ovarian Reserve

eSET – Elective Single Embryo Transfer

FET/TEC – Frozen-Thawed Embryo Transfer

FGR – Fetal Growth Restriction

Fresh ET – Fresh Embryo Transfer

GDM – Gestational Diabetes Mellitus

HBW – High Birth Weight

HDP – Hypertensive Disorders of Pregnancy

HELLP – Hemolysis, Elevated Liver enzymes, Low Platelet count

ICP – Intrahepatic Cholestasis of Pregnancy

ICSI – Intracytoplasmic Sperm Injection

IVF – *In Vitro* Fertilization

LBW – Low Birth Weight

LGA – Large for Gestational Age

OHSS/SHO – Ovarian Hyperstimulation Syndrome

PGDM – Pre-gestational Diabetes Mellitus

PIH – Pregnancy-Induced Hypertension

SD – Standard Deviation

SET – Single Embryo Transfer

SGA – Small for Gestational Age

TESE – Testicular Sperm Extraction

ULSSA – Unidade Local de Saúde de Santo António

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INTRODUCTION

Infertility and Assisted Reproductive Technology

Infertility is defined as a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce. [1] Primary infertility is the inability to have any pregnancy, while secondary infertility is the inability to have a pregnancy after previously successful conception. [2]

Nowadays, one in six couples experience fertility issues at least once during their reproductive lifetime and nearly 10% of couples worldwide are subfertile. [2, 3] Infertility may occur due to female, male, both or unexplained causes. However, lifestyle factors such as smoking, excessive alcohol intake and obesity, along with environmental contaminants including endocrine-disrupting chemicals and air pollution, have also been linked to reduced fertility and negative impacts in pregnancy and fetal development. [2, 4]

In the female reproductive system, infertility may be caused by a range of abnormalities affecting the ovaries, uterus, fallopian tubes or the endocrine system. In the male reproductive system, infertility is most commonly linked to impairments in semen ejaculation or production, which may result in poor semen quality, manifesting as low sperm concentration, a complete absence of sperm (azoospermia), poor motility or abnormal morphology. [2, 5] Recently, the number of couples with unexplained infertility has increased, partly attributed to the growing trend of postponing motherhood, with maternal age playing an increasingly relevant role in assisted reproductive technology (ART) outcomes.[5]

Beyond its medical implications, this condition often acts as a disruptive factor in couple and family dynamics and is recognized as a significant source of psychological stress, frequently associated with emotional distress, anxiety, depression, social stigmatization, reduced self-esteem, marital conflict, and an increased risk of divorce. [2]

ART comprises all interventions or procedures that include the *in vitro* handling of human gametes or embryos for the purpose of reproduction. This encompasses, but is not limited to, *in vitro* fertilization (IVF) and embryo transfer, intracytoplasmic sperm injection (ICSI), embryo biopsy, preimplantation genetic testing, assisted hatching, gamete intrafallopian transfer, zygote intrafallopian transfer, gamete and embryo cryopreservation, and oocyte, sperm and embryo donation, as well as gestational carrier cycles. Thus, assisted insemination using either partner or donor sperm is explicitly excluded. [1]

Over the past decades, ART has become an increasingly well-established therapeutic approach for infertility, with steadily improving success rates and more than 3 million cycles performed annually worldwide. [6] The continuous growth in its prevalence has raised concerns regarding the obstetrical and perinatal safety of pregnancies achieved through these techniques, particularly given the still limited and sometimes inconsistent scientific evidence available on this topic. [7]

Fresh versus Frozen-Thawed Embryo Transfer

Conventional IVF and ICSI cycles consist of controlled ovarian stimulation (COS), oocyte retrieval, *in vitro* fertilization, and embryo culture, followed by embryo transfer, while surplus viable embryos may be cryopreserved for transfer in subsequent cycles. Fresh and frozen-thawed embryo transfer represent two key strategies with distinct physiological and clinical implications. In fresh embryo transfer (Fresh ET), embryos are transferred within the same COS cycle, occurring in a supra-physiological hormonal environment induced by stimulation, which may have an impact on endometrial receptivity and early placentation. In contrast, frozen-thawed embryo transfer (FET) involves transfer in a subsequent cycle after cryopreservation, typically performed in a more

physiologic hormonal setting, potentially mitigating these effects and contributing to differences in pregnancy outcomes. [1, 5]

Since the first live birth after FET in 1983, major advances in cryopreservation techniques, particularly vitrification, have markedly enhanced post-thaw embryo survival. As a result, FET has become increasingly common in clinical practice, with pregnancy and cumulative live birth rates now comparable to those following Fresh ET. [8, 9] This progress has prompted a shift toward more individualized transfer strategies based not only on implantation potential but also on overall obstetric and perinatal safety profiles, an area that remains under active investigation. [5, 9]

COS, particularly in high responders, can lead to ovarian hyperstimulation syndrome (OHSS), a serious complication characterized by increased ovarian volume, fluid shifts from blood vessels to the extravascular space, which can result in high risk of thrombosis and decreased perfusion to organs such as kidneys and liver, causing significant maternal morbidity and potentially life-threatening outcomes. This condition is particularly pronounced when pregnancy occurs in the same stimulated cycle, a scenario inherent to Fresh ET. [10-12]

The freeze-all strategy, in which all viable embryos are cryopreserved for transfer in subsequent cycles without ovarian stimulation, allows embryo transfer to be deferred to a non-stimulated cycle, mitigating OHSS risk and avoiding the potential adverse effects of COS on the endometrial environment, which could negatively impact implantation and subsequent obstetric and perinatal outcomes. [5, 9-15]

Obstetric Outcomes

The most robust evidence comparing obstetric outcomes according to embryo transfer comes from systematic reviews and meta-analyses, including those synthesized in recent Cochrane reviews. [5] Overall, while cumulative live birth rates appear comparable between strategies, maternal complications during pregnancy seem to differ. [5, 13]

Hypertensive disorders of pregnancy (HDP) represent the most consistently reported difference between the two approaches. Several meta-analyses suggest that pregnancies following FET are associated with an increased risk of pregnancy-induced hypertension (PIH) and pre-eclampsia compared with Fresh ET, particularly in singleton pregnancies. [13, 16-19] A proposed explanation is the corpus luteum hypothesis, whereby programmed FET cycles rely exclusively on exogenous estrogen and progesterone, creating a non-physiological hormonal environment, whereas natural and modified-natural cycles preserve corpus luteum function. The absence of corpus luteum-derived vasoactive mediators such as relaxin may impair cardiovascular adaptation and early placentation, contributing to increased HDP risk. In addition, prolonged or supra-physiological exposure to exogenous progesterone may also influence trophoblast differentiation and spiral artery remodeling, potentially affecting placental development. [5, 13, 20, 21]

Evidence regarding placenta-related complications is less uniform. Most pooled analyses report no significant differences in the incidence of placenta previa between fresh and frozen cycles. [16] However, some studies suggest a higher risk of placenta accreta following FET. [14, 15, 18, 19]

The association between embryo transfer strategy and gestational diabetes mellitus (GDM) remains less consistent. Some cohort studies suggest a higher GDM rates after FET, but adjusted analyses, for maternal age, body mass index, and other confounders, generally do not confirm a significant or clinically meaningful difference. [5, 17, 18, 22]

Data on intrahepatic cholestasis of pregnancy (ICP) in ART-conceived pregnancies are scarce, and very few studies compare its frequency between transfer strategies, leaving the relative risk uncertain. [23, 24]

Collectively, while HDP have been more consistently reported after FET, the available evidence, especially for other obstetric outcomes of interest, remains heterogeneous and of moderate to low quality, highlighting the need for further investigation. [5, 13]

Perinatal Outcomes

Perinatal outcomes following Fresh ET and FET have been widely investigated, although findings remain heterogeneous. In regard to gestational age at delivery, several cohort studies and meta-analyses suggest that FET may be associated with a lower risk of preterm delivery compared with Fresh ET. [9, 25-27] However, randomized evidence has not consistently confirmed this difference, and the overall certainty of evidence remains limited. [5]

Fetal growth patterns show the most consistent differences. Literature suggests that FET is consistently associated with higher mean birth weight, increased rates of large for gestational age (LGA) and macrosomia, whereas Fresh ET is more often linked to low birth weight (LBW). Evidence regarding small for gestational age (SGA) is mixed, with observational studies suggesting lower rates after FET but no clear confirmation from randomized trials. These differences have been documented across multiple cohort studies and meta-analyses, and are thought to reflect variations in early placentation, endometrial preparation, and hormonal exposure at implantation. [5, 9, 25-27]

Apgar scores and congenital anomalies show no clinically relevant differences in most studies. [5, 12] However, some cohort studies have suggested slightly higher rates of congenital malformations after cryopreservation. [9, 25, 28]

Furthermore, embryo developmental stage may also influence perinatal outcomes. Blastocyst-stage transfers have higher implantation potential and generally exhibit more favorable implantation dynamics and endometrial synchrony. These factors have been associated with differences in gestational age, risk of preterm birth, and fetal growth patterns, including slightly higher birth weight and increased rates of LGA infants. In contrast, cleavage-stage transfers occur earlier in embryonic development and may follow different implantation trajectories, which potentially explain part of the variability observed in neonatal outcomes across studies. [29-32]

Multiple Gestations

Multiple gestations represent a major concern in ART due to their strong association with adverse obstetric and perinatal outcomes, including preterm birth, low birth weight, HDP and neonatal morbidity and malformations. Their incidence is primarily determined by the number of embryos transferred. Historically, transferring multiple embryos was common practice to maximize pregnancy rates, which led to high twin rates in ART populations. [33, 34]

However, the widespread implementation of elective single embryo transfer (eSET), particularly at the blastocyst stage, has substantially reduced the incidence of twin pregnancies while maintaining high cumulative live birth rates and leaving more embryos available for cryopreservation. [25, 33, 34]

In this context, given the heterogeneity and limited robustness of the current aforementioned literature, the present study aims to compare obstetric and perinatal outcomes in pregnancies conceived through ART following fresh versus frozen-thawed embryo transfer, in order to better clarify their potential impact on maternal and neonatal health.

METHODS

Study Design and Population

A retrospective cohort study was conducted at the Center of Assisted Medical Procreation of Centro Materno-Infantil do Norte (CMIN) to compare obstetric and perinatal outcomes between Fresh ET and FET in ART pregnancies from January 2019 to December 2024.

Prior to data collection, the Department of Education, Training and Research and the Ethics Committee of the Unidade Local de Saúde de Santo António (ULSSA) approved this study, with registration number N/REF.ª 2025.286 (244-CAC-244-CE). The written informed consent was waived due to the retrospective study design.

To ensure confidentiality and prevent patient re-identification, hospital record numbers were replaced by unique alphanumeric codes. The correspondence linking these codes to patient records was stored separately under restricted access, and the analytical database did not contain direct personal identifiers.

The study population comprised IVF and ICSI pregnancies resulting in live births after Fresh ET or FET, obtained from the Center of Assisted Medical Procreation database. Both blastocyst-stage (day 5-6) and cleavage-stage (day 2-3) transfers were eligible, as well as single and double embryo transfers and multiple pregnancies. Pregnancies conceived using donor sperm were also included.

Exclusion criteria encompassed pregnancies achieved using donor oocytes or donated embryos, cycles using testicular sperm extraction (TESE), mixed insemination cycles, and cases with incomplete relevant obstetric or neonatal data. Non-evolving pregnancies, ectopic pregnancies, and miscarriages are not captured in the database and were therefore not represented in the study population. The selection process is illustrated in Figure 1.

Outcome Measurements

General characteristics of included patients

Patients' baseline characteristics, obtained from the aforementioned database, were used to describe the study population and allow adjustment for potential confounders in the statistical analysis, including maternal age, body mass index (BMI), smoking habits, duration of infertility, type of infertility (primary or secondary), infertility diagnosis, fertilization method (IVF or ICSI), donor sperm use, cryopreservation origin (freeze-all or surplus embryos from a previous fresh cycle), number of cycles, number of embryos transferred (single or double embryo transfer), embryo stage at transfer (blastocyst or cleavage stage) and implantation rate (number of embryos with confirmed cardiac activity divided by the total number of embryos transferred).

Smoking status was classified as smoker or non-smoker at the time of ART treatment, regardless of subsequent cessation during pregnancy.

Regarding infertility etiology, the male factor group covered abnormalities in semen parameters, neurological, testicular, neoplastic and genetic conditions. The female factor included endocrine, tubal, ovulatory, uterine and cervical alterations as well as diminished ovarian reserve, and endometriosis. Combined infertility was defined as the coexistence of both male and female factors, and unexplained when no identifiable cause could be determined after clinical evaluation. Infertility diagnoses were not mutually exclusive, and each patient could be assigned more than one diagnosis simultaneously.

Obstetric and Perinatal Outcomes

Obstetric and perinatal outcomes were obtained from clinical records using the *SClínico Hospitalar* computer program.

The obstetric outcomes of the study included hypertensive disorders of pregnancy (including chronic hypertension, PIH, preeclampsia, eclampsia and HELLP syndrome), placenta-related complications (placenta previa, placenta accreta spectrum and placental abruption), diabetes in pregnancy (both pre-existing and gestational diabetes mellitus), intrahepatic cholestasis of pregnancy (ICP), multiple pregnancy and chorionicity.

Regarding the perinatal outcomes measurements used were gestational age at birth, preterm birth (delivery before 37 weeks of gestation), mode of delivery, birth weight including low birth weight (< 2500g) and high birth weight (> 4000g), fetal growth restriction (FGR) (according to delphi criteria [35]), Apgar scores at 1 and 5 minutes after birth, major congenital anomalies and neonatal death.

Mode of delivery was classified as eutocic (vaginal delivery without instrumentation) or dystocic (any delivery requiring medical intervention, including instrumental vaginal delivery and cesarean section).

Major congenital anomalies were defined as structural abnormalities with significant medical or surgical consequences, including congenital heart defects, neural tube defects, genitourinary, gastrointestinal and musculoskeletal malformations, and chromosomal or genetic syndromes. Newborns with multiple defects could be assigned to more than one category. Minor anomalies with limited clinical significance, such as ankyloglossia, isolated inguinal hernias and single umbilical artery, were excluded from the analysis, in accordance with the EUROCAT classification. [36]

Statistical analysis

Descriptive statistics were employed to summarize maternal baseline characteristics, reproductive variables, and obstetric and perinatal outcomes. Continuous variables are presented as means ± standard deviations (SD) or medians (range), while categorical variables are presented as frequencies and percentages.

The Kolmogorov-Smirnov test was used to evaluate normal distribution. Comparisons between the Fresh ET and the FET groups were performed using independent samples t-tests for normally distributed continuous variables or Mann-Whitney U test when data were not normally distributed, and chi-square test or Fisher's exact test for categorical variables, as appropriate.

To adjust for potential confounding factors, logistic regression was used for categorical outcomes, such as hypertensive disorders, GDM, birth weight classification, and mode of delivery, and linear regression was applied for continuous outcomes, such as birth weight and gestational age. Adjusted odds ratio (aOR) with 95% confidence intervals (CI) were reported. Potential confounders considered in the models included BMI, maternal age, smoking habits, number of embryos transferred, embryo developmental stage and ART technique (IVF or ICSI). Multivariable regression analyses were performed for outcomes that were statistically significant in univariable analysis and considered clinically relevant.

Missing values were handled using complete-case analysis and were therefore excluded from statistical calculations. Additionally, due to the small number of cases in each congenital anomaly subtype, no inferential statistical analysis was performed for individual subcategories.

All statistical tests were two-sided, and statistical significance was set at $p < 0.05$ with a 95% confidence interval. Data analysis was conducted using The Statistical Package for the Social Sciences software (IBM SPSS Statistics, version 30).

RESULTS

Baseline Characteristics

A total of 491 pregnancies were included in the analysis, as detailed in Figure 1, of which 246 (50.1%) resulted from FET and 245 (49.9%) from Fresh ET.

Baseline maternal characteristics of both groups are summarized in Table I. The two groups were comparable across most demographic and clinical variables. Mean maternal age was similar between the FET and Fresh ET groups (33.99 ± 3.88 versus 34.47 ± 3.42 years, p = 0.153), as was the proportion of women of advanced maternal age (≥ 35 years) (49.2% versus 53.9%, p = 0.298).

Maternal BMI was significantly higher in the FET group (24.93 ± 4.75 versus 23.99 ± 4.21 kg/m², p = 0.018), although no differences were observed in the proportions of underweight (BMI < 19 kg/m²; 7.3% versus 4.9%, p = 0.263) or obese women (BMI ≥ 30 kg/m²; 15.4% versus 11.4%, p = 0.192). Smoking status was also similar, with approximately one quarter being active smokers in each group (23.2% versus 24.9%, p = 0.654).

Regarding infertility characteristics, the duration of infertility did not differ between groups (45.82 ± 23.82 versus 47.11 ± 26.94 months, p = 0.692) nor did the type of infertility (primary: 84.1% versus 79.2%; secondary: 15.9% versus 20.8%, p = 0.162).

Among infertility diagnoses, DOR was significantly more prevalent in the Fresh ET group (10.6% versus 2.0%, p < 0.001). A trend toward a higher prevalence of ovulatory disorders (54.5% versus 46.5%, p = 0.078) and endocrine factors (11.0% versus 6.1%, p = 0.055) was observed in the FET group, although these differences did not reach statistical significance. The remaining infertility diagnoses, including male factor (62.6% versus 69.0%, p = 0.136), tubal factor (13.8% versus 14.3%, p = 0.882), uterine factor (12.6% versus 9.0%, p = 0.196), endometriosis (15.0% versus 19.2%, p = 0.223), cervical factor (1.2% versus 1.2%, p = 1.000), combined causes (44.3% versus 44.9%, p = 0.896) and unexplained infertility (3.7% versus 2.4%, p = 0.436), were similarly distributed between groups.

Characteristics of ART procedures are presented in Table II. Within the FET group, most embryos originated from a freeze-all strategy (62.6%), while 37.4% were surplus embryos from a previous fresh cycle.

Fertilization methods differed significantly between groups, with a higher proportion of IVF in the FET group (56.5% versus 46.5%) and a higher proportion of ICSI in the Fresh ET group (53.5% versus 43.5%, p = 0.027). Donor sperm use was comparable (5.3% versus 7.3%, p = 0.347). The distribution of treatment cycles was similar (p = 0.942), with approximately two-thirds of patients in both groups achieving pregnancy in their first cycle (65.0% versus 67.6%), followed by a second cycle (27.2% versus 23.7%) and three or more cycles (7.7% versus 9.0%).

Two key differences were observed regarding embryo transfer characteristics. Single embryo transfer (SET) was significantly more frequent in the FET group (60.6% versus 43.3), whereas double embryo transfer (DET) predominated in the Fresh ET group (39.4% versus 56.7%%, p < 0.001). Blastocyst-stage transfer was markedly more common in the FET group (87.8% versus 38.8%), whereas cleavage-stage transfer was predominantly performed in the Fresh ET group (12.2% versus 61.2%, p < 0.001)

Implantation rate was comparable between groups, with a higher rate observed in the FET group (278/343, 81.0% versus 289/384, 75.3%, p = 0.060).

Obstetric Outcomes

Obstetric outcomes are summarized in Table III. Data were available for a total of 421 pregnancies, of which 216 (51.3%) were from FET and 205 (48.7%) from Fresh ET, with 70 cases lost to follow-up. No statistically significant differences were observed between groups for any of the obstetric outcomes assessed.

Regarding hypertensive disorders of pregnancy, the overall rate was comparable between groups (9.7% versus 9.3%, p = 0.874). Rates of chronic hypertension (1.9% versus 1.0%, p = 0.686), PIH (3.7% versus 1.5%, p = 0.150), preeclampsia (3.7% versus 6.3%, p = 0.214), and HELLP syndrome (0.5% versus 0.5%, p = 1.000) were comparable. No cases of eclampsia were reported.

Placenta-related complications were similarly distributed, with no significant differences in placenta previa (3.2% versus 4.9%, p = 0.394) or placental abruption (2 cases in the FET group versus none in the Fresh ET group, p = 0.499). No cases of placenta accreta were observed.

The prevalence of diabetes mellitus, including GDM (11.6% versus 8.3%) and pre-gestational diabetes (PGDM) (0.9% versus 0.5%), was comparable between groups (p = 0.452). ICP was also comparable (1.4% versus 3.4%, p = 0.211).

Pregnancy and Birth Outcomes

Pregnancy and birth outcomes are represented in Table IV. A total of 491 deliveries were analyzed, of which 246 (50.1%) resulted from FET and 245 (49.9%) from Fresh ET.

The rate of multiple pregnancy was lower in the FET group compared with the Fresh ET group (10.6% versus 15.5%, p = 0.104), though not statistically significant. Among twin pregnancies, all FET cases were dichorionic (100.0%), compared with the Fresh ET group in which 89.5% were dichorionic and 10.5% monochorionic, with no statistically significant difference between groups (p= 0.140).

Total live births were slightly higher in the Fresh ET group (282 versus 272). The distribution of singleton and twin births differed significantly between the two groups (p = 0.036), with a higher proportion of twins following Fresh ET (19.1% versus 26.6%) and a correspondingly lower proportion of singletons (80.9% versus 73.4%). One case of selective fetal reduction in the Fresh ET group resulted in a singleton live birth.

Mean gestational age at delivery was similar between groups (37.33 ± 2.23 weeks in FET versus 37.48 ± 2.47 weeks in Fresh ET, p = 0.141), as was the rate of preterm birth (26.8% versus 26.5%, p = 0.940).

Given the significant difference in the distribution of singleton and twin pregnancies, perinatal outcomes were analyzed separately for singleton and twin gestations to better delineate the potential differential impact of embryo transfer strategy on neonatal health.

Perinatal outcomes in singleton pregnancies

Perinatal outcomes among singleton pregnancies are illustrated in Table V. A total of 427 singletons were included, 220 (51.5%) following FET and 207 (48.5%) after Fresh ET.

Mean gestational age at delivery was significantly lower in the FET group (37.72 ± 1.85 weeks versus 38.02 ± 1.73 weeks, p = 0.026), although the rate of preterm birth was similar between groups (18.6% versus 16.9%, p = 0.641). Regarding mode of delivery, dystocic deliveries were significantly more frequent following FET (80.0% versus 68.1%, p = 0.005), with a correspondingly lower rate of eutocic deliveries (20.0% versus 31.9%). Neonatal sex distribution was comparable (male: 50.5% versus 52.7%; female: 49.5% versus 47.3%, p = 0.649).

Mean birth weight was significantly higher in the FET group (3241.35 ± 541.56 g versus 3023.98 ± 540.97 g, $p < 0.001$). Birth weight classification differed marginally between strategies ($p = 0.050$), with a higher proportion of LBW infants in the Fresh ET group (13.5% versus 7.7%) and a higher proportion of high birth weight (HBW) infants in the FET group (5.5% versus 2.4%).

Apgar scores at 1 and 5 minutes were high and comparable between groups at both time points (1st minute: 9 (9-10) versus 9 (8-10), $p = 0.403$; 5th minute: 10 (10-10) versus 10 (9-10), $p = 0.455$). Low Apgar scores (< 7) were rare and did not differ significantly at either the 1st minute (4.1% versus 4.3%, $p = 0.895$) or the 5th minute (0.5% versus 0.0%, $p = 1.000$).

Data on FGR, congenital anomalies, and neonatal death were unavailable for some cases due to losses to follow-up or incomplete neonatal records. Therefore, a complete-case analysis was performed for these outcomes.

FGR incidence was lower in the FET group (4.2% versus 8.0%, $p = 0.122$), although this difference was not statistically significant. Major congenital anomalies occurred in 11 (5.9%) FET and 9 (5.2%) Fresh ET singletons, with no statistically significant difference ($p = 0.806$). Subtype distribution of congenital anomalies is presented descriptively. Cardiac defects were the most frequent (8 (72.7%) versus 4 (44.4%)), followed by genitourinary anomalies (3 (27.3%) versus 1 (11.1%)), gastrointestinal malformations (1 (9.1%) in each group), musculoskeletal malformations (1 (9.1%) versus 2 (22.2%)), neural tube defects were observed only in the Fresh ET group (2 (22.2%)), whereas genetic syndromes occurred only in the FET (1 (9.1%)). There was one case of neonatal death reported in the Fresh ET group (0.6%) and none after FET ($p = 0.477$).

Perinatal outcomes in twin pregnancies

Perinatal outcomes among twin pregnancies are presented in Table VI. A total of 127 twin live births were analyzed, including 52 (40.9%) following FET and 75 (59.1%) after Fresh ET.

Mean gestational age was significantly lower in the FET group (34.00 ± 2.47 versus 34.55 ± 3.63 weeks, $p = 0.024$). Although preterm birth rates tended to be higher after FET (96.2% versus 78.9%, $p = 0.071$), this difference did not reach statistical significance. Dystocic delivery was frequent in both groups and comparable (75.0% versus 74.7%, $p = 0.966$), with eutocic delivery rates being lower (25.0% versus 25.3%). Neonatal sex distribution was also similar (male: 48.1% versus 60.8%; female: 51.9% versus 39.2%, $p = 0.157$).

Mean birth weight did not differ significantly between groups (2133.85 ± 578.91 g versus 2160.88 ± 572.30 g, $p = 0.734$). The proportion of LBW infants was nearly identical (71.2% versus 70.3%, $p = 0.915$), with no cases of HBW recorded.

Apgar scores were comparable at both time points (1st minute: 9 (3-10) versus 9 (3-10), $p = 0.319$; 5th minute: 10 (4-10) versus 10 (6-10), $p = 0.392$), with low scores (<7) similarly distributed (1st minute: 13.5% versus 12.2%, $p = 0.829$; 5th minute: 1.9% versus 2.7%, $p = 1.000$).

Data on fetal growth restriction, congenital anomalies, and neonatal death were incomplete for a small number of cases due to missing data resulting from loss to follow-up, and analyses for these outcomes were performed on available cases.

FGR rates were similar between groups (32.0% versus 27.9%, $p = 0.636$). Major congenital anomalies occurred in 3 (6.0%) FET and 8 (13.1%) Fresh ET, with no statistically significant difference ($p = 0.339$). Among these, cardiac defects were the most frequent, observed exclusively in the Fresh ET group (7 (87.5%)), followed by genitourinary anomalies (1 (33.3%) versus 2 (25.0%)), gastrointestinal malformations (1 (33.3%) versus none), and genetic syndromes (1 (33.3%) versus 2 (25.0%)). No musculoskeletal or neural tube defects were identified. Neonatal death occurred in two (3.3%) cases after Fresh ET and in none after FET ($p = 0.500$).

Multivariable Analysis

Multivariable regression analyses were performed for selected outcomes, as detailed in Tables VII-X. The significant differences in embryo stage and number of embryos transferred between groups were confirmed in the singleton subgroup ($p < 0.001$), justifying their inclusion as covariates in the multivariable analyses, although these differences were not significant in the twin subgroup ($p = 0.265$).

Regarding obstetric outcomes, FET was not identified as an independent predictor of HDP (aOR = 1.399, 95% CI 0.602-3.251, $p = 0.435$), preeclampsia (aOR = 0.733, 95% CI 0.240-2.238, $p = 0.586$) or GDM (aOR = 1.359, 95% CI 0.595-3.107, $p = 0.467$) after multivariable adjustment. Maternal age and BMI were the main independent predictors of HDP and GDM, while BMI was the only significant predictor of preeclampsia. Notably, the number of embryos transferred was identified as an independent predictor of HDP, with DET associated with significantly higher odds compared with SET (aOR = 2.377, 95% CI 1.104-5.114, $p = 0.027$) (Table VII).

In singleton pregnancies, FET remained independently associated with significantly higher birth weight after adjustment for gestational age, BMI, maternal age, fertilization method, embryo stage, number of embryos transferred, and smoking (B = +266.86g, 95% CI 177.12-356.61, $p < 0.001$) (Table VIII). In the multivariable model adjusted for gestational age, BMI, maternal age, fertilization method, and smoking, Fresh ET was independently associated with higher odds of LBW compared with FET (aOR = 3.388, 95% CI 1.352-8.493, $p = 0.009$) (Table IXa).

However, this association was weakened and no longer reached statistical significance after additional adjustment for embryo stage and number of embryos transferred (aOR = 2.260, 95% CI 0.724–7.054, $p = 0.160$) (Table IXb), suggesting that the observed effect may be mediated by embryo stage at transfer rather than cryopreservation itself.

Furthermore, FET remained independently associated with a higher odd of dystocic delivery (aOR = 1.653, 95% CI 1.029-2.653, $p = 0.037$), with birth weight also emerging as a significant predictor (aOR = 1.001, 95% CI 1.000-1.001, $p = 0.037$) (Table X).

Multivariable linear regression analysis was performed for gestational age in both singleton and twin pregnancies. However, as the overall models did not reach statistical significance in either subgroup (singletons: $p = 0.164$; twins: $p = 0.286$), these results were not included in the final analysis.

DISCUSSION

Baseline Characteristics

The two groups were broadly comparable across maternal demographics and most infertility diagnoses, suggesting that the allocation to Fresh ET or FET was not driven by these factors. The mean maternal age was approximately 34 years in both groups, with similar proportions of advanced maternal age, which is reassuring given its established impact on ART outcomes. [37]

Although BMI was higher in the FET group ($p = 0.018$), the absolute difference was less than 1 kg/m² and both groups fell within the normal-to-overweight range, with similar proportions of underweight and obese women, limiting clinical relevance. Nevertheless, BMI was included as a covariate, given its well-established role in obstetric complications namely GDM and HDP. [38, 39]

DOR was significantly more prevalent in the Fresh ET group ($p < 0.001$), a clinically expected finding given that women with DOR typically yield fewer oocytes and consequently fewer surplus embryos for cryopreservation, making fresh transfer the more viable option. This rationale is supported by recent evidence showing higher live birth rates with Fresh ET in women with low prognosis. [40]

Three significant procedural differences were identified, reflecting the evolution of ART clinical practice toward blastocyst culture, SET, and freeze-all strategies. [6, 41, 42] Conventional IVF was more frequent in the FET group while ICSI predominated in the Fresh ET group, possibly reflecting the higher proportion of male factor infertility in the latter, given that ICSI is the preferred technique for severe male factor. [43] Recent randomized controlled trials have demonstrated that this choice does not significantly affect obstetric or perinatal outcomes. [44-46] However, both methods were included as covariates in the multivariable analysis.

SET was more common in the FET group, consistent with the freeze-all strategy favoring eSET, thereby reducing multiple pregnancy risk. [42] The number of embryo transfer was included as a covariate given the established association of DET with adverse perinatal outcomes, specifically preterm birth, low birth weight, and neonatal death, even in singleton pregnancies. [47, 48]

Blastocyst-stage transfer was also more prevalent in FET cycles, reflecting its link to cryopreservation strategies and improved embryo-endometrium synchrony. [41] As blastocyst transfer has been associated with higher LGA rates and preterm birth risk, embryo stage was similarly included as a covariate. [31, 49] Moreover, the implantation rate was numerically higher in the FET group, suggesting a trend towards improved implantation in frozen cycles, consistent with the proposed beneficial effect of avoiding ovarian stimulation on endometrial receptivity. [15]

Obstetric Outcomes

Obstetric outcomes were comparable between the FET and Fresh ET groups, with no statistically significant differences observed in any of the variables assessed. These findings contrast with the existing literature, which has consistently reported an increased risk of HDP, particularly preeclampsia, following FET. [7, 13, 50] As previously discussed, this increased risk has been largely attributed to the absence of a corpus luteum in programmed FET cycles and appears specific to these protocols rather than to FET *per se*. [7, 51, 52]

In the present study, preeclampsia tended to be more frequent in the Fresh ET group, a direction contrary to most of the literature. However, this difference did not reach statistical significance which may reflect limited statistical power and the lack of data on endometrial preparation protocols. As FET cycles likely included both natural and programmed regimens, the absence of an increased HDP risk in our cohort may reflect a non-predominance of programmed cycles. [51, 52] However, although available data from a subset of more recent FET cycles in our center (47/246, 19.1%) suggested a clear predominance of programmed protocols (93.6%) over modified-natural

cycles (6.4%), the limited number of cases precludes definitive interpretation. Under this assumption, an increased HDP risk in the FET group would have been expected. This finding is therefore reassuring and supports the obstetric safety of FET in our clinical practice. Nonetheless, the inability to systematically differentiate between endometrial preparation protocols represents an important limitation of the present study.

Regarding GDM, its prevalence was numerically higher in the FET group, consistent with the literature suggesting a modestly increased risk after FET, particularly after blastocyst-stage transfer. [9, 22, 53, 54] This trend should be interpreted with caution, as the FET group also exhibited slightly higher BMI and a significantly greater proportion of blastocyst transfers, both recognized risk factors for GDM and potential sources of residual confounding. [53, 55]

Placenta-related complications and ICP were comparably distributed between groups. Regarding placenta previa specifically, this finding is consistent with the majority of pooled analyses, while data specifically comparing ICP between transfer strategies remain scarce. [16, 23, 24] Given the low number of events, multivariable analysis was not performed for these outcomes.

Multivariable analysis confirmed that FET was not an independent predictor of any obstetric outcome. BMI and maternal age emerged as the main independent predictors of both HDP and GDM, consistent with their well-established role. [38, 39] Notably, DET was independently associated with HDP, consistent with the well-documented link between multiple gestation and hypertensive disorders, and further supporting the clinical relevance of eSET policies. [42, 47, 48] These findings reinforce the interpretation that the absence of significant differences in obstetric outcomes may reflect a true lack of effect of FET *per se*, rather than solely insufficient statistical power.

Pregnancy and Birth Outcomes

In the present study, the distribution of singleton and twin pregnancies differed significantly, with a higher proportion of twin gestations following Fresh ET. This finding likely reflects procedural differences between transfer strategies, as evidenced by the higher prevalence of DET in the Fresh ET group, consistent with the ongoing paradigm shift toward eSET, facilitated by advances in vitrification and blastocyst culture, which permit elective deferred embryo transfer without compromising cumulative live birth rates. [34, 42, 47] These current clinical patterns have contributed to the steady decline in twin gestations observed globally in ART, which is clinically relevant given the well-established association of multiple gestation with adverse maternal and neonatal outcomes, as aforementioned. [34, 48, 56] Consequently, the slightly lower rate of twin gestations after FET in this study reinforces the strategy's potential contribution to improved obstetric and perinatal safety profiles.

The predominantly dichorionic twin population in both groups is clinically relevant, as monochorionic twins carry intrinsically higher risks of adverse perinatal outcomes, including twin-to-twin transfusion syndrome, preterm birth, and perinatal mortality, independently of the mode of conception, this reduces the likelihood that chorionicity-related complications confounded the comparison between strategies. [57]

Regarding gestational age and preterm birth, the overall analysis showed comparable mean gestational age and preterm birth rates between strategies. When analyzed separately, singletons in the FET group delivered at a slightly earlier gestational age ($p = 0.026$), although the absolute difference was less than two weeks and both groups delivered at term on average, with comparable preterm birth rates, suggesting that the transfer strategy did not meaningfully influence the risk of preterm delivery. In twin pregnancies, gestational age was also significantly lower in the FET group ($p = 0.024$), with both groups falling within the preterm range, reflecting the inherently higher obstetric risk of multiple gestation particularly related to preterm delivery. [58] Alongside this, a non-

significant trend toward higher preterm birth rates in the FET group (p = 0.071), a pattern similar to that observed in singleton pregnancies.

These findings partly contrast with the broader literature, where FET has generally been associated with comparable or longer gestational duration. [7, 9, 26] However, this latter association has been shown to attenuate or disappear when all pregnancies are combined, as noted in our cohort. [9, 13, 26] Moreover, randomized controlled trials have not demonstrated clinically meaningful differences in gestational duration or preterm birth rates between transfer strategies. [17, 18] The literature specifically addressing twin pregnancies following FET versus Fresh ET is considerably more limited than for singletons, and existing evidence is not entirely consistent. [26, 59] Thus, the observed trend toward shorter gestational age and higher preterm birth rates in FET twins likely reflects the limited sample size of this subgroup rather than a true biological effect.

Perinatal Outcomes in Singleton Pregnancies

Among singleton pregnancies, the most clinically significant finding of the present study was the significantly higher mean birth weight observed in the FET group, an association that was confirmed and strengthened by multivariable linear regression analysis (B = +266.86 g, 95% CI 177.12-356.61, p < 0.001). This demonstrates that FET remained independently associated with higher birth weight after adjustment, reinforcing that this difference cannot be attributed to other variables alone. This finding is consistent with an extensive and growing body of literature linking FET to higher birth weight, LGA neonates and macrosomia when compared to Fresh ET. [5, 7, 9, 25-27, 60]

The mechanisms underlying this phenomenon remain incompletely understood but are likely multifactorial. Epigenetic alterations induced by the cryopreservation have been proposed as a key contributor, with vitrification shown to affect methylation of imprinted genes such as H19 and IGF2, key regulators of fetal growth, potentially resulting in a pattern resembling "Large Offspring Syndrome". [61, 62] Additionally, programmed FET cycles have been associated with significantly higher fetal weight and LGA compared with natural cycle FET, suggesting that the lack of corpus luteum-derived vasoactive mediators may favor fetal overgrowth. [63] The unavailability of endometrial preparation data in our cohort precludes further stratification of this effect, representing a relevant limitation. Notably, the apparent predominance of programmed cycles in our center, as previously discussed, is consistent with the higher birth weight observed in the FET group. Furthermore, the significantly higher proportion of blastocyst-stage transfers in the FET group may have also contributed, as blastocyst transfer has been independently associated with higher birth weight. [31, 49]

Regarding birth weight classification, the distribution differed marginally between groups (p = 0.050), with a higher proportion of LBW infants in the Fresh ET group and a higher proportion of HBW infants in the FET group. This finding is consistent with the literature, which has consistently demonstrated that Fresh ET is associated with higher rates of LBW and SGA, while FET is associated with increased rates of HBW and LGA. [7, 60]

In multivariable analysis, Fresh ET was associated with higher odds of LBW (aOR = 3.388, 95% CI 1.352-8.493, p = 0.009) (Table IXa). However, after additional adjustment for embryo stage and number of embryos transferred, this association was weakened (aOR = 2.260, 95% CI 0.724-7.054, p = 0.160) (Table IXb), suggesting that the protective effect of FET against LBW may be partly mediated by the higher proportion of blastocyst-stage transfers rather than cryopreservation *per se*. This has important clinical implications, as it suggests that the shift toward blastocyst culture, independent of the cryopreservation strategy, may be a key driver of increased birth weight outcomes. [31, 41, 49]

Dystocic deliveries were significantly more frequent following FET (p = 0.005), consistent with the higher caesarean section rates reported in the literature after FET. [7, 13] This finding is likely

multifactorial, as the higher birth weight, macrosomia and LGA rates in FET may directly contribute to intrapartum complications requiring operative delivery. [7, 60] Besides, programmed FET cycles have been independently associated with higher caesarean section rates, potentially mediated by the same corpus luteum-deficient hormonal environment implicated in placental dysfunction and fetal overgrowth, previously discussed, an observation consistent with the apparent predominance of programmed cycles in our center. [51] Notably, ART pregnancies in general carry a higher baseline risk of operative delivery, partly attributable to obstetric risk factors inherent to underlying infertility and to clinical decision-making in the context of a "precious pregnancy". [64] Multivariable analysis confirmed FET as an independent predictor of dystocic delivery (aOR = 1.653, 95% CI 1.029-2.653, p = 0.037), with birth weight also emerging as a significant predictor (aOR = 1.001, 95% CI 1.000-1.001, p = 0.037), supporting a mechanistic pathway whereby higher birth weight contributes to, but does not fully explain, the increased rate of operative delivery. [51, 64]

Apgar scores, FGR, and major congenital anomalies were comparable between groups, in line with the literature showing no meaningful differences between transfer strategies. Specifically, similar Apgar scores reinforce the absence of significant differences in neonatal adaptation, while the non-significant trend toward lower FGR rates in the FET group is biologically coherent with the observed higher birth weights and consistent with the lower SGA rates reported following FET. [7, 9, 26, 65] Cardiac defects were the most frequent anomaly subtype in both groups, consistent with recent evidence suggesting that while ART is associated with a modestly higher risk of congenital heart defects compared with spontaneous conception, no significant difference has been observed between FET and Fresh ET. [66] One case of neonatal death occurred in the Fresh ET group, from which no meaningful conclusion can be drawn given the very low absolute numbers, consistent with the current evidence. [7, 9]

Perinatal Outcomes in Twin Pregnancies

Birth weight, LBW rates, Apgar scores, FGR and mode of delivery were comparable between groups, consistent with the available evidence. [22, 25, 26, 59] Cardiac defects were numerically more frequent among Fresh ET twins, a finding that warrants extreme caution given the very small numbers involved and should be regarded as a chance finding, once the existing literature does not support a differential risk of cardiac anomalies between transfer type. [7, 60] Neonatal death occurred exclusively in the Fresh ET group, attributable to extreme prematurity rather than the transfer strategy, consistent with the literature. [7, 9]

Overall, the twin subgroup findings should be interpreted with particular caution given the small sample size, and the limited statistical power inherent to subgroup analyses. Larger prospective studies specifically designed to address perinatal outcomes in twin pregnancies following different transfer strategies are warranted.

Finally, it is worth noting that the majority of FET cycles in the present study originated from a freeze-all strategy, reflecting the growing adoption of this approach in clinical practice. [6, 41] However, for the freeze-all strategy to be justified as a default approach, it must demonstrate clear clinical and cost-effectiveness advantages over Fresh ET. [12] While it significantly reduces the risk of OHSS and may improve endometrial receptivity, it inherently involves a delay in achieving pregnancy and additional clinical visits, which may represent a significant burden for patients. [10, 11] The findings of the present study contribute to this debate by supporting the obstetric safety of FET, while highlighting that procedural factors such as embryo stage and number of embryos transferred may be key drivers of perinatal outcomes, independent of the freeze-all strategy itself.[5]

Strengths and Limitations

The strengths of this study include a substantial cohort of 491 ART pregnancies from a single tertiary referral center over a six-year period, with well-balanced groups (FET n = 246 versus Fresh ET n = 245), allowing direct comparison of obstetric and perinatal outcomes between transfer strategies with consistent clinical protocols. Additionally, the separate analysis of singleton and twin pregnancies, along with multivariable adjustment for key confounders, selected based on their established influence on each specific outcome according to the literature, strengthens the robustness of the findings.

Nevertheless, several limitations should be acknowledged, such as the retrospective design, which limits the ability to control for unmeasured confounders. Although the analysis was adjusted to minimize confounding, selection bias could not be entirely excluded. The relatively small twin subgroup, albeit clinically desirable, may have limited statistical power to detect differences in less frequent outcomes within this subset. The unavailability of systematic data on endometrial preparation protocols precluded stratification by natural versus programmed cycles, despite their recognized influence on obstetric and perinatal outcomes. Beyond this, some outcomes had incomplete data due to losses to follow-up, partly because many couples travel long distances to access ART treatment and often continue pregnancy surveillance closer to home.

Moreover, lifestyle factors such as physical activity and nutritional habits, which may also influence obstetric and perinatal outcomes, were not systematically assessed. This is particularly relevant given the likely interrelation between these complications, which often share common risk factors and may co-occur, potentially influencing their observed frequencies. Lastly, the long-term health of newborns conceived through different embryo transfer strategies was beyond the scope of this study and warrants further investigation.

CONCLUSION

As the global utilization of ART continues to expand, a comprehensive understanding of the comparative impact of different embryo transfer strategies on maternal and neonatal outcomes has become increasingly important for optimizing clinical practice and ensuring informed patient counselling.

This cohort study found that FET does not confer a meaningful increase in obstetric risk compared with Fresh ET, even after adjustment for potential confounders. Notably, DET was independently associated with HDP, reinforcing the clinical relevance of eSET policies in reducing obstetric risk.

In singleton pregnancies, FET was independently associated with higher birth weight and a higher rate of dystocic delivery. The protective effect against LBW appeared to be partly mediated by the higher proportion of blastocyst-stage transfers rather than a direct effect of cryopreservation *per se*. Although a statistically significant difference in gestational age was observed, this was of limited clinical magnitude, with both groups delivering at term on average and no significant difference in preterm birth rates. Remaining perinatal outcomes, including Apgar scores, FGR, and congenital anomalies, were comparable between groups, suggesting that neonatal adaptation was not influenced by the transfer strategy employed.

In twin pregnancies, outcomes were broadly comparable between groups, with the exception of a shorter gestational age in the FET group, likely reflecting the limited sample size of this subgroup rather than a true biological effect, with both groups delivering in the preterm range on average and comparable preterm birth rates. Nonetheless, considering the limitations of the present study, these findings require cautious interpretation.

Overall, the decision between FET and Fresh ET should be individualized rather than guided by a universal freeze-all approach. The ongoing shift toward blastocyst-stage transfer and eSET appears to be a key driver of perinatal outcomes, independent of the freeze-all strategy itself. While FET offers clear advantages in selected populations, particularly in high responders at risk of OHSS, it inherently involves a delay in achieving pregnancy and additional clinical visits, which may impact patients' quality of life and represent a significant lifestyle burden. These considerations highlight the importance of optimizing embryo transfer strategy to maximize both reproductive success and perinatal safety for each patient.

Looking ahead, larger prospective studies and well-designed randomized controlled trials are warranted to further elucidate the independent effect of embryo transfer strategy on maternal and neonatal health, with particular attention to twin pregnancies, which remain an underrepresented subgroup in the existing literature.

APPENDIX

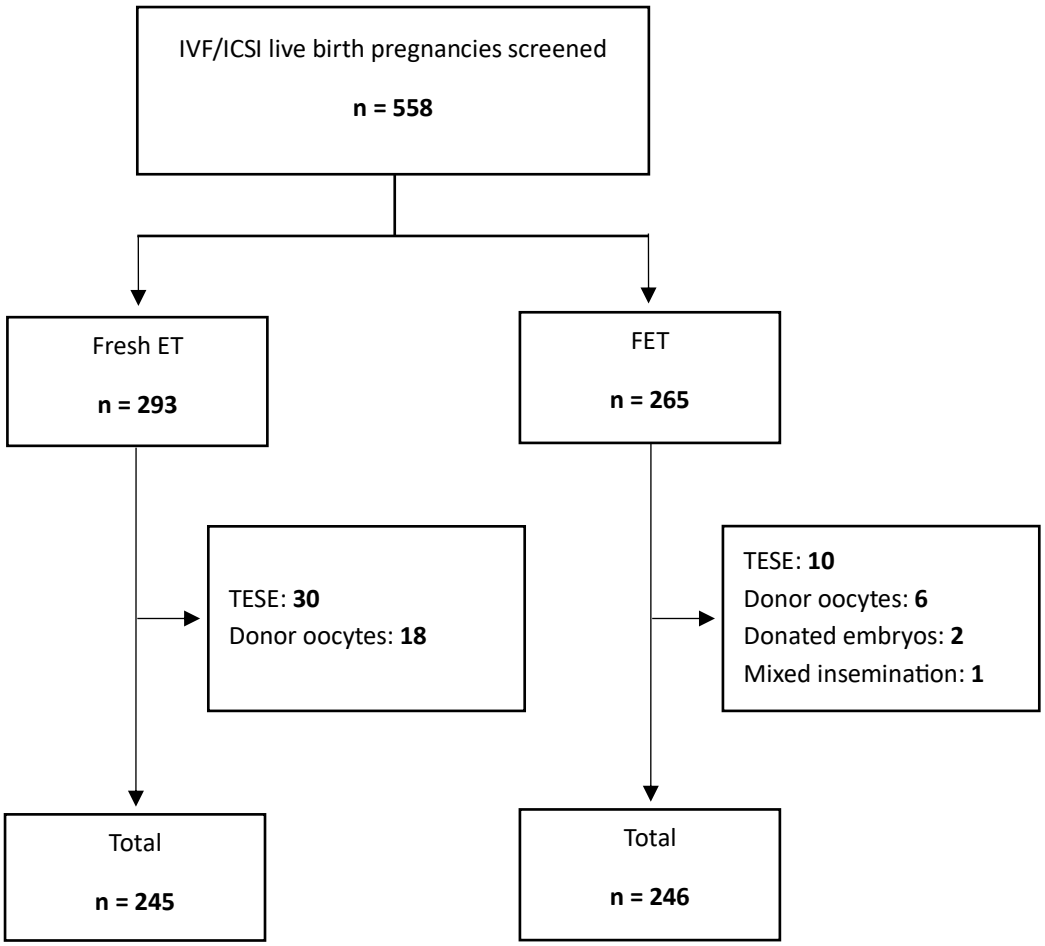

Figure 1 Flowchart of the study population.

Table I Maternal background characteristics according to embryo transfer strategy. Data are presented as mean ± SD, or number (percentage), as appropriate.

	FET (n = 246)	**Fresh ET (n = 245)**	***p*- value**
Maternal age (years)	33.99 ± 3.88	34.47 ± 3.42	0.153
Advanced maternal age (≥ 35 years), n (%)	121 (49.2)	132 (53.9)	0.298
Maternal BMI (kg/m^2)	24.93 ± 4.75	23.99 ± 4.21	**0.018**
BMI < 19 kg/m^2, n (%)	18 (7.3)	12 (4.9)	0.263
BMI ≥ 30 k g/m^2, n (%)	38 (15.4)	28 (11.4)	0.192
Smoking habits, n (%)			0.654
Non-smoker	189 (76.8)	184 (75.1)	
Smoker	57 (23.2)	61 (24.9)	
Duration of infertility (months)	45.82 ± 23.82	47.11 ± 26.94	0.692
Type of Infertility, n (%)			0.162
Primary	206 (84.1)	194 (79.2)	
Secondary	39 (15.9)	51 (20.8)	
Infertility diagnosis, n (%)			
Male	154 (62.6)	169 (69.0)	0.136
Ovulatory	134 (54.5)	114 (46.5)	0.078
DOR	5 (2.0)	26 (10.6)	**<0.001**
Endocrine	27 (11.0)	15 (6.1)	0.055
Tubal	34 (13.8)	35 (14.3)	0.882
Uterine	31 (12.6)	22 (9.0)	0.196
Endometriosis	37 (15.0)	47 (19.2)	0.223
Cervical	3 (1.2)	3 (1.2)	1.000
Combined	109 (44.3)	110 (44.9)	0.896
Unexplained	9 (3.7)	6 (2.4)	0.436

SD – Standard Deviation; IQR – Interquartile Range; BMI – Body Mass Index; FET – Frozen- thawed Embryo Transfer; Fresh ET – Fresh Embryo Transfer; DOR – Diminished Ovarian Reserve.

Table II Baseline characteristic of ART procedures according to embryo transfer strategy. Data are represented as number (percentage) or n/N (%).

	FET (n = 246)	Fresh ET (n = 245)	*p*- value
Cryopreservation origin, n (%)			
Freeze-all	154 (62.6)		
Surplus embryos	92 (37.4)		
Fertilization method, n (%)			**0.027**
IVF	139 (56.5)	114 (46.5)	
ICSI	107 (43.5)	131 (53.5)	
Donor sperm, n (%)	13 (5.3)	18 (7.3)	0.347
Number of cycles, n (%)			0.942
1 cycle	160 (65.0)	165 (67.3)	
2 cycles	67 (27.2)	58 (23.7)	
3 or more cycles	19 (7.7)	22 (9.0)	
No. of embryos transferred, n (%)			**<0.001**
SET	149 (60.6)	106 (43.3)	
DET	97 (39.4)	139 (56.7)	
Embryo stage at transfer, n (%)			**<0.001**
Cleavage	30 (12.2)	150 (61.2)	
Blastocyst	216 (87.8)	95 (38.8)	
Implantation rate, n/N (%)	278/343 (81.0)	289/384 (75.3)	0.060

IVF – *In Vitro* Fertilization; ICSI – Intracytoplasmic Sperm injection; SET – Single Embryo Transfer; DET – Double Embryo Transfer.

Table III Obstetric outcomes according to embryo transfer strategy. Data are represented as number (percentage).

	FET (n = 216)*	Fresh ET (n = 205)*	*p*- value
Hypertensive disorders, n (%)	21 (9.7)	19 (9.3)	0.874
Chronic hypertension	4 (1.9)	2 (1.0)	0.686
PIH	8 (3.7)	3 (1.5)	0.150
Preeclampsia	8 (3.7)	13 (6.3)	0.214
HELLP syndrome	1 (0.5)	1 (0.5)	1.000
Placenta-related complications, n (%)			
Placenta previa	7 (3.2)	10 (4.9)	0.394
Placental abruption	2 (0.9)	0 (0.0)	0.499
Diabetes Mellitus, n (%)			0.452
Pre-gestational diabetes	2 (0.9)	1 (0.5)	
Gestational diabetes	25 (11.6)	17 (8.3)	
Intrahepatic cholestasis of pregnancy, n (%)	3 (1.4)	7 (3.4)	0.211

* Missing values excluded. PIH – Pregnancy Induced Hypertension; HELLP – Hemolysis, Elevated Liver enzymes, Low Platelet count.

Table IV Pregnancy and birth outcomes according to embryo transfer strategy. Data are presented as mean ± SD, or number (%), as appropriate.

	FET	Fresh ET	*p*- value
No. of deliveries	246	245	
Multiple pregnancies, n (%)	26 (10.6)	38 (15.5)	0.104
Chorioniciy, n (%)			0.140
Monochorionic	0 (0.0)	4 (10.5)	
Dichorionic	26 (100.0)	34 (89.5)	
No. of live births, n (%)	272	282	
Singletons	220 (80.9)	207 (73.4)	**0.036**
Twins	52 (19.1)	75 (26.6)	
Selective fetal reduction, n (%)	0 (0.0)	1 (2.6)	1.000
Gestational age (weeks)	37.33 ± 2.23	37.48 ± 2.47	0.141
Preterm birth, n (%)	66 (26.8)	65 (26.5)	0.940

SD – Standard Deviation; IQR – Interquartile Range. Gestational age and preterm birth reported for the overall population (singletons and twins combined).

Table V Perinatal outcomes in singleton pregnancies according to embryo transfer strategy. Data are presented as mean ± SD, median (range), or number (%), as appropriate.

Singletons	**FET (n = 220)**	**Fresh ET (n = 207)**	***p*- value**
Gestational age (weeks)	37.72 ± 1.85	38.02 ± 1.73	**0.026**
Preterm birth, n (%)	41 (18.6)	35 (16.9)	0.641
Mode of delivery, n (%)			**0.005**
Eutocic	44 (20.0)	66 (31.9)	
Dystocic	176 (80.0)	141 (68.1)	
Gender, n (%)			0.649
Male	111 (50.5)	109 (52.7)	
Female	109 (49.5)	98 (47.3)	
Birth weight (g)	3241.35 ± 541.56	3023.98 ± 540.97	**< 0.001**
LBW (< 2500 g)	17 (7.7)	28 (13.5)	
HBW (> 4000 g)	12 (5.5)	5 (2.4)	**0.050**
Apgar score			
1st minute	9 (9-10)	9 (8-10)	0.403
5th minute	10 (10-10)	10 (9-10)	0.455
1st minute (< 7), n (%)	9 (4.1)	9 (4.3)	0.895
5th minute (< 7), n (%)	1 (0.5)	0 (0.0)	1.000
	FET (n = 191)*	**Fresh ET (n = 174)***	
Fetal growth restriction, n (%)	8 (4.2)	14 (8.0)	0.122
Major congenital anomalies, n (%)	11 (5.9)	9 (5.2)	0.806
Heart defects	8 (72.7)	4 (44.4)	
Neural Tube	0 (0.0)	2 (22.2)	
Genitourinary	3 (27.3)	1 (11.1)	
Gastrointestinal	1 (9.1)	1 (11.1)	
Musculoskeletal	1 (9.1)	2 (22.2)	
Genetic Syndromes	1 (9.1)	0 (0.0)	
Neonatal death, n (%)	0 (0.0)	1 (0.6)	0.477

* Missing values excluded. SD – Standard Deviation; LBW – Low Birth Weight; HBW – High Birth Weight.

Table VI Perinatal outcomes in twin pregnancies according to embryo transfer strategy. Data are presented as median (IQR), median (range), or number (%), as appropriate.

Twins	**FET (n = 52)**	**Fresh ET (n = 75)**	***p*- value**
Gestational age (weeks)	34.00 ± 2.47	34.55 ± 3.63	**0.024**
Preterm birth, n (%)	25 (96.2)	30 (78.9)	0.071
Mode of delivery, n (%)			0.966
Eutocic	13 (25)	19 (25.3)	
Dystocic	39 (75.0)	56 (74.7)	
Gender, n (%)*			0.157
Male	25 (48.1)	45 (60.8)	
Female	27 (51.9)	29 (39.2)	
Birth weight (g)*	2133.85 ± 578.91	2160.88 ± 572.30	0.734
LBW (< 2500 g)	37 (71.2)	52 (70.3)	0.915
Apgar score*			
1st minute	9 (3-10)	9 (3-10)	0.319
5th minute	10 (4-10)	10 (6-10)	0.392
1st minute (< 7), n (%)	7 (13.5)	9 (12.2)	0.829
5th minute (< 7), n (%)	1 (1.9)	2 (2.7)	1.000
	FET (n = 50)*	**Fresh ET (n = 61)***	
Fetal growth restriction, n (%)	16 (32.0)	17 (27.9)	0.636
Major congenital anomalies, n (%)	3 (6.0)	8 (13.1)	0.339
Heart defects	0 (0.0)	7 (87.5)	
Genitourinary	1 (33.3)	2 (25.0)	
Gastrointestinal	1 (33.3)	0 (0.0)	
Genetic Syndromes	1 (33.3)	2 (25.0)	
Neonatal death, n (%)	0 (0.0)	2 (3.3)	0.500

* Missing values excluded. SD – Standard Deviation; LBW – Low Birth Weight. Gestational age and preterm birth are reported per pregnancy (FET n = 26; Fresh ET n = 38). Remaining outcomes are reported per neonate.

Table VII Multivariable logistic regression analysis for obstetric outcomes. Nagelkerke R^2: HDP = 0.158; Preeclampsia = 0.105; GDM = 0.122.

	aOR	95% CI	*p*- value
Hypertensive Disorders of Pregnancy			
Embryo transfer strategy			
Fresh ET	Reference		
FET	1.399	0.602-3.251	0.435
BMI (kg/m²)	1.161	1.085-1.242	**<0.001**
Maternal age (years)	1.128	1.015-1.255	**0.026**
Embryo stage			
Cleavage	Reference		
Blastocyst	0.616	0.265-1.433	0.261
No. of embryos transferred			
SET	Reference		
DET	2.377	1.104-5.114	**0.027**
Fertilization method			
IVF	Reference		
ICSI	1.053	0.523-2.118	0.886
Preeclampsia			
Embryo transfer strategy			
Fresh ET	Reference		
FET	0.733	0.240-2.238	0.586
BMI (kg/m²)	1.129	1.036-1.230	**0.006**
Maternal age (years)	1.101	0.960-1.263	0.169
Embryo stage			
Cleavage	Reference		
Blastocyst	0.626	0.212-1.846	0.395
No. of embryos transferred			
SET	Reference		
DET	1.525	0.570-4.082	0.401
Fertilization method			
IVF	Reference		
ICSI	2.058	0.791-5.354	0.139
Gestational Diabetes Mellitus			
Embryo transfer strategy			
Fresh ET	Reference		
FET	1.359	0.595-3.107	0.467
BMI (kg/m²)	1.137	1.065-1.214	**<0.001**
Maternal age (years)	1.151	1.038-1.276	**0.008**
Embryo stage			
Cleavage	Reference		
Blastocyst	0.798	0.341-1.872	0.604
No. of embryos transferred			
SET	Reference		
DET	0.879	0.435-1.776	0.719
Fertilization method			
IVF	Reference		
ICSI	0.605	0.303-1.208	0.154

aOR – adjusted Odds Ratio; CI – Confidence Interval; HDP – Hypertensive Disorders of Pregnancy; GDM – Gestational Diabetes Mellitus; FET – Frozen- thawed Embryo Transfer; Fresh ET – Fresh Embryo Transfer; BMI – Body Mass Index; IVF – *In vitro* fertilization; ICSI – Intracytoplasmic Sperm Injection; SET – Single Embryo Transfer; DET – Double Embryo Transfer.

Table VIII Multivariable linear regression analysis for birth weight in singleton pregnancies. Adjusted $R^2 = 0.481$.

Birth weight	B (g)	95% CI	*p*- value
Embryo transfer strategy			
Fresh ET	Reference		
FET	+266.86	177.12-356.61	**< 0.001**
Gestational age (weeks)	+ 204.85	183.51-226.18	**< 0.001**
BMI (kg/m²)	+ 9.45	1.03-17.88	**0.028**
Maternal age (years)	-5.50	-16.32-5.33	0.319
Smoking habits			
Non-smokers	Reference		
Smokers	+0.88	-88.80-90.55	0.985
Embryo stage			
Cleavage	Reference		
Blastocyst	-9.47	-104.83-85.89	0.845
No. of embryos transferred			
SET	Reference		
DET	-22.07	-103.59-59.45	0.595
Fertilization method			
IVF	Reference		
ICSI	-3.15	-80.92-74.61	0.937

B – unstandardized regression coefficient; CI – Confidence Interval; FET – Frozen-thawed Embryo Transfer; Fresh ET – Fresh Embryo Transfer; BMI – Body Mass Index; IVF – *In Vitro* Fertilization; ICSI – Intracytoplasmic Sperm Injection; SET – Single Embryo Transfer; DET – Double Embryo Transfer.

Table IXa Multivariable regression analysis for birth weight classification in singleton pregnancies - Model 1: adjusted for gestational age, BMI, maternal age, smoking, and IVF vs ICSI, without adjustment for embryo stage or number of embryos transferred. Nagelkerke R^2 = 0.502. Reference category: Normal birth weight.

Birth weight classification	aOR	95% CI	*p*- value
LBW vs Normal			
Embryo transfer strategy			
FET	Reference		
Fresh ET	3.388	1.352-8.493	**0.009**
Gestational age (weeks)	0.247	0.172-0.356	**< 0.001**
BMI (kg/m²)	0.931	0.843-1.029	0.160
Maternal age (years)	1.115	0.981-1.266	0.095
Smoking habits			
Smokers	Reference		
Non-smokers	1.216	0.419-3.535	0.719
Fertilization method			
ICSI	Reference		
IVF	0.576	0.232-1.432	0.235
HBW vs Normal			
Embryo transfer strategy			
FET	Reference		
Fresh ET	0.370	0.121-1.129	0.081
Gestational age (weeks)	2.014	1.224-3.313	**0.006**
BMI (kg/m²)	1.053	0.946-1.171	0.345
Maternal age (years)	0.907	0.796-1.033	0.142
Smoking habits			
Smokers	Reference		
Non-smokers	1.785	0.472-6.749	0.393
Fertilization method			
ICSI	Reference		
IVF	0.856	0.303-2.415	0.769

aOR – adjusted Odds Ratio; CI – Confidence Interval; LBW – Low Birth Weight (< 2500 g); HBW – High Birth Weight (> 4000 g); FET – Frozen-thawed Embryo Transfer; Fresh ET – Fresh Embryo Transfer; BMI – Body Mass Index; IVF – *In vitro* fertilization; ICSI – Intracytoplasmic Sperm Injection. SET – Single Embryo Transfer; DET – Double Embryo Transfer.

Table IXb Multivariable regression analysis for birth weight classification in singleton pregnancies - Model 2: additionally adjusted for embryo stage and number of embryos transferred. Nagelkerke $R^2 = 0.507$. Reference category: Normal birth weight.

Birth weight classification	aOR	95% CI	*p*- value
LBW vs Normal			
Embryo transfer strategy			
FET	Reference		
Fresh ET	2.260	0.724-7.054	0.160
Gestational age (weeks)	0.245	0.170-0.353	**< 0.001**
BMI (kg/m²)	0.935	0.846-1.032	0.182
Maternal age (years)	1.094	0.955-1.253	0.197
Smoking habits			
Smokers	Reference		
Non-smokers	1.323	0.445-3.936	0.615
Fertilization method			
ICSI	Reference		
IVF	0.601	0.239-1.509	0.278
Embryo stage			
Blastocyst	Reference		
Cleavage	2.167	0.654-7.179	0.206
No. of embryos transferred			
DET	Reference		
SET	1.210	0.470-3.112	0.693
HBW vs Normal			
Embryo transfer strategy			
FET	Reference		
Fresh ET	0.351	0.102-1.210	0.097
Gestational age (weeks)	1.997	1.208-3.302	**0.007**
BMI (kg/m²)	1.054	0.946-1.173	0.340
Maternal age (years)	0.906	0.795-1.034	0.144
Smoking habits			
Smokers	Reference		
Non-smokers	1.804	0.474-6.858	0.387
Fertilization method			
ICSI	Reference		
IVF	0.845	0.297-2.404	0.752
Embryo stage			
Blastocyst	Reference		
Cleavage	1.113	0.302-4.105	0.872
No. of embryos transferred			
DET	Reference		
SET	0.953	0.305-2.976	0.933

aOR – adjusted Odds Ratio; CI – Confidence Interval; LBW – Low Birth Weight (< 2500 g); HBW – High Birth Weight (> 4000 g); FET – Frozen- thawed Embryo Transfer; Fresh ET – Fresh Embryo Transfer; BMI – Body Mass Index; IVF – *In Vitro* Fertilization; ICSI – Intracytoplasmic Sperm Injection. SET – Single Embryo Transfer; DET – Double Embryo Transfer.

Table X Multivariable logistic regression analysis for mode of delivery in singleton pregnancies. Nagelkerke $R^2 = 0.048$. Reference category: Eutocic delivery.

Mode of delivery	aOR	95% CI	*p*- value
Embryo transfer strategy			
Fresh ET	Reference		
FET	1.653	1.029-2.653	**0.037**
Birth weight (g)	1.001	1.000-1.001	**0.037**
Gestational age (weeks)	0.884	0.744-1.051	0.162
BMI (kg/m²)	0.970	0.925-1.018	0.221
Maternal age (years)	1.021	0.961-1.086	0.497
Fertilization method			
IVF	Reference		
ICSI	0.893	0.570-1.399	0.621

aOR – adjusted Odds Ratio; CI – Confidence Interval; FET – Frozen- thawed Embryo Transfer; Fresh ET – Fresh Embryo Transfer; BMI – Body Mass Index; IVF – *In Vitro* Fertilization; ICSI – Intracytoplasmic Sperm Injection.

REFERENCES

1. Zegers-Hochschild F, Adamson GD, Dyer S, et al. The International Glossary on Infertility and Fertility Care, 2017. Fertil Steril. 2017;108(3):393–406.

2. World Health Organization. Infertility. Geneva: WHO; Available from: https://www.who.int/news-room/fact-sheets/detail/infertility. Last accessed 2026/05/31.

3. Gnoth C, Godehardt E, Frank-Herrmann P, Friol K, Tigges J, Freundl G. Definition and prevalence of subfertility and infertility. Hum Reprod. 2005;20(5):1144–7.

4. Giudice LC. Environmental toxicants: hidden players on the reproductive stage. Fertil Steril. 2016;106(4):791–4.

5. Zaat T, Zagers M, Mol F, Goddijn M, van Wely M, Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database of Systematic Reviews. 2021(2):CD011184.

6. Baker VL, Dyer S, Chambers GM, et al. International Committee for Monitoring Assisted Reproductive Technologies (ICMART): world report for cycles conducted in 2017-2018. Hum Reprod. 2025;40(6):1110–26.

7. Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? Hum Reprod Update. 2018;24(1):35–58.

8. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature. 1983;305(5936):707–9.

9. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertility and Sterility. 2012;98(2):368–77.e9.

10. Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. Hum Reprod. 2011;26(10):2593–7.

11. Mourad S, Brown J, Farquhar C. Interventions for the prevention of OHSS in ART cycles: an overview of Cochrane reviews. Cochrane Database Syst Rev. 2017;1(1):Cd012103.

12. Maheshwari A, Bell JL, Bhide P, et al. Elective freezing of embryos versus fresh embryo transfer in IVF: a multicentre randomized controlled trial in the UK (E-Freeze). Hum Reprod. 2022;37(3):476–87.

13. Roque M, Valle M, Sampaio M, Geber S. Obstetric outcomes after fresh versus frozen-thawed embryo transfers: A systematic review and meta-analysis. JBRA Assist Reprod. 2018;22(3):253–60.

14. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfers in high responders. Fertil Steril. 2011;96(2):516–8.

15. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. Fertil Steril. 2011;96(2):344–8.

16. Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. Fertil Steril. 2014;101(1):128–33.

17. Wei D, Liu JY, Sun Y, et al. Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial. Lancet. 2019;393(10178):1310–8.

18. Vuong LN, Dang VQ, Ho TM, et al. IVF Transfer of Fresh or Frozen Embryos in Women without Polycystic Ovaries. N Engl J Med. 2018;378(2):137–47.

19. Chen ZJ, Shi Y, Sun Y, et al. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome. N Engl J Med. 2016;375(6):523–33.

20. Conrad KP. Emerging role of relaxin in the maternal adaptations to normal pregnancy: implications for preeclampsia. Semin Nephrol. 2011;31(1):15–32.

21. Saito K, Kuwahara A, Ishikawa T, et al. Endometrial preparation methods for frozen-thawed embryo transfer are associated with altered risks of hypertensive disorders of pregnancy, placenta accreta, and gestational diabetes mellitus. Hum Reprod. 2019;34(8):1567–75.

22. Zhang B, Wei D, Legro RS, et al. Obstetric complications after frozen versus fresh embryo transfer in women with polycystic ovary syndrome: results from a randomized trial. Fertil Steril. 2018;109(2):324–9.

23. Williamson C, Geenes V. Intrahepatic cholestasis of pregnancy. Obstet Gynecol. 2014;124(1):120–33.

24. Yang J, Gao J, Zhou D, et al. Comparing risk factors and neonatal outcomes in women with intrahepatic cholestasis of pregnancy between assisted reproductive technology and spontaneous conception. Int J Gynaecol Obstet. 2025;168(2):663–72.

25. Spijkers S, Lens JW, Schats R, Lambalk CB. Fresh and Frozen-Thawed Embryo Transfer Compared to Natural Conception: Differences in Perinatal Outcome. Gynecol Obstet Invest. 2017;82(6):538–46.

26. Raja EA, Bhattacharya S, Maheshwari A, McLernon DJ. Comparison of perinatal outcomes after frozen or fresh embryo transfer: separate analyses of singleton, twin, and sibling live births from a linked national in vitro fertilization registry. Fertil Steril. 2022;118(2):323–34.

27. Pinborg A, Loft A, Aaris Henningsen AK, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995-2006. Fertil Steril. 2010;94(4):1320–7.

28. Pelkonen S, Hartikainen AL, Ritvanen A, et al. Major congenital anomalies in children born after frozen embryo transfer: a cohort study 1995-2006. Hum Reprod. 2014;29(7):1552–7.

29. Alviggi C, Conforti A, Carbone IF, Borrelli R, de Placido G, Guerriero S. Influence of cryopreservation on perinatal outcome after blastocyst- vs cleavage-stage embryo transfer: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2018;51(1):54–63.

30. Maheshwari A, Kalampokas T, Davidson J, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of blastocyst-stage versus cleavage-stage embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril. 2013;100(6):1615–21.e1–10.

31. Glujovsky D, Cornelisse S, Blake D, Quinteiro Retamar AM, Alvarez Sedo CR, Ciapponi A. Blastocyst-stage versus cleavage-stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev. 2026;1(1):CD002118.

32. Martins WP, Nastri CO, Rienzi L, van der Poel SZ, Gracia CR, Racowsky C. Obstetrical and perinatal outcomes following blastocyst transfer compared to cleavage transfer: a systematic review and meta-analysis. Hum Reprod. 2016;31(11):2561–9.

33. Gerris J, De Neubourg D, Mangelschots K, et al. Elective single day 3 embryo transfer halves the twinning rate without decrease in the ongoing pregnancy rate of an IVF/ICSI programme. Hum Reprod. 2002;17(10):2626–31.

34. Thurin A, Hausken J, Hillensjö T, et al. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. N Engl J Med. 2004;351(23):2392–402.

35. Gordijn SJ, Beune IM, Thilaganathan B, et al. Consensus definition of fetal growth restriction: a Delphi procedure. Ultrasound Obstet Gynecol. 2016;48(3):333–9.

36. European Concerted Action on Congenital Anomalies and Twins (EUROCAT). EUROCAT Guide 1.5: Instructions for the Registration of Congenital Anomalies. European Commission, Joint Research Centre; 2022.

37. Wang SF, Seifer DB. Age-related increase in live-birth rates of first frozen thaw embryo versus first fresh transfer in initial assisted reproductive technology cycles without PGT. Reprod Biol Endocrinol. 2024;22(1):42.

38. Sole KB, Staff AC, Laine K. Maternal diseases and risk of hypertensive disorders of pregnancy across gestational age groups. Pregnancy Hypertens. 2021;25:25–33.

39. Yong HY, Mohd Shariff Z, Mohd Yusof BN, et al. Independent and combined effects of age, body mass index and gestational weight gain on the risk of gestational diabetes mellitus. Sci Rep. 2020;10(1):8486.

40. Wei D, Sun Y, Zhao H, et al. Frozen versus fresh embryo transfer in women with low prognosis for in vitro fertilisation treatment: pragmatic, multicentre, randomised controlled trial. Bmj. 2025;388:e081474.

41. Practice Committee of the American Society for Reproductive Medicine, Practice Committee of the Society for Assisted Reproductive Technology. Blastocyst culture and transfer in clinically assisted reproduction: a committee opinion. Fertility and Sterility. 2018;110(7):1246–52.

42. Practice Committee of the Society for Reproductive Endocrinology and Infertility, Quality Assurance Committee of the Society for Assisted Reproductive Technology, Practice Committee of the American Society for Reproductive Medicine. Multiple gestation associated with infertility therapy: a committee opinion. Fertility and Sterility. 2022;117(3):498–511.

43. Practice Committees of the American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. Intracytoplasmic sperm injection (ICSI) for non-male factor indications: a committee opinion. Fertility and Sterility. 2020;114(2):239–45.

44. Dang VQ, Vuong LN, Luu TM, et al. Intracytoplasmic sperm injection versus conventional in-vitro fertilisation in couples with infertility in whom the male partner has normal total sperm count and motility: an open-label, randomised controlled trial. Lancet. 2021;397(10284):1554–63.

45. Keyhan S, Truong T, Li YJ, Jackson-Bey T, Eaton JL. Preterm Delivery and Low Birth Weight Among Neonates Conceived With Intracytoplasmic Sperm Injection Compared With Conventional In Vitro Fertilization. Obstet Gynecol. 2018;131(2):262–8.

46. Wang Y, Li R, Yang R, et al. Intracytoplasmic sperm injection versus conventional in-vitro fertilisation for couples with infertility with non-severe male factor: a multicentre, open-label, randomised controlled trial. Lancet. 2024;403(10430):924–34.

47. Ma S, Peng Y, Hu L, et al. Comparisons of benefits and risks of single embryo transfer versus double embryo transfer: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2022;20(1):20.

48. Rodriguez-Wallberg KA, Palomares AR, Nilsson HP, Oberg AS, Lundberg F. Obstetric and Perinatal Outcomes of Singleton Births Following Single- vs Double-Embryo Transfer in Sweden. JAMA Pediatrics. 2023;177(2):149–59.

49. Marconi N, Allen CP, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes of singleton pregnancies after blastocyst-stage embryo transfer compared with those after cleavage-stage embryo transfer: a systematic review and cumulative meta-analysis. Hum Reprod Update. 2022;28(2):255–81.

50. Moreno Sepulveda JA, Santucci P, Checa MA. Higher risk of hypertensive disorders of pregnancy and preeclampsia in pregnancies following frozen embryo transfer: a systematic review and meta-analysis. GREM Gynecological and Reproductive Endocrinology & Metabolism. 2021;2(2):74–84.

51. Gu F, Wu Y, Tan M, et al. Programmed frozen embryo transfer cycle increased risk of hypertensive disorders of pregnancy: a multicenter cohort study in ovulatory women. Am J Obstet Gynecol MFM. 2023;5(1):100752.

52. von Versen-Höynck F, Schaub AM, Chi YY, et al. Increased Preeclampsia Risk and Reduced Aortic Compliance With In Vitro Fertilization Cycles in the Absence of a Corpus Luteum. Hypertension. 2019;73(3):640–9.

53. Chen H, Ma S, Liu Y, et al. Embryo Culture Duration Is an Independent Risk Factor for Gestational Diabetes in Frozen Embryo Transfer Pregnancies. J Endocr Soc. 2026;10(2):bvaf161.

54. Sha T, Yin X, Cheng W, Massey IY. Pregnancy-related complications and perinatal outcomes resulting from transfer of cryopreserved versus fresh embryos in vitro fertilization: a meta-analysis. Fertil Steril. 2018;109(2):330–42.e9.

55. Zhang S, Liu H, Li N, et al. Relationship between gestational body mass index change and the risk of gestational diabetes mellitus: a community-based retrospective study of 41,845 pregnant women. BMC Pregnancy and Childbirth. 2022;22(1):336.

56. Smeenk J, Wyns C, De Geyter C, et al. ART in Europe, 2019: results generated from European registries by ESHRE†. Hum Reprod. 2023;38(12):2321–38.

57. Mascarenhas M, Kamath MS, Muthukumar K, Mangalaraj AM, Chandy A, Aleyamma T. Obstetric outcomes of monochorionic pregnancies conceived following assisted reproductive technology: A retrospective study. J Hum Reprod Sci. 2014;7(2):119–24.

58. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75–84.

59. Shavit M, Miller N, Schreiber H, et al. Twin pregnancies and perinatal outcomes: a comparison between fresh and frozen embryo transfer: a two-centre study. Reprod Biomed Online. 2019;38(2):241–8.

60. Tocariu R, Niculae LE, Niculae A, Carp-Velișcu A, Brătilă E. Fresh versus Frozen Embryo Transfer in In Vitro Fertilization/Intracytoplasmic Sperm Injection Cycles: A Systematic Review and Meta-Analysis of Neonatal Outcomes. Medicina (Kaunas). 2024;60(8).

61. Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique? Hum Reprod. 2014;29(3):618–27.

62. Wang Z, Xu L, He F. Embryo vitrification affects the methylation of the H19/Igf2 differentially methylated domain and the expression of H19 and Igf2. Fertil Steril. 2010;93(8):2729–33.

63. Rosalik K, Carson S, Pilgrim J, et al. Effects of different frozen embryo transfer regimens on abnormalities of fetal weight: a systematic review and meta-analysis. Hum Reprod Update. 2021;28(1):1–14.

64. Lodge-Tulloch NA, Elias FTS, Pudwell J, et al. Caesarean section in pregnancies conceived by assisted reproductive technology: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2021;21(1):244.

65. Terada S, Habara T, Terada R, et al. Risks of neonatal anomalies and obstetric complications in 7378 singleton births after frozen-thawed and fresh embryo transfers in Japan: An analysis using doubly robust estimation. Reprod Med Biol. 2025;24(1):e12623.

66. Sargisian N, Petzold M, Furenäs E, et al. Congenital heart defects in children born after assisted reproductive technology: a CoNARTaS study. Eur Heart J. 2024;45(45):4840–58.

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