Research Article

Pre-Transfer Circulating Progesterone and Clinical Pregnancy in HRT-FET Cycles

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Abstract

Objectives: To investigate the association between circulating progesterone concentrations measured one day prior to Frozen Embryo Transfer (FET) in Hormone Replacement Therapy (HRT) cycles and clinical pregnancy outcomes, and to determine the optimal progesterone threshold for predicting clinical pregnancy.

Methods: A prospective cohort study was conducted at Da Nang Women and Children's Hospital from January 2023 to October 2024. A total of 243 women undergoing HRT-FET cycles were enrolled. Eligible participants had a body mass index (BMI) ≤25 kg/m^2, were aged 18–45 years, had an endometrial thickness >7 mm, and underwent transfer of one or two high-quality blastocysts. Circulating progesterone concentrations were measured on the fourth day of progesterone supplementation, one day prior to embryo transfer, and categorized into quartiles. Clinical pregnancy was defined by ultrasound confirmation of fetal cardiac activity at 7 weeks of gestation. Statistical analyses included t-tests, chi-square tests, multivariate regression, and receiver operating characteristic (ROC) curve analysis.

Results: Clinical pregnancy rates across increasing progesterone quartiles were 31.7% (<9.1 ng/mL), 49.2% (9.1–<11.0 ng/mL), 50.8% (11.0–<13.5 ng/mL), and 57.4% (≥13.5 ng/mL). Women with progesterone concentrations <9.1 ng/mL had a significantly lower clinical pregnancy rate compared with those in higher quartiles (31.7% vs. 52.5%, p = 0.033). ROC analysis demonstrated moderate predictive performance (AUC 0.65; 95% CI: 0.58–0.72), with an optimal progesterone cut-off value of 10.35 ng/mL (sensitivity 80.9%, specificity 52.3%).

Conclusions: Lower serum progesterone concentrations measured one day prior to embryo transfer are associated with reduced clinical pregnancy rates in HRT-FET cycles, supporting the clinical value of progesterone monitoring. However, given the single-centre design and moderate predictive performance, the proposed threshold should be interpreted with caution and validated in larger, more diverse populations.

Keywords: clinical pregnancy, frozen embryo transfer, hormone replacement therapy, progesterone.

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INTRODUCTION

In Vitro Fertilization (IVF) is a complex but effective infertility treatment that has enabled many couples to conceive and experience parenthood[1]. The combination of IVF and frozen embryo transfer (FET) has been shown to improve clinical pregnancy rates[2-4].

Endometrial preparation using exogenous Hormone Replacement Therapy (HRT) is a widely adopted approach in FET cycles, particularly in programmed cycles without corpus luteum activity. Despite its routine use, there is no universally accepted standard for progesterone (P4) dosing, and the optimal serum P4 concentration required to support implantation and pregnancy remains under investigation. Vaginal administration of micronized progesterone at doses ranging from 600 to 800 mg/day is the most commonly used regimen due to its ease of administration and targeted endometrial effect. However, a substantial proportion of patients fail to achieve optimal serum progesterone levels, which may adversely affect reproductive outcomes. Previous studies have reported that up to 25% of patients using vaginal progesterone in FET cycles do not reach circulating P4 concentrations considered sufficient for adequate endometrial receptivity[5].

Emerging evidence suggests a threshold effect for serum progesterone in artificial cycles, below which pregnancy rates are significantly reduced. Prospective studies have shown that patients with higher P4 concentrations (>10 ng/mL) achieve better reproductive outcomes, including higher live birth rates (41.1% vs. 25.7%) and clinical pregnancy rates (48.6% vs. 33.0%)[6]. Additionally, 63% of patients with higher P4 concentrations on the day before FET demonstrated an increased likelihood of live birth (OR 1.05, 95% CI [1.02–1.09])6. These findings highlight the importance of individualized luteal phase support. However, many existing studies are retrospective, with heterogeneous methodologies and inconsistent findings, underscoring the need for well-designed prospective studies.

Furthermore, data on serum P4 concentrations measured one day prior to embryo transfer in HRT-FET cycles remain limited, particularly in Asian populations, including Vietnam. Given the potential implications for clinical practice and IVF success rates, this prospective cohort study was designed with two primary objectives; to investigate circulating progesterone concentrations measured one day prior to embryo transfer and their association with clinical pregnancy outcomes in HRT-FET cycles; and to determine the optimal serum progesterone threshold for predicting clinical pregnancy.

METHODS

This prospective cohort study was conducted at the Infertility Department, Da Nang Women and Children's Hospital, between January 2023 and October 2024. A total of 243 infertile patients underwent frozen embryo transfer (FET) using a hormonally prepared endometrial priming protocol. Eligible participants were women aged 18–45 years with a body mass index (BMI) ≤25 kg/m^2, no systemic medical conditions, and an endometrial thickness >7 mm on the day of embryo transfer. Only patients who received one or two high-quality blastocysts and provided written informed consent were included. Embryo grading was performed in the embryology laboratory using the Gardner blastocyst grading system. High-quality blastocysts were defined as those with expansion grades of 3–6 and trophectoderm (TE) and inner cell mass (ICM) scores of at least BB, including AA, AB, BA, and BB[7].

Patients with a history of recurrent miscarriage, repeated implantation failure, or uterine abnormalities such as submucosal fibroids, endometrial polyps, congenital uterine malformations, or intrauterine fluid were excluded. Patients who declined participation were also excluded.

The sample size was calculated using an area under the curve (AUC)-based method to evaluate the predictive value of serum progesterone concentration for clinical pregnancy. Based on a previously reported AUC of 0.595, with a two-sided alpha error of 0.05 (95% confidence interval) and a margin of error of 0.05, the minimum required sample size was 241 patients. Eligible patients were consecutively recruited from January 2023 to October 2024 until the target sample size was reached.

Data collection began when patients presented on day 2 of menstruation to initiate endometrial preparation. At this visit, all participants underwent a comprehensive evaluation, including medical history, physical examination, and transvaginal ultrasound. Eligible patients were invited to participate and subsequently provided written informed consent.

Serum Progesterone (P4) concentrations were measured one day prior to embryo transfer, corresponding to the fourth day of progesterone administration. To ensure consistency, blood samples were collected at a standardized time point, 4 hours after the most recent vaginal progesterone dose. Subsequent stages of the treatment cycle, including embryo transfer, serum β-hCG testing, ultrasound confirmation of clinical pregnancy at 7 weeks, and early pregnancy monitoring, were conducted according to standard clinical protocols and systematically recorded. Circulating P4 concentrations were measured in the hospital laboratory using the COBAS immunoassay analyzer, in accordance with the manufacturer's instructions (Roche Diagnostics, Germany). All procedures were performed in compliance with the approved study protocol, and participants were fully informed about the study objectives, procedures, and potential implications.

Protocol for Endometrial Preparation in HRT-Based FET Cycles

Patients received oral estrogen starting on cycle day 2, at doses ranging from 4–8 mg daily, divided into 2–4 doses. On cycle day 7,

transvaginal ultrasound was performed to assess endometrial thickness and morphology. The estrogen dose was maintained or increased based on ultrasound findings, up to a maximum of 16 mg/day. Follow-up ultrasound examinations were performed every 3–5 days, depending on endometrial response.

Once the endometrial thickness reached ≥7 mm with a trilaminar pattern, embryo transfer was scheduled. A minimum of 12 days of estrogen administration was required before initiating progesterone. The timing of progesterone initiation was determined according to the developmental stage of the frozen embryo.

For day-5 blastocyst transfer, progesterone was administered for 5 days prior to embryo transfer. Serum P4 was measured on the fourth day of progesterone administration, 4 hours after the most recent dose. Micronized progesterone was administered vaginally at a dose of 800 mg/day. Estrogen was continued at a reduced dose (equivalent to the initial dose at the beginning of the cycle) until pregnancy testing. If pregnancy was achieved, progesterone supplementation was continued until 12 weeks of gestation.

Statistical Analysis

Data were analyzed using SPSS Statistics version 24. Serum progesterone levels were categorized into quartiles, and clinical pregnancy rates were calculated and compared across groups. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as proportions. Differences between categorical variables were analyzed using the chi-square test, and continuous variables between two groups were compared using the independent t-test. Multivariate logistic regression analysis was performed to evaluate the association between patient characteristics and pre-embryo transfer progesterone levels. The predictive performance of serum progesterone for clinical pregnancy was assessed using receiver operating characteristic (ROC) curve analysis and the area under the curve (AUC).

Ethical Approval

This study was approved by the Ethics Committee of Da Nang Women and Children's Hospital (No. 126/BVPSN-ĐN/HĐYD/2022).

RESULTS

Baseline and Cycle Characteristics

A total of 243 infertile women undergoing HRT-based FET were prospectively enrolled in the study. A dominant proportion of participants were women of reproductive age (mean 33.7 ± 4.4 years) and had normal BMI averaging 21.1 ± 1.7 kg/m^2. The mean length of the infertility period was 3.0 years. Primary infertility was more prevalent than secondary infertility, accounting for 55.6% and 44.4%, respectively. Male factor infertility represented the leading indication for IVF, observed in 43.6% of participants. Mean circulating P4 concentration measured one day prior to embryo transfer was 11.41 ± 2.26 ng/mL (range: 8.5–22.0 ng/mL). Endometrial thickness on the day of transfer averaged 10.65 ± 1.31 mm (range: 7–14 mm). The number of embryos transferred averaged 1.52 ± 0.52 (range: 1–3) (table 1).

Table 1. Baseline and Cycle Characteristics

Characteristic	*P-value*
Baseline characteristics	
Mean age ± SD (min-max), years	33.7 ± 4.4 (20-42)
BMI, kg/m^2	21.1 ± 1.7
Duration of infertility, years	3.0 ± 2.5
Type of infertility	
Primary	55.6 (135/243)
Secondary	44.4 (108/243)
Indications for IVF	
Male factor	43.6 (106/243)
Tubal disease	22.2 (54/243)
Unexplained	16.5 (40/243)
Endometriosis	9.5 (23/243)
Others	8.2 (20/243)
FET cycle characteristics	
Serum progesterone level one day before embryo transfer, ng/mL	11.41 ± 2.26 (8.5-22.0)
Endometrial thickness, mm	10.65 ± 1.31 (7-14)
Number of embryos transferred	1.52 ± 0.52 (1-3)

Progesterone Quartiles and Clinical Outcomes

To explore the link between circulating P4 concentrations and clinical outcomes, patients were assigned into quartiles depending on progesterone concentration measured one day prior to embryo transfer. The distribution is summarized in Table 2. The quartile distribution of serum progesterone was as follows: <9.1 ng/mL (Quartile 1), 9.1–<11.0 ng/mL (Quartile 2), 11.0–<13.5 ng/mL (Quartile 3), ≥13.5 ng/mL (Quartile

4). All groups had comparable mean embryo transfer numbers (p = 0.508). The rate of positive β-hCG increased progressively from Quartile 1 to Quartile 4 with no significant difference observed (p = 0.328). However, a significant difference in clinical pregnancy rate was documented among the quartiles (p=0.033). Specifically, patients in Quartile 1 (< 9.1 ng/mL) exhibited a significantly reduced clinical pregnancy rate (31.7%) relative to those in quartiles 2 through 4 combined (52.5%) (table 2).

Table 2. Progesterone Quartiles and Clinical Outcomes

Quartile	P4 range (ng/mL)	P4 level (ng/mL), mean ± SD	N	Mean no. of embryos transferred, mean ± SD	Positive β-hCG rate, % (n/N), 95% CI	Clinical pregnancy rate, % (n/N), 95% CI
Q1	<9.1	8.87 ± 0.14	60	1.48 ± 0.50	45.0 (27/60), 33.1-57.5	31.7 (19/60), 21.3-44.2
Q2	9.1–<11.0	10.23 ± 0.61	61	1.52 ± 0.50	57.4 (35/61), 44.9-69.0	49.2 (30/61), 37.1-61.4
Q3	11.0–<13.5	12.11 ± 0.80	61	1.59 ± 0.50	57.4 (35/61), 44.9-69.0	50.8 (31/61), 38.6-62.9
Q4	≥13.5	14.40 ± 1.49	61	1.48 ± 0.57	60.7 (37/61), 48.1-71.9	57.4% (35/61), 44.9-69.0
P-value				0.508	0.328	0.033*

The p-value: comparison between the first quartile of serum progesterone levels and quartiles 2-4.

Predictive Value of Progesterone for Clinical Pregnancy

Analysis of ROC curve revealed that circulating progesterone concentrations measured one day before embryo transfer had predictive value for clinical pregnancy, as reflected by an AUC of 0.65 (95% CI: 0.58–0.72). The best-performing circulating progesterone cut-off for clinical pregnancy prediction was 10.35 ng/mL (80.9% sensitivity; 52.3% specificity) (Figure 1).

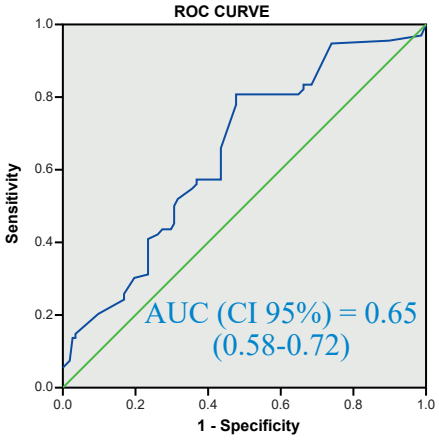

Figure 1. ROC curve of progesterone level one day before embryo transfer in predicting clinical pregnancy. Multivariate Assessment of Clinical Pregnancy–Related Factors

Factors independently related to clinical pregnancy were examined using multivariate logistic regression. Progesterone concentrations and BMI were found to be significant predictors. Specifically, each 1 ng/mL increase in serum progesterone concentration corresponded to a 3.89-fold rise in the likelihood of clinical pregnancy (OR = 3.89; 95% CI: 2.65–5.70; p < 0.001). Each 1 kg/m^2 increase in BMI was linked to a 70% diminished likelihood of clinical pregnancies (OR = 0.30; 95% CI: 0.19–0.49; p < 0.001) (table 3).

Table 3. Multivariate Assessment of Clinical Pregnancy–Related Factors

Factor	OR (95%CI)	*P-value*
Age	0.89 (0.77-1.03)	0.111
BMI	0.30 (0,19-0,49)	<0.001
Endometrial Thickness	0.79 (0.62-1.02)	0.066
No. of Embryos Transferred	1.74 (0,95-3.19)	0.073
Progesterone Level	3.89 (2.65-5.70)	<0.001

DISCUSSION

The results of the present study indicate that patients with circulating progesterone (P4) concentrations <9.1 ng/mL one day prior to embryo transfer in HRT cycles had a significantly lower clinical pregnancy rate (Table 2). Receiver operating characteristic (ROC) curve analysis demonstrated that circulating progesterone measured one day before embryo transfer had

predictive value for clinical pregnancy, with an AUC of 0.65 (95% CI: 0.58–0.72) (Figure 1). These findings were observed in a cohort limited to transfers of high-quality day-5 blastocysts, minimizing the influence of embryo quality on clinical outcomes. Furthermore, all patients received the same dose and duration of progesterone prior to embryo transfer.

Although serum progesterone demonstrated statistically significant predictive value, its moderate AUC and specificity suggest limitations as a standalone predictor of clinical pregnancy. Nevertheless, it remains a clinically relevant marker, particularly for identifying patients who may benefit from individualized luteal phase support.

The mean serum progesterone concentration in our cohort was 11.41 ± 2.26 ng/mL, with most values clustering around 10 ng/mL. These findings are consistent with previous studies using vaginal progesterone, which reported similar mean circulating levels ranging from 11.3 to 12.7 ng/mL[8-10]. In contrast, studies using intramuscular (IM) progesterone have reported substantially higher serum concentrations (mean 33.2 ± 23 ng/mL), likely due to pharmacokinetic differences[11]. Vaginal administration results in higher local endometrial exposure with lower systemic absorption, whereas IM administration leads to higher circulating levels. These differences highlight the influence of the route of administration on serum progesterone concentrations and potentially on clinical outcomes[11].

The optimal circulating progesterone cut-off for predicting clinical pregnancy in our study was 10.35 ng/mL (sensitivity 80.9%; specificity 52.3%) (Figure 1). These findings are consistent with several previous studies, despite variations in blood sampling timing and treatment protocols. Multiple studies have identified progesterone thresholds ranging from 8.8 to 10.0 ng/mL, below which clinical outcomes were significantly reduced[5,8,10]. For instance, a study of 277 FET cycles reported that circulating P4 <10 ng/mL was associated with lower pregnancy and live birth rates8. Similarly, a 2021 prospective study involving 1,205 patients reported that a progesterone cut-off of 8.8 ng/mL on the day of embryo transfer was associated with reduced ongoing pregnancy rates[10]. More recent studies have shown that women with progesterone levels in the lowest range (7–8 ng/mL) on the day of transfer have markedly reduced clinical pregnancy and live birth rates in HRT cycles[11-14]. Although these studies measured progesterone on the day of embryo transfer, whereas our study assessed it one day earlier, the consistent threshold range of 9–11 ng/mL supports the validity of our identified cut-off of 10.35 ng/mL as a clinically meaningful minimum level. In addition, circadian variations in serum progesterone levels around the time of embryo transfer have been reported, which may affect the interpretation of progesterone measurements[15].

Our findings are also consistent with studies evaluating pre-transfer progesterone levels. A retrospective cohort study reported that circulating P4 <10.64 ng/mL one day prior to FET was associated with poorer reproductive outcomes[9]. In that study, age and endometrial thickness were not significantly associated with progesterone levels. Similarly, in our cohort, the mean patient age was 34 years, with most patients aged 31–35 years, and endometrial thickness ranged from ≥8 mm to <14 mm (Table 1).

Comparable findings have also been reported in natural-cycle FET, where low progesterone levels one day prior to embryo transfer were associated with poorer outcomes[6]. These observations suggest that progesterone levels measured before embryo transfer may serve not only as a prognostic marker but also as an indicator of suboptimal endometrial preparation. By identifying a pre-transfer progesterone threshold, our study extends existing evidence to a different clinical setting and population, supporting the use of progesterone assessment to stratify patients at risk of inadequate endometrial receptivity in HRT-FET cycles. Recent studies have explored intervention strategies to correct low progesterone levels at the time of FET to improve pregnancy outcomes. Comparable pregnancy rates have been observed when patients with low progesterone received additional supplementation[16]. Moreover, the live birth rate in patients receiving 25 mg IM progesterone supplementation was reported to be 1.37 times higher than in those without supplementation[17].

In another study using a daily progesterone dose of 600 mg, cycles were divided into two groups: 348 cycles with P4 >10.6 ng/mL and 226 cycles with P4 <10.6 ng/mL, the latter receiving individualized 25 mg subcutaneous progesterone supplementation[17]. If post-supplementation progesterone remained <10.6 ng/mL, the cycle was canceled; if it exceeded this threshold,

embryo transfer proceeded. No significant differences were observed in clinical pregnancy, ongoing pregnancy, or live birth rates between groups[17].

Multivariate logistic regression analysis in our study showed that BMI and pre-transfer progesterone levels were associated with clinical pregnancy outcomes (Table 4). These findings are consistent with a French cohort study of 915 HRT-FET cycles, which reported that higher BMI, non-European ethnicity, and parity were independently associated with lower progesterone levels on the day of embryo transfer (≤9.8 ng/mL)18. Lower progesterone levels in that study were also associated with reduced live birth rates and increased miscarriage risk[18].

Collectively, these findings suggest a potential mechanism whereby elevated BMI may reduce progesterone bioavailability, thereby impairing endometrial receptivity and implantation success. The consistency across studies underscores the importance of monitoring serum progesterone and considering patient characteristics, such as BMI, when individualizing luteal phase support in HRT-FET cycles[19,20].

Routine measurement of serum progesterone prior to embryo transfer may help identify patients at risk of implantation failure due to inadequate hormonal support[21,22]. In patients with low progesterone levels, strategies such as dose escalation, addition of IM or subcutaneous progesterone, or postponement of embryo transfer may be considered to optimize outcomes[23,24]. However, further high-quality randomized studies are needed to confirm the effectiveness of these interventions.

This study has several limitations. First, embryo ploidy status was not assessed, although the use of high-quality day-5 blastocysts helps mitigate this limitation. Second, endometrial pattern was not evaluated, although current evidence suggests it has a limited impact on pregnancy outcomes in FET cycles. Third, as a single-center observational study, residual confounding cannot be excluded, and the findings may reflect local laboratory practices, patient selection, and progesterone measurement protocols. Therefore, the generalizability of the 10.35 ng/mL threshold to other settings and populations should be interpreted with caution until externally validated.

In conclusion, our findings highlight the clinical importance of circulating progesterone levels in HRT-FET cycles and support individualized monitoring to optimize luteal phase support. Future studies should evaluate whether proactive progesterone supplementation in women with low levels can improve clinical outcomes.

CONCLUSION

Circulating Progesterone (P4) concentrations measured one day prior to embryo transfer are associated with clinical pregnancy outcomes in HRT-based FET cycles. Patients in the lowest quartile (<9.1 ng/mL) exhibited a significantly lower clinical pregnancy rate (31.7%) compared with those in quartiles 2–4 combined (52.5%). The optimal progesterone cut-off for predicting clinical pregnancy was 10.35 ng/mL, with a sensitivity of 80.9% and a specificity of 52.3%. These findings support the clinical relevance of pre-transfer progesterone measurement. However, given the single-center design and the potential for residual confounding, further studies are needed to determine whether enhanced progesterone support can improve outcomes in patients with low circulating P4 levels.

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