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Cristiana Filipa Ribeiro e Silva

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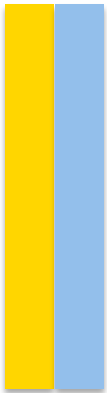

Association between serum progesterone levels on the day of frozen-thawed embryo transfer and pregnancy and neonatal outcomes

Dissertação de candidatura ao grau de Mestre em Medicina, submetida ao Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

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RESUMO

Introdução: Os níveis séricos de progesterona (P) no dia da transferência de embriões criopreservados (TEC) parecem estar associados ao sucesso do tratamento. Alguns estudos sugerem que baixos níveis de P podem ter um efeito prejudicial na evolução da gravidez, enquanto outros descrevem um efeito deletério perante valores elevados de P. Apesar do limiar de 10 ng/mL ser o mais relatado, por ser o valor atualmente aceite para a função fisiológica do corpo lúteo, ainda não é evidente o limiar ideal de P no dia da TEC em relação aos desfechos, possivelmente devido às diferenças dos protocolos entre centros.

Objetivos: O estudo tem como principal objetivo determinar um valor preditivo de P sérico em ciclos de TEC, a partir do qual as taxas de gravidez não diminuem. Pretende ainda estabelecer uma associação entre os níveis séricos de P no dia da TEC e as características basais e os desfechos gestacionais e neonatais. Visa também identificar as complicações obstétricas mais frequentes.

Métodos: Um estudo retrospetivo incluindo 313 mulheres que realizaram TEC e para as quais se determinou o P sérico no dia da TEC, entre novembro de 2021 e julho de 2023, foi realizado no Centro de Procriação Medicamente Assistida do Centro Materno-Infantil do Norte. Três limiares diferentes foram considerados na análise da relação entre os níveis de P e os desfechos da TEC: (1) 10 ng/mL; (2) quatro quartis (Q) de acordo com os percentis 25, 50 e 75 (Q1<7.30; Q2:7.30-10.26; Q3:10.27–13.42; Q4>13.42 ng/mL); e (3) valor limiar ideal de P para predição da taxa de gravidez clínica obtido a partir de uma curva ROC (9.34 ng/mL). Os testes T-test ou One-Way ANOVA foram utilizados para comparar variáveis contínuas. Utilizou-se o teste qui-quadrado ou o teste de Fisher para comparar as variáveis categóricas. Realizou-se ainda uma regressão logística multivariada.

Resultados: Para o limiar de P de 10 ng/mL, mulheres com P <10 ng/mL tiveram menores taxas de teste β-hCG positivo (p=0.020), de implantação (p=0.002) e de gravidez clínica (p=0.019). Avaliando os níveis de P em Q, não se obteve resultados significativos. A comparação dos desfechos de acordo com P <9.34 ng/mL e ≥9.34 ng/mL revelou resultados significativos na taxa de teste β-hCG positivo (38.5% vs. 52.8%, p=0.012), taxa de implantação (30.0% vs. 45.5%, p=0.002), taxa de gravidez clínica (36.3% vs. 50.0%, p=0.016), taxa de gravidez evolutiva (24.4% vs. 37.6%, p=0.013) e taxa de nados-vivos (24.4% vs. 37.6 %, p=0.013). A análise multivariada mostrou resultados significativos para P ≥9.34 ng/mL como fator prognóstico independente de desfechos gestacionais. O abortamento precoce, o parto pré-termo e as anomalias placentárias foram as complicações mais frequentes.

Conclusão: Níveis séricos de P no dia da TEC ≥9.34 ng/mL, um limiar semelhante aos relatados previamente, conferiram uma melhoria estatisticamente significativa nos desfechos gestacionais.

Palavras-chave: Progesterona, Transferência de embriões criopreservados, Desfechos reprodutivos.

ABSTRACT

Introduction: Serum progesterone (P) levels on the day of frozen-thawed embryo transfer (FET) seem to be associated with treatment success. Some studies suggest that low P levels may have a detrimental effect on pregnancy evolution, while others describe a deleterious effect of high P values. Despite of P threshold of 10 ng/mL being the most reported, as it is the currently accepted level for physiological corpus luteal function, it is still unclear the optimal P cut-off point on the FET day in relation to outcomes, possibly due to the protocols disparities between centers.

Objectives: The main aim of this study is to determine a predictive value of serum P in FET cycles, from which pregnancy rates do not decrease. It aims to establish the association between serum P levels on the FET day and patients' baseline characteristics, and pregnancy and neonatal outcomes. Additionally, it aims to identify the most frequent obstetric complications.

Methods: A retrospective cohort study comprising 313 women who underwent FET and collected serum P on the FET day, between November 2021 and July 2023, was performed at the Centre of Assisted Medical Procreation of *Centro Materno-Infantil do Norte*. Three different thresholds were considered in assessing the relationship between P levels and FET outcomes: (1) 10 ng/mL; (2) four quartiles according to the 25th, 50th and 75th percentiles (Q1<7.30; Q2:7.30-10.26; Q3:10.27–13.42; Q4>13.42 ng/mL); (3) the optimum P cut-off value for prediction of the clinical pregnancy rate obtained from a ROC curve (9.34 ng/mL). T-test or One-Way ANOVA were used to compare continuous variables. Chi-square test or Fisher's exact test were used to compare categorical variables between groups. The relationship between serum P level and pregnancy outcomes was evaluated by univariate and multivariate logistic regression analysis.

Results: Considering the P threshold of 10 ng/mL, patients with P <10 ng/mL had a significantly lower positive β-hCG test rate (p=0.020), implantation rate (p=0.002), and clinical pregnancy rate (p=0.019). By assessing the P levels into Q, no significant results were found. The comparison of FET outcomes according to P levels <9.34 ng/mL and ≥9.34 ng/mL showed significant results in positive β-hCG test rate (38.5% vs. 52.8%, p=0.012), implantation rate (30.0% vs. 45.5%, p=0.002), clinical pregnancy rate (36.3% vs. 50.0%, p=0.016), ongoing pregnancy rate (24.4% vs. 37.6%, p=0.013), and live birth rate (24.4% vs. 37.6%, p=0.013). Multivariate analysis demonstrated a significant result for P levels ≥9.34 ng/mL as an independent prognostic factor for pregnancy outcomes. Early miscarriage, preterm birth and placental disorders were the most frequent obstetric complications.

Conclusions: This study shows that P levels on day of FET ≥9.34 ng/mL, a similar cutoff point to those previously reported, conferred a statistically significant improvement in pregnancy outcomes.

Keywords: Progesterone, Frozen embryo transfer, Reproductive outcomes.

LIST OF ABBREVIATIONS

ART	Assisted reproductive technology
AUC	Area under the curve
BMI	Body mass index
CI	Confidence interval
CPR	Clinical pregnancy rate
FET/TEC	Frozen-thawed embryo transfer
hCG	Human chorionic gonadotrophin
HRT	Hormone replacement therapy
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilization
LBR	Live birth rate
LBW	Low birthweight
OPR	Ongoing pregnancy rate
OR	Odds ratio
P	Progesterone
PTB	Preterm birth
Q	Quartile
ROC	Receiver operating characteristic

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Infertility affects millions of individuals worldwide, with approximately one in six people experiencing infertility in their lifetimes[1]. It is commonly described as a disease of the male or female reproductive systems defined by the failure to achieve a pregnancy after, at least, 12 months of regular unprotected sexual intercourse[2]. Given the global burden of infertility, namely the devastating societal and health consequences, including social stigma, economic hardship, and poor physical and mental wellbeing, there is an urgent need for improved efforts to address it[3].

Over the last few decades, the use of assisted reproductive technology (ART) has increased globally and has made pregnancy possible for many couples[4]. ART comprises all fertility treatments in which oocytes are surgically removed from the ovary, fertilized with spermatozoa, and transferred to the uterus[5]. Current ART procedures include *in vitro* fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI), and there are situational indications for either fresh or frozen embryo transfer[5].

Frozen-thawed embryo transfer (FET) has been widely used in ART since the adoption of more advanced cryopreservation techniques[6]. FET enables the excess embryos derived from IVF or ICSI treatment to be stored and utilized at a later date[7]. This minimizes wastage and increases the likelihood of conceiving after one cycle of ovarian stimulation and oocyte retrieval, besides from improving clinical outcomes[7]. The "freeze-all'' strategy followed by FET is a promising approach to avoid embryo-endometrial asynchrony in fresh cycles, minimize the risk of ovarian hyperstimulation syndrome, implement preimplantation genetic testing, increase cumulative pregnancy rates, and achieve high live birth rates (LBR)[8]. Nowadays, the FET procedure accounts for 25% of births resulting from ART, and high-quality embryos are crucial to significantly increase the chance of successful pregnancy and live birth in FET cycles besides good coordination between the transferred embryo and the endometrium in the pre-implantation cycle[9].

Endometrial preparation is an important stage in FET cycles and it has received increased attention as clinicians seek to improve pregnancy outcomes[6,8]. Several cycle regimens are available, including true natural cycle with spontaneous ovulation, modified natural cycle using human chorionic gonadotrophin (hCG) to induce ovulation, hormone replacement therapy (HRT) cycle with or without gonadotropin-releasing hormone agonist downregulation, and ovarian stimulation cycle with or without letrozole[8]. HRT is one of the most used endometrial preparation protocols for FET and it aims to reproduce the physiological hormonal milieu transforming the endometrium into a secretory one through sequential administration of estrogen and progesterone (P)[6,10]. Artificial FET cycles are oftentimes adopted over natural cycles for medical reasons, such as in the case of

anovulation, and for practical benefits, as they are more flexible and convenient, allowing an easier programming of the embryo thawing and transfer and resulting in a lower cancellation rate compared to natural cycles[6,8]. However, HRT cycles have been associated with an increased risk of some adverse maternal and fetal outcomes, such as pregnancy-related hypertensive disorders, preterm or post-term delivery and macrosomia, caused by exogenous estrogen supplementation and the absence of corpus luteum as a result of anovulation[8,11]. Therefore, women capable of ovulation are recommended to prioritize natural cycle regimen[8].

P is the main hormone in the luteal phase and it is required for an adequate endometrial transformation, embryo implantation and maintenance of the pregnancy in ART cycles[10,12]. Patients undergoing FET cycles are unable to provide sufficient endogenous P and need P supplementation to establish and maintain a functional secretory endometrium and pregnancy[13]. Different routes of P administration are available, including oral, intramuscular, vaginal, retal and subcutaneous preparation. However, the efficacy, safety, and tolerability of exogenous P depend on the route of administration[8]. Over the past decades, vaginal and intramuscular routes have been preferentially used, whereas oral P, despite being convenient, has been generally avoided due to low bioavailability caused by hepatic first-pass effect and reduced efficacy in ART[14]. However, dydrogesterone, a synthetic progestin with enhanced oral bioavailability, has been frequently used as a complement to other administration routes to improve pregnancy outcomes[8,14]. Intramuscular injection is often used in FET cycles to achieve both a high serum level and a high clinical pregnancy rate (CPR)[9] but most patients prefer vaginal over intramuscular P administration for greater convenience, ease of use, and less pain[8,14]. Despite the unquestionable importance of P on artificial endometrial preparation, there is still no standard protocol for HRT[7,15] due to the lack of consensus on the optimal route of delivery, type, dose, and duration of P supplementation to increase the success of FET, or how to monitor its efficacy[6,16].

It has been reported that serum P levels around the time of FET seem to be associated with treatment success[13,17]. Recent studies suggest that low serum P levels on the FET day may have a significant detrimental effect on pregnancy evolution, and some of them postulate that women may benefit from additional P supplementation to achieve optimal luteal phase support[17,18]. However, other studies showed a deleterious effect of high serum P levels on LBR[13,19]. Despite of P threshold of 10 ng/mL being the most reported in literature, as it is the currently accepted indicator of adequate corpus luteal function[20], it is still unclear the optimal serum P cut-off point on the day of embryo transfer in relation to pregnancy rates and early pregnancy loss among women undergoing FET cycles[6,12,18]. Many factors could account for the heterogeneous findings. Specifically, the lack of uniformity in P supplementation protocols between centers may impact the P levels obtained[6], suggesting each center should define its own threshold.

Given the heterogeneity and limited strength of the literature available in this area, the current study aims to investigate a predictive value of serum P in cryopreserved embryo transfer cycles in our center, from which pregnancy rates do not decrease. To this end, we aim to determine the association between serum P levels, measured on the day of FET, and pregnancy and neonatal outcomes, in women undergoing FIV or ICSI techniques with transfer of frozen-thawed embryos at different stages of development. Furthermore, we aim to evaluate patient's baseline characteristics according to serum P levels on the FET day. Lastly, the most frequent obstetric complications during pregnancy, childbirth and postpartum will be identified.

MATERIALS AND METHODS

Study design

A retrospective cohort study was performed at the Centre of Assisted Medical Procreation of Centro Materno-Infantil do Norte Dr. Albino Aroso, comprising all FET between November 2021 and July 2023. All participants provided informed consent after counselling for infertility treatments and routine IVF/ICSI procedures and after being informed about the possible anonymous use of their clinical data in scientific studies. The study was approved by the Department of Education, Training and Research and the Ethics Committee of Unidade Local de Saúde de Santo António, with the registration number N/REF.ª2023-238 (201-DEFI/190-CE).

Study population

A total of 417 FET cycles were performed in the described period, 313 of them meeting the inclusion criteria. Inclusion criteria comprise patients who underwent HRT-FET with their own oocytes and had serum P levels measured on the FET day. Exclusion criteria include patients with known uterine abnormalities. Likewise, cases with missing data were excluded.

Study protocol

Endometrial preparation

Endometrial preparation was induced with sequential provision of oral estradiol (Zumenon® or Estrofem®) 2mg/8h, starting 15 days after the subcutaneous administration of goserelin acetate (Zoladex®) 3,6mg/24h in the midluteal phase of the preceding cycle. After 7 days of estrogen therapy, a transvaginal ultrasound scan was performed to assess endometrial thickness. In patients whose endometrial thickness was <8 mm, oral estradiol 2mg/6h was extended for as long as 4 days if required. If a triple-line endometrium of ≥8 mm thickness was observed, exogenous P supplementation was initiated as micronised P vaginal capsules (Cyclogest®) 400mg/12h. Due to the absence of a corpus luteum and endogenous sex steroid production, exogenous hormone therapy was continued until 12 completed weeks of gestation.

Embryo transfer

Embryos were previously generated from IVF or ICSI cycles and vitrified on day 3 or at the blastocyst stage. The quality of the embryos was determined through morphological assessment methods, with the embryologist's decision being the last step in choosing the embryo to be

transferred. Embryo transfer was performed 3 or 5-6 days after initial P administration depending on the embryo stage. A blood sample was obtained between 8 am and 10 am on the FET day for serum P measurement. Preferably, one embryo was transferred in each FET cycle. Embryo transfer was performed under ultrasound guidance. The number of embryos transferred and the embryo stage at transfer were recorded. An hCG test was performed 14 days after FET and ultrasound examination was routinely performed 28 days after FET.

General characteristics of included patients

The patients' baseline characteristics were obtained from the database of the Centre of Assisted Medical Procreation. These included maternal age, maternal body mass index (BMI), duration of infertility, type of infertility (primary, secondary), infertility diagnosis (female factor, male factor, combined or unexplained), fertilization method (IVF, ICSI or IVF+ICSI), number of FET cycles, P level on FET day, number of embryos transferred (single or double), and embryo stage at transfer (cleavage or blastocyst). The duration of infertility was calculated in months, from the time the couple started trying to get pregnant until the day of the FET. Primary infertility was considered when a pregnancy has never been achieved by the couple, and secondary infertility when at least one prior pregnancy has been achieved. Regarding the infertility diagnosis, the classification "Female factor" was assigned to all women who had exclusively female factors, namely tubal factor, endometriosis, ovulatory factor, oocyte factor and uterine factor excluding uterine abnormalities. The “Male factor” group included cases of oligoasthenoteratozoospermia and paraplegia. All cases in which there was at least one female factor and one male factor contributing to the infertility were considered in the “Combined” group. Cases in which there was no apparent cause to justify the couple's infertility were classified as “Unexplained”. However, considering the small number of cases with unexplained infertility, without statistical significance, these were excluded to allow a more accurate analysis of the remaining three categories of infertility causes.

Clinical outcomes

The same database was accessed to obtain the pregnancy and neonatal outcomes needed for this study.

Regarding the pregnancy outcomes, the primary endpoint was the relationship between P levels on the FET day and the LBR per cycle, defined as the proportion of the number of deliveries that resulted in at least one live birth (> 24 weeks gestation) among all transfer cycles. Secondary endpoints included positive β-hCG test rate, biochemical pregnancy rate, implantation rate, CPR, early miscarriage rate and ongoing pregnancy rate (OPR). A β-hCG test was considered positive if

β-hCG was > 25 IU/L. The biochemical pregnancy rate was defined as the proportion of patients with a positive β-hCG test but no gestational sac visible on the ultrasound. The CPR was defined as the proportion of patients with an ultrasound confirmation of at least one intrauterine gestational sac after 6 weeks of gestation, among all embryo transfer cycles. The implantation rate was defined as the number of gestational sacs per transferred embryos. The early miscarriage rate was defined as the proportion of patients with pregnancy lost before 12 completed weeks of gestation. No late miscarriages were found. The OPR was defined as the proportion of patients with a gestational sac with fetal heart activity assessed by ultrasound examination at 13 weeks of gestation, among all embryo transfer cycles.

The neonatal outcomes of the study included the number of neonates (singletons or twins), gestational age at birth, birthweight, preterm birth (PTB), low birthweight (LBW), major congenital malformations and neonatal death. PTB was defined as a birth takes place before 37 completed weeks of gestation. LBW was defined as a birthweight below 2500g. The percentages were determined considering the number of births.

Obstetric complications

The obstetric complications described during pregnancy, childbirth and postpartum for each pregnant woman were obtained from consultation of the respective clinical file, using the "SClínico Hospitalar" computer program. The obstetric complications considered were early miscarriage, hypertensive disorders (gestational hypertension, preeclampsia), endocrine disorders (gestational diabetes, intrahepatic cholestasis of pregnancy), hematological changes (gestational thrombocytopenia, severe anemia), maternal infections, multiple pregnancy, placental disorders (placenta previa, placenta accrete, abruptio placentae), uteroplacental insufficiency (fetal growth restriction, oligohydramnios), cervical insufficiency, preterm premature rupture of membranes, PTB, LBW, congenital abnormalities, neonatal death and maternal postpartum complications (hemorrhage, severe anemia, infections).

Maternal hospitalization during pregnancy and cesarean section were also assessed.

Statistical analysis

Three different thresholds were considered in assessing the relationship between P levels and both baseline characteristics and FET outcomes: (1) 10 ng/mL; (2) four quartiles according to the 25^{th}, 50^{th} and 75^{th} percentiles; and (3) the optimum P cut-off value for prediction of the CPR, obtained by plotting a receiver operating characteristic (ROC) curve and comparing sensitivity and specificity.

Continuous variables were presented as the mean ± standard deviation, and the T-test or One-Way ANOVA were used to compare continuous variables. APGAR was presented as median (interquartile range). Categorical variables were described as the frequency with the percentage, and between-group differences were assessed by Chi-square test or Fisher's exact test. The relationship between serum P level and pregnancy outcomes was evaluated by univariate and multivariate logistic regression analysis. To adjust for potential confounders, those variables were introduced into the regression model. These included maternal age, maternal BMI, duration of infertility, infertility diagnosis, P level on FET day, number of embryos, and embryo stage at transfer. The obstetric complications were assessed through descriptive analysis.

All P values were based on two-sided tests, and $p<0.05$ was considered statistically significant.

The statistical analysis was performed using IBM SPSS Statistics version 29.0 software.

RESULTS

A total of 313 cycles were analyzed in terms of demographic characteristics and reproductive outcomes. Patient's mean age was 34.2 ± 3.9 years and had a BMI of 24.8 ± 4.6 kg/m^2. The main indication for FET was freeze-all cycles. There was a wide range of observed P levels on the day of FET (0.12–39.57 ng/mL), with a mean value of 10.98 ± 6.37 ng/mL.

Considering the P threshold of 10 ng/mL, almost no statistical results were found in patients' baseline characteristics (Table I). The two groups, P<10 ng/mL versus P≥10 ng/mL, differed significantly in terms of maternal BMI (25.37 ± 4.71 versus 24.27 ± 4.38 (p=0.034)). No significant differences were found when the remaining characteristics were analyzed. The mean serum P values on the FET day were 6.49±2.62 and 15.38±5.87 ng/mL in the P<10 ng/mL and P≥10 ng/mL groups, respectively (p<0.001). Statistical results were also found in pregnancy outcomes (Table II). Patients with serum P levels on the FET day that were <10 ng/mL had a significantly lower positive β-hCG test rate, implantation rate and CPR than the other patients (P≥10 ng/mL): (62/155, 40.0%) versus (84/158, 59.5%) (p=0.020); (60/193, 31.1%) versus (91/197, 46.2%) (p=0.002); (58/155, 37.4%) versus (80/158, 50.6%) (p=0.019), respectively. No significant differences were found in biochemical pregnancy rate, early miscarriage rate, OPR and LBR between the two cohorts. Additionally, no significant differences were found when neonatal outcomes were analyzed (Table III).

The serum P intervals for each quartile were as follows: Q1 < 7.30 ng/mL; Q2: 7.30–10.26 ng/mL; Q3: 10.27–13.42 ng/mL; and Q4 > 13.42 ng/mL. The mean serum P values on the FET day were 4.41±2.07, 8.63±0.82, 11.79±0.97 and 19.20±6.43 ng/mL in the Q1, Q2, Q3 and Q4 groups, respectively (p<0.001). By assessing the P levels into quartiles, no significant results were found between them when baseline characteristics and reproductive outcomes were analyzed (Tables IV, V and VI).

Thereafter, a ROC curve showed a significant predictive value of serum P levels on the day of embryo transfer for CPR, with an AUC (95% CI) = 0.587 (0.523–0.650) (Figure 1). The optimal cut-off value for prediction of the CPR was a P level of 9.34 ng/mL (64.5% sensitivity, 49.1% specificity).

Considering the P threshold of 9.34 ng/mL, almost no statistical results were found in patients' baseline characteristics (Table VII). The two groups, P<9.34 ng/mL versus P≥9.34 ng/mL, differed significantly in terms of maternal BMI (25.53 ± 4.77 versus 24.27 ± 4.34 (p=0.015)). No

significant differences were found when the remaining characteristics were analyzed. The mean serum P values on the FET day were 6.02±2.49 and 14.73±5.83 ng/mL in the P<9.34 ng/mL and P≥9.34 ng/mL groups, respectively (p<0.001).

Several statistically significant differences were found when assessing the pregnancy outcomes according to the serum P threshold of 9.34 ng/mL (Table VIII). Patients with serum P levels on the FET day that were <9.34 ng/mL had a significantly lower favorable pregnancy outcomes than the other patients (P≥9.34 ng/mL): positive β-hCG test rate ((52/135, 38.5%) versus (94/178, 52.8%), p=0.012); implantation rate ((51/170, 30.0%) versus (100/220, 45.5%), p=0.002); CPR ((49/135, 36.3%) versus (89/178, 50.0%), p=0.016), OPR ((33/135, 24.4%) versus (67/178, 37.6%), p=0.013), LBR ((33/135, 24.4%) versus (67/178, 37.6%), p=0.013). No significant results were found when biochemical pregnancy rate and early miscarriage rate were analyzed.

In the multivariate analysis, the factors significantly associated with positive β-hCG test were infertility diagnosis combining female and male factors (adjusted OR=2.51, 95% CI: 1.29-4.87, p=0.007), number of embryos transferred (adjusted OR=1.95, 95% CI: 1.08–3.53, p=0.028), and P levels above 9.34 ng/mL on day of FET (adjusted OR=2.04, 95% CI: 1.23–3.37, p=0.006) (Table IX). Regarding CPR, maternal age (adjusted OR = 0.94, 95% CI: 0.88-1.01, p=0.042), infertility diagnosis combining female and male factors (adjusted OR=2.50, 95% CI: 1.28-4.85, p=0.007), number of embryos transferred (adjusted OR=1.98, 95% CI: 1.09–3.58, p=0.024), and P levels above 9.34 ng/mL (adjusted OR=1.92, 95% CI: 1.16–3.19, p=0.011) were significantly associated with this pregnancy outcome. Additionally, the multivariate analysis demonstrated that the factors significantly associated with LBR were maternal age (adjusted OR = 0.92, 95% CI: 0.86-1.00, p=0.036), number of embryos transferred (adjusted OR=2.73, 95% CI: 1.47–5.10, p=0.002), and P levels above 9.34 ng/mL on day of FET (adjusted OR=2.10, 95% CI: 1.20–3.67, p=0.009). Thus, the multivariate analysis demonstrated significant results for P levels above 9.34 ng/mL on day of FET as an independent predictive factor of pregnancy outcomes.

Regarding the neonatal outcomes of live born infants according to the serum P threshold of 9.34 ng/mL, no statistically significant differences were found for any outcome (Table X).

The obstetric complications assessed in this study are presented in Figure 2. In 64.5% of clinical pregnancies there was at least one obstetric complication during pregnancy, childbirth or postpartum. Early pregnancy loss was the most frequent complication (26.0%) followed by PTB (13.7%); placental disorders (9.6%); LBW (8.9%); endocrine disorders (6.8%); maternal infections (6.2%), namely urinary tract infections; maternal postpartum complications (6.2%); uteroplacental insufficiency (4.8%); hypertensive disorders (4.1%); multiple pregnancy (4.1%); cervical insufficiency and/or preterm premature rupture of membranes (3.4%); congenital abnormalities

(2.7%), namely trisomy 21 and congenital heart defect; hematological changes (2.1%); and neonatal death (1.4%).

During pregnancy, 0.07% of pregnant women required hospitalization in the Maternal-Fetal Medicine Unit in the context of selective fetal growth restriction of one of the fetuses, abundant metrorrhagia in the 2nd or 3rd trimester and threatened preterm labor.

Cesarean section was performed in 52% of the deliveries, mostly due to placental disorders, abnormal fetal presentation, non-reassuring fetal status, cephalopelvic disproportion, failed labor induction and inertia of labor.

DISCUSSION

The results of the present study showed that there was an association between serum P levels on the FET, after an artificial endometrial preparation cycle with vaginal micronized P, and pregnancy outcomes. A serum P threshold of 10 ng/mL was firstly analyzed based on previous studies that determined such a P level to be an indicator of appropriate corpus luteal function[6,20]. Univariate analyses showed that patients with serum P levels ≥ 10 ng/mL on the FET day had a significantly higher positive β-hCG test rate, implantation rate and CPR. A further analysis looking at P threshold of 9.34 ng/mL on day of FET showed that patients with serum P levels above this threshold had a significantly increased positive β-hCG test, implantation rate, CPR, OPR and LBR. Regarding the early miscarriage rate, although not statistically significant, there appears to be a tendency for patients with P levels ≥ 9.34 ng/mL to have a reduced early miscarriage rate. After adjusting for potential confounders, a significant relationship between serum P levels on the day of FET at or above 9.34 ng/mL and the likelihood of a positive β-hCG test, a clinical pregnancy and a live birth was observed. Thus, P levels above 9.34 ng/mL on day of FET are a significant factor in predicting pregnancy outcomes. These results are in line with what is currently available in the literature[10,21,22].

Although the use of FET has increased globally, the success rate of FET cycles remains relatively low, with fewer than one in three women achieving a live birth after FET treatment worldwide[18]. In this study, among the 313 women who underwent HRT-FET, only 100 of them achieved a live birth, which is consistent with what has been reported. Thus, there has been a growing body of research aimed at finding ways to predict and enhance FET outcomes[18]. It is undebatable the importance of luteal phase P supplementation for the establishment and maintenance of pregnancy, and it has been widely accepted that the success of FET cycle is dependent on adequate luteal phase support[14]. Nevertheless, the ideal serum P level around the time of FET is still poorly understood and has not been consistent[14].

Different cut-off levels have been suggested when using intravaginal P. A previous study by Yovich et al. indicated that the optimal P range needed to achieve a live birth was found to be 22-31 ng/mL, while P concentrations outside this range were associated with decreased implantation rates[19]. On the other hand, Gaggiotti-Marre et al. showed that serum P levels ≤ 10.64 ng/mL one day before FET were associated with a lower pregnancy and LBR following FET of euploid embryos[10]. Similarly, a study conducted by Cedrin-Durnerin et al. found that the optimal serum P value around the time of FET was ≥ 10 ng/mL[21]. In line with the above study, a study in the oocyte donation cycle context by Labarta et al. showed that patients with serum P < 9.2 ng/mL on the day of embryo

transfer after an artificial endometrial preparation cycle with vaginal micronized P had a significantly reduced OPR[22]. In another study, Labarta et al. showed that serum P levels < 8.8 ng/mL on the day of embryo transfer significantly decreased OPR, in both own and donated oocyte cycles[23]. Despite the variations in the optimal serum P level across several studies using intravaginal P supplementation, the results indeed suggest that a minimal threshold of serum P on the day of FET needs to be reached in HRT cycles. As FET protocols differ between centers, which may impact the P levels obtained[6], it has been suggested that each center should define its own threshold.

Furthermore, a debate exists regarding the optimal dose and route of P administration in relation to reproductive outcomes, in addition to the ideal serum P levels on the FET day among patients undergoing FET cycles[10]. In our center, vaginal route for P supplementation has been used given its better steady-state serum level compared to oral and intramuscular administration, as well as higher P levels at the site of the endometrium, and higher implantation rates in FET cycles[10]. Although all patients received the same dose of vaginal P, it is known that the uptake, absorption and metabolism of hormones can vary among women[14,19]. For this reason, studies support the need for a careful monitoring of serum P levels during luteal phase to perform a rescue P protocol in case of low serum P levels[24]. Some studies support the increment of the vaginal P supplementation if serum P levels were deemed inadequate[6]. Other studies defend the use of a combination of oral dydrogesterone and vaginal P to reduce the vaginal dose to a level that is more comfortable for patients, while ensuring P support for patients with possible poor vaginal absorption[14]. As recently available evidence indicates that in HRT cycles there might be a significant inter-personal variation in serum P levels with an impact on reproductive outcomes, despite the use of the same dose and route of P administration[24], recent studies point to the need of individualizing luteal phase support according to the needing of each patient[12].

Some studies support that neonatal outcomes, in addition to pregnancy outcomes, should also be considered when evaluating the optimal P level during HRT-FET cycles[8]. In this study, we did not find statistically significant differences when neonatal outcomes, such as PTB, LBW, major congenital malformations and early neonatal death, were analyzed according to the three established thresholds. These results are similar to another study developed by Zhu et al. who found no statistically significant differences in the neonatal outcomes, across the serum P quartiles, when using a combination of oral dydrogesterone and vaginal progesterone for luteal phase support[14]. Further investigation on this subject must be performed.

Regarding other predictive factors of pregnancy outcomes, previous studies have reported that an advanced maternal age is associated with a lower live birth rate, which is in line with our

study[25,26]. On the other hand, despite the increased single embryo transfers over time, studies showed that the transfer of two frozen embryos enhance the probability of a live birth, which is also in line with this study[27,28].

Concerning the obstetric complications, in 64.5% of clinical pregnancies there was at least one complication during pregnancy, childbirth or postpartum. Although studies showed that FET significantly improves clinical outcomes, HRT-FET cycles have been associated with an increased risk of pregnancy-related hypertensive disorders, gestational diabetes mellitus, placental disorders, preterm and post-term delivery, macrosomia, and other adverse obstetrical or prenatal outcomes[8,11,29]. As a result, some scholars advocate the prioritization of natural cycle FET in women capable of ovulation[8].

Early miscarriage was the most frequent complication. It should be noted that 27.5% of clinical pregnancies did not progress, which represents a considerable percentage of ART failure.

Although multiple pregnancy is one of the most important complications of ART as it is associated with other obstetric complications, with the increase of one single embryo transfers, the occurrence of multifetal pregnancies has been decreasing, as it was seen in this study in which only 6 cases of twin pregnancies occurred.

Cesarean section was the most common type of delivery, having been performed in 52 out of the 100 women that delivered. In fact, the incidence of cesarean delivery is higher in pregnancies conceived by ART, mainly due to the fact that different obstetric complications may arise and require a cesarean section[30].

The main limitation of this study is the retrospective nature of the data, which precludes to conclude on how to enhance reproductive outcomes in women with low serum P values. Also, the small sample size due to the short period of the study and the exclusion of some cases due to missing data, limited the power of the study. Although the analysis was adjusted to reduce the probability of confounding, selection bias could not be entirely excluded. Furthermore, embryo aneuploidy is associated to implantation failure, but the influence of embryo quality on this finding was not ruled out. In the current study, endometrial thickness and pattern were not assessed, although a previous study found no influence on implantation or pregnancy rates in FET[31]. Finally, the obstetric complications identified in this study may have several associations and influences on each other, meaning that the frequency of a certain complication may overestimate the frequency of another.

CONCLUSION

ART has proven to be crucial for millions of infertile women all over the world to achieve a successful pregnancy. Although the use of FET has steadily increased over the past decade, research on the optimal endometrial preparation protocol continues.

The present study demonstrated that serum P levels at or above 9.34 ng/mL on the day of FET correlate with the likelihood of a clinical pregnancy and live birth in artificial cycles when using intravaginal P for luteal phase support. Our finding suggests that a minimum P value on the day of FET appears to be associated with improved pregnancy outcomes.

Considering this finding, several approaches could be assessed in the future to evaluate their impact on increasing serum P levels and enhancing reproductive outcomes, such as increasing P dose supplementation or adding a different route of P administration, when very low serum P levels are observed. Thus, further research is required to characterize the ideal P replacement and its role in monitoring artificial cycles after additional supplementation.

APPENDIX

Tables

Table I Baseline characteristics according to the serum P threshold of 10 ng/mL on the day of FET. Data are expressed as the mean ± standard deviation or number (percentage).

	P<10	P≥10	*p*-value
No. of cycles	155	158	
Maternal age (years)	34.28 ± 3.62	34.11 ± 4.12	0.699
Maternal BMI (kg/m^2)	25.37 ± 4.71	24.27 ± 4.38	**0.034**
Duration of infertility (months)	51.20 ± 22.82	51.70 ± 24.49	0.851
Type of infertility, n (%)			0.394
Primary	133 (85.8)	130 (82.3)	
Secondary	22 (14.2)	28 (17.7)	
Infertility diagnosis, n (%)			0.391
Female factor	42 (28.2)	54 (35.5)	
Male factor	33 (22.1)	31 (20.4)	
Combined	74 (49.7)	67 (44.1)	
Fertilization method, n (%)			0.650
IVF	54 (34.8)	62 (39.3)	
ICSI	75 (48.4)	74 (46.8)	
IVF+ICSI	26 (16.8)	22 (13.9)	
Number of FET cycles n (%)			0.170
1 cycle	116 (74.8)	103 (65.2)	
2 cycles	28 (18.1)	38 (24.0)	
3 or more cycles	11 (7.1)	17 (10.8)	
Progesterone level on embryo transfer day (ng/mL)	6.49 ± 2.62	15.38 ± 5.87	**<0.001**
No. of embryos transferred, n (%)			0.973
Single	117 (75.5)	119 (75.3)	
Double	38 (24.5)	39 (24.7)	
Embryo stage at transfer, n (%)			0.969
Cleavage	8 (5.2)	8 (5.1)	
Blastocyst	147 (94.8)	150 (94.9)	

BMI - body mass index; FET - frozen-thawed embryo transfer; ICSI - intracytoplasmic sperm injection IVF - in vitro fertilization; P - progesterone.

Table II Pregnancy outcomes according to the serum P threshold of 10 ng/mL on the day of FET. Data are expressed as the frequency (percentage).

	P<10	P≥10	*p*-value
Positive β-hCG test, n/N (%)	62/155 (40.0)	84/158 (59.5)	**0.020**
Biochemical pregnancy rate, n/N (%)	4/62 (6.5)	4/84 (4.8)	0.723
Implantation rate, n/N (%)	60/193 (31.1)	91/197 (46.2)	**0.002**
Clinical pregnancy rate, n/N (%)	58/155 (37.4)	80/158 (50.6)	**0.019**
Early miscarriage rate, n/N (%)	16/58 (27.6)	22/80 (27.5)	0.992
Ongoing pregnancy rate, n/N (%)	42/155 (27.1)	58/158 (36.7)	0.068
Live birth rate, n/N (%)	42/155 (27.1)	58/158 (36.7)	0.068

hCG - human chorionic gonadotrophin; P - progesterone.

Table III Neonatal outcomes of live born infants according to the serum P threshold of 10 ng/mL on the day of FET. Data are expressed as mean ± standard deviation or number (percentage). APGAR is presented as median (interquartile range).

	P<10	**P≥10**	***p*-value**
No. of births	42	58	
No. of neonates, n (%)			1.000
Singletons	40 (90.9)	54 (97.1)	
Twins	4 (9.1)	8 (12.9)	
Gestational age (weeks)	37.81 ± 2.32	37.95 ± 2.66	0.787
Birthweight (g)	3074.79 ± 599.29	3103.19 ± 615.86	0.818
Preterm birth, n (%)	8 (19.0)	12 (20.7)	0.839
Low birthweight, n (%)	7 (16.7)	6 (10.3)	0.354
APGAR			
1st minute	9 (9;9)	9 (8;9)	0.975
5th minute	10 (10;10)	10 (9;10)	0.990
Major congenital malformations, n (%)	2 (4.8)	2 (3.4)	1.000
Early neonatal death, n (%)	0 (0.0)	2 (3.4)	0.508

P - progesterone.

Table IV Baseline characteristics according to serum P quartiles (ng/mL) on the day of FET. Data are expressed as the mean ± standard deviation or number (percentage).

	Q1 (<7.30)	**Q2 (7.30-10.26)**	**Q3 (10.27-13.42)**	**Q4 (>13.42)**	***p*-value**
No. of cycles	78	79	79	77	
Maternal age (years)	34.23 ± 3.54	34.28 ± 3.68	33.72 ± 4.18	34.55 ± 4.09	0.606
Maternal BMI (kg/m^2)	25.15 ± 4.75	25.59 ± 4.80	23.92 ± 4.18	24.59 ± 4.42	0.113
Duration of infertility (months)	54.35 ± 25.53	48.44 ± 19.60	53.85 ± 24.94	49.16 ± 23.91	0.264
Type of infertility, n (%)					0.599
Primary	69 (88.5)	66 (83.5)	66 (83.5)	62 (80.5)	
Secondary	9 (11.5)	13 (16.5)	13 (16.5)	15 (19.5)	
Infertility diagnosis, n (%)					0.329
Female factor	18 (24.3)	24 (31.2)	25 (33.8)	29 (38.2)	
Male factor	18 (24.3)	16 (20.8)	11 (14.9)	19 (25.0)	
Combined	38 (51.4)	37 (48.0)	38 (51.3)	28 (36.8)	
Fertilization method, n (%)					0.331
IVF	29 (37.2)	25 (31.6)	28 (35.4)	34 (44.2)	
ICSI	34 (43.6)	42 (53.2)	36 (45.6)	37 (48.0)	
IVF+ICSI	15 (19.2)	12 (15.2)	15 (19.0)	6 (7.8)	
Number of FET cycles n (%)					0.067
1 cycle	52 (66.7)	66 (83.5)	54 (68.4)	47 (61.0)	
2 cycles	19 (24.3)	9 (11.4)	19 (24.0)	19 (24.7)	
3 or more cycles	7 (9)	4 (5.1)	6 (7.6)	11 (14.3)	
Progesterone level on embryo transfer day (ng/mL)	4.41 ± 2.07	8.63 ± 0.82	11.79 ± 0.97	19.20 ± 6.43	**<0.001**
No. of embryos transferred, n (%)					0.998
Single	59 (75.6)	60 (75.9)	59 (74.7)	58 (75.3)	
Double	19 (24.4)	19 (24.1)	20 (25.3)	19 (24.7)	
Embryo stage at transfer, n (%)					0.474
Cleavage	3 (3.8)	5 (6.3)	6 (7.6)	2 (2.6)	
Blastocyst	75 (96.2)	74 (93.7)	73 (92.4)	75 (97.4)	

BMI - body mass index; FET - frozen-thawed embryo transfer; ICSI - intracytoplasmic sperm injection IVF - in vitro fertilization; P – progesterone; Q - quartile.

Table V Pregnancy outcomes according to serum P quartiles (ng/mL) on the day of FET. Data are expressed as the frequency (percentage).

	Q1 (<7.30)	Q2 (7.30-10.26)	Q3 (10.27-13.42)	Q4 (>13.42)	*p*-value
Positive β-hCG test, n/N (%)	29/78 (37.2)	34/79 (43.0)	39/79 (49.4)	44/77 (57.1)	0.076
Biochemical pregnancy rate, n/N (%)	0/29 (0.0)	4/34 (11.8)	1/39 (2.5)	3/44 (6.8)	0.167
Implantation rate, n/N (%)	30/97 (30.9)	31/98 (31.6)	44/99 (44.4)	46/96 (47.9)	0.055
Clinical pregnancy rate, n/N (%)	29/78 (37.2)	30/79 (38.0)	38/79 (48.1)	41/77 (53.2)	0.119
Early miscarriage rate, n/N (%)	10/29 (34.5)	6/30 (20.0)	10/38 (26.3)	12/41 (29.3)	0.649
Ongoing pregnancy rate, n/N (%)	19/78 (24.4)	24/79 (30.4)	28/79 (35.4)	29/77 (37.7)	0.289
Live birth rate, n/N (%)	19/78 (24.4)	24/79 (30.4)	28/79 (35.4)	29/77 (37.7)	0.289

hCG - human chorionic gonadotrophin; P - progesterone; Q - quartile.

Table VI Neonatal outcomes of live born infants according to the serum P quartiles (ng/mL) on the day of FET. Data are expressed as the mean ± standard deviation or number (percentage). APGAR is presented as median (interquartile range).

	Q1 (<7.30)	Q2 (7.30-10.26)	Q3 (10.27-13.42)	Q4 (>13.42)	*p*-value
No. of births	19	24	28	29	
No. of neonates, n (%)					0.966
Singletons	18 (90.0)	23 (92.0)	26 (86.7)	27 (87.1)	
Twins	2 (10.0)	2 (8.0)	4 (13.3)	4 (12.9)	
Gestational age (weeks)	38.53 ± 1.54	37.25 ± 2.64	38.32 ± 2.45	37.59 ± 2.89	0.260
Birthweight (g)	3200.79 ± 502.02	2995.46 ± 658.60	3123.39 ± 615.74	3067.76 ± 630.22	0.725
Preterm birth, n (%)	2 (10.5)	6 (25.0)	5 (17.9)	7 (24.1)	0.608
Low birthweight, n (%)	2 (10.5)	5 (20.8)	2 (7.1)	4 (13.8)	0.518
APGAR					
1st minute	9 (9;9)	9 (9;9)	9 (8;9)	9 (8;9)	0.973
5th minute	10 (10;10)	10 (10;10)	10 (9;10)	10 (9;10)	0.978
Major congenital malformations, n (%)	0 (0.0)	2 (8.3)	2 (7.1)	0 (0.0)	0.273
Early neonatal death, n (%)	0 (0.0)	0 (0.0)	1 (3.6)	1 (3.4)	0.673

P - progesterone; Q - quartile.

Table VII Baseline characteristics according to the serum P threshold of 9.34 ng/mL on the day of FET. Data are expressed as the mean ± standard deviation or number (percentage).

	P<9.34	**P≥9.34**	***p*-value**
No. of cycles	135	178	
Maternal age (years)	34.30 ± 3.62	34.11 ± 4.07	0.678
Maternal BMI (kg/m^2)	25.53 ± 4.77	24.27 ± 4.34	**0.015**
Duration of infertility (months)	50.96 ± 22.71	51.83 ± 24.38	0.746
Type of infertility, n (%)			0.424
Primary	116 (85.9)	147 (82.6)	
Secondary	19 (14.1)	31 (17.4)	
Infertility diagnosis, n (%)			0.213
Female factor	36 (27.7)	60 (35.1)	
Male factor	33 (25.4)	31 (18.1)	
Combined	61 (46.9)	80 (46.8)	
Fertilization method, n (%)			0.343
IVF	46 (34.1)	70 (39.3)	
ICSI	64 (47.4)	85 (47.8)	
IVF+ICSI	25 (18.5)	23 (12.9)	
Number of FET cycles n (%)			0.500
1 cycle	99 (73.3)	120 (67.4)	
2 cycles	26 (19.3)	40 (22.5)	
3 or more cycles	10 (7.4)	18 (10.1)	
P level on embryo transfer day (ng/mL)	6.02 ± 2.49	14.73 ± 5.83	**<0.001**
No. of embryos transferred, n (%)			0.635
Single	100 (74.1)	136 (76.4)	
Double	35 (25.9)	42 (23.6)	
Embryo stage at transfer, n (%)			0.959
Cleavage	7 (5.2)	9 (5.1)	
Blastocyst	128 (94.8)	169 (94.9)	

BMI - body mass index; FET - frozen-thawed embryo transfer; ICSI - intracytoplasmic sperm injection IVF - in vitro fertilization; P - progesterone.

Table VIII Pregnancy outcomes according to the serum P threshold of 9.34 ng/mL on the day of FET. Data are expressed as the frequency (percentage).

	P<9.34	**P≥9.34**	***p*-value**
Positive β-hCG test, n/N (%)	52/135 (38.5)	94/178 (52.8)	**0.012**
Biochemical pregnancy rate, n/N (%)	3/52 (5.8)	5/94 (5.3)	1.000
Implantation rate, n/N (%)	51/170 (30.0)	100/220 (45.5)	**0.002**
Clinical pregnancy rate, n/N (%)	49/135 (36.3)	89/178 (50.0)	**0.016**
Early miscarriage rate, n/N (%)	16/49 (32.7)	22/89 (24.7)	0.318
Ongoing pregnancy rate, n/N (%)	33/135 (24.4)	67/178 (37.6)	**0.013**
Live birth rate, n/N (%)	33/135 (24.4)	67/178 (37.6)	**0.013**

hCG - human chorionic gonadotrophin; P - progesterone.

Table IX Multivariate logistic regression analysis of factors related to pregnancy outcomes, P threshold 9.34 ng/mL

	Adjusted OR (95% CI)	*p*-value
Positive β-hCG test		
Maternal age (years)	0.95 (0.89-1.01)	0.119
Maternal BMI (kg/m^2)	0.99 (0.94-1.05)	0.742
Duration of infertility (months)	1.00 (0.99-1.01)	0.576
Infertility diagnosis		
Female factor	Reference	
Male factor	1.34 (0.61-2.91)	0.466
Combined	2.51 (1.29-4.87)	**0.007**
No. of embryos transferred		
Single	Reference	
Double	1.95 (1.08-3.53)	**0.028**
Embryo stage at transfer		
Cleavage	Reference	
Blastocyst	2.53 (0.66-9.74)	0.178
P ≥ 9.34 ng/mL	2.04 (1.23-3.37)	**0.006**
Clinical pregnancy rate		
Maternal age (years)	0.94 (0.88-1.01)	**0.042**
Maternal BMI (kg/m^2)	0.98 (0.92-1.04)	0.446
Duration of infertility (months)	1.00 (0.98-1.01)	0.507
Infertility diagnosis		
Female factor	Reference	
Male factor	1.18 (0.54-2.60)	0.675
Combined	2.50 (1.28-4.85)	**0.007**
No. of embryos transferred		
Single	Reference	
Double	1.98 (1.09-3.58)	**0.024**
Embryo stage at transfer		
Cleavage	Reference	
Blastocyst	2.23 (0.58-8.54)	0.243
P ≥ 9.34 ng/mL	1.92 (1.16-3.19)	**0.011**
Live birth rate		
Maternal age (years)	0.92 (0.86-1.00)	**0.036**
Maternal BMI (kg/m^2)	0.96 (0.90-1.02)	0.238
Duration of infertility (months)	0.99 (0.98-1.00)	0.178
Infertility diagnosis		
Female factor	Reference	
Male factor	1.26 (0.53-2.97)	0.599
Combined	1.93 (0.95-3.91)	0.070
No. of embryos transferred		
Single	Reference	
Double	2.73 (1.47-5.10)	**0.002**
Embryo stage at transfer		
Cleavage	Reference	
Blastocyst	4.93 (0.60-40.52)	0.138
P ≥ 9.34 ng/mL	2.10 (1.20-3.67)	**0.009**

BMI - body mass index; CI - confidence interval; hCG - human chorionic gonadotrophin; OR - odds ratio; P - progesterone.

Table X Neonatal outcomes of live born infants according to the serum P threshold of 9.34 ng/mL on the day of FET. Data are expressed as the mean ± standard deviation or number (percentage). APGAR is presented as median (interquartile range).

	P<9.34	**P≥9.34**	***p*-value**
No. of births	33	67	
No. of neonates, n (%)			1.000
Singletons	31 (88.6)	63 (88.7)	
Twins	4 (11.4)	8 (11.3)	
Gestational age (weeks)	37.70 ± 2.53	37.99 ± 2.51	0.592
Birthweight (g)	3054.12 ± 656.84	3109.55 ± 583.74	0.669
Preterm birth, n (%)	7 (21.2)	13 (19.4)	0.832
Low birthweight, n (%)	7 (21.2)	6 (8.9)	0.115
APGAR			
1st minute	9 (9;9)	9 (8;9)	0.999
5th minute	10 (10;10)	10 (9;10)	0.964
Major congenital malformations, n (%)	1 (3.0)	3 (4.5)	1.000
Early neonatal death, n (%)	0 (0.0)	2 (3.0)	1.000

P - progesterone.

Figures

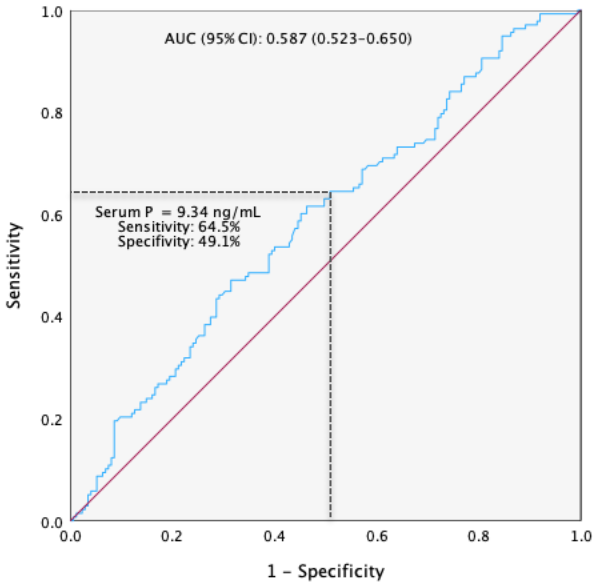

Figure 1 Receiver operating characteristic (ROC) curve for prediction of the CPR using serum P levels on the day of FET. AUC - area under the curve

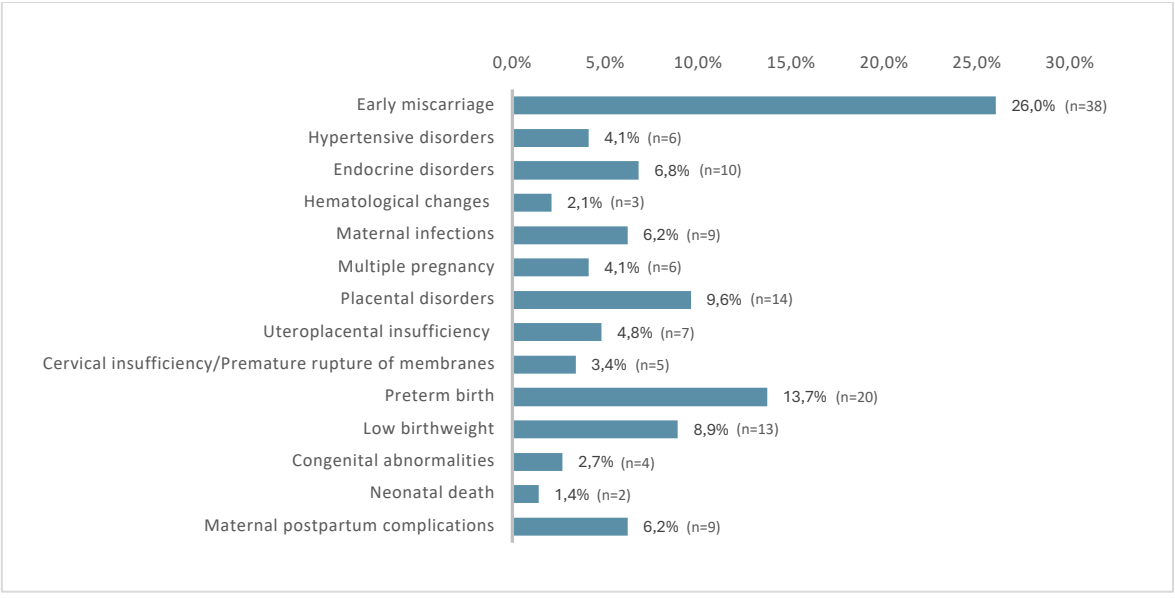

Figure 2 Obstetric complications (n=146). Data are expressed as the percentage (number).

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