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Original Article

Efficacy of Daily 50 mg Intramuscular Progesterone for Luteal Phase Support in Frozen Embryo Transfer Cycles: Analysis of Serum Levels and Pregnancy Outcomes

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Abstract

Objective: To evaluate the efficacy of 50 mg/day intramuscular (IM) progesterone in achieving optimal serum P4 levels during endometrial preparation and investigate the association between serum P4 levels on embryo transfer (ET) day and subsequent fertility outcomes in hormone replacement therapy (HRT) frozen embryo transfer (FET) cycles.

Materials and methods: This prospective cohort study included 121 women (aged 22-45 years) undergoing HRT-FET at Imam Khomeini Hospital Complex from December 2022 to January 2024. Endometrial preparation began with oral estradiol valerate (6 mg/day) on cycle day 2. Once an endometrial thickness of ≥8 mm was achieved, daily IM P4 (50 mg) was initiated. Serum P4 levels were measured on ET day, and oral dydrogesterone (20 mg/day) was added for patients with P4 levels <10.0 ng/mL. Primary outcomes included chemical pregnancy, clinical pregnancy, miscarriage, and ongoing pregnancy rates.

Results: The mean serum P4 level on ET day was 22.8 ± 10.1 ng/mL, with 78.5% of participants achieving the target range (10-32.5 ng/mL) following IM P4 administration. Overall chemical pregnancy, clinical pregnancy, and ongoing pregnancy rates were 23.1%, 18.2%, and 14.1%, respectively, with a miscarriage rate of 5.0%. Multivariate analysis revealed that P4 levels >27.8 ng/mL were associated with reduced odds of chemical pregnancy (OR = 0.20; 95% CI: 0.05-0.86; p = 0.03), while no significant differences were observed in other pregnancy outcomes across P4 quartiles.

Conclusion: Daily administration of 50 mg IM P4 effectively achieved optimal serum P4 levels in most patients. While higher P4 levels (>27.8 ng/mL) were associated with reduced chemical pregnancy rates, the absence of significant correlations with other pregnancy outcomes highlights the multifactorial nature of embryo implantation success. These findings emphasize the need for further research to refine P4 thresholds and identify additional predictive factors influencing pregnancy outcomes in FET cycles.

Keywords: Frozen Embryo Transfer; Luteal Phase Support; Intramuscular Progesterone; Dydrogesterone; Pregnancy-Related Outcomes; Hormone Replacement Therapy

Introduction

Frozen embryo transfer (FET) has undergone remarkable transformation with advancements in vitrification techniques, revolutionizing assisted reproductive technology (ART) by offering enhanced

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pregnancy rates and improved clinical outcomes (1). By optimizing embryo transfer conditions, FET provides clinicians and patients with a more flexible and potentially more successful approach to assisted reproduction.

Endometrial preparation represents a critical determinant of reproductive success, with multiple protocols available, including natural cycle (NC), modified natural cycle (MNC), artificial cycle with hormone replacement therapy (AC-HRT), and mild ovarian stimulation (2). There is no sufficient evidence that shows which endometrium preparation protocol is the best. While natural menstrual cycles involve corpus luteum production of approximately 25-50 mg of progesterone daily, ART cycles require exogenous supplementation to support endometrial gland formation and successful trophoblast invasion (3, 4).

Luteal phase support (LPS) in FET cycles has predominantly been studied through retrospective research, revealing significant knowledge gaps (5). Recent studies have highlighted the critical importance of serum progesterone (P4) levels on the day of embryo transfer (ET) (6-8). Emerging evidence suggests remarkable variability in serum P4 levels among patients receiving identical hormonal protocols, with these variations potentially correlating with differential live birth rates. Most research identifies a threshold range between 10-20 ng/mL as pivotal for successful pregnancy, with levels below 5-12 ng/mL or above 30-32.5 ng/mL associated with reduced clinical pregnancy rates and increased miscarriage risks (6-13).

Patients have traditionally received P4 supplementation uniformly, adopting a "one size fits all" approach that ignores potential inter-individual physiological variations that could impact reproductive success (14, 15). Factors such as patient age, body mass index (BMI), metabolic characteristics, and individual hormonal profiles contribute to these complex interactions (9). Multiple administration routes exist for P4 supplementation, including intramuscular, vaginal, oral, subcutaneous, and trans-rectal, each associated with distinct serum and endometrial tissue responses (12, 16, 17). Comparative studies have revealed potential differences in outcomes between various P4 administration routes, with some research suggesting that combined or IM approaches might offer superior results compared to vaginal administration alone (17-20). Additionally, Dydrogesterone (DYD), a stereoisomer of P4, seems to be a good alternative for the treatment of LPS in an artificial cycle, especially in combination with a high dose of IM P4 during the course of oocyte donation (21-24).

Given the intricate relationship between P4 levels and reproductive outcomes, this prospective cohort study aims to comprehensively investigate two primary objectives: first, to determine whether IM P4 administration can effectively elevate serum P4 concentrations to the optimal range during endometrial preparation; and second, to examine the correlation between these serum P4 levels on embryo transfer day and subsequent fertility outcomes in HRT-FET protocols. By elucidating these nuanced relationships, we seek to advance personalized approaches in assisted reproductive interventions and challenge the conventional uniform approach to LPS.

Materials and methods

Study design and ethical considerations: This prospective cohort study was conducted at the Infertility Clinic of Imam Khomeini Hospital Complex (IKHC), a teaching center affiliated with Tehran University of Medical Sciences (TUMS) in Tehran, Iran, from December 2022 to January 2024. The study protocol received approval from the Institutional Review Board and Ethics Committee of Tehran University of Medical Sciences, with a comprehensive informed consent process implemented for all participants (approval code: IR.TUMS.IKHC.REC.1401.204). This study was performed in accordance with the Declaration of Helsinki. Clinical trial number is not applicable due to study design.

Study Population: The study included 121 eligible women aged 22 to 45 years who were candidates for frozen embryo transfer (FET) using hormone replacement therapy (HRT). Participants were carefully selected based on specific inclusion and exclusion criteria. Inclusion criteria comprised willingness to participate, normal uterine anatomy, infertility requiring FET, and achieving an appropriate endometrial thickness of ≥8 mm prior to starting the treatment. Patients with recurrent miscarriage, repeated implantation failure, uterine abnormalities, metabolic disorders, antiphospholipid syndrome, severe male factor infertility, or the presence of hydrosalpinx were excluded.

Embryo morphology assessment: About 55% of the patients were undergoing their first FET cycle. Embryos were derived from intracytoplasmic sperm injection (ICSI) cycles. Embryo quality assessment

utilized the standardized Gardner classification system (Gardner and Schoolcraft, 1999) and categorized as excellent, good, average, or poor. In the present, study patients who had poor-level embryos, according to the Gardner classification, were excluded.

Endometrial preparation and progesterone administration protocol: Endometrial preparation commenced on the second day of the menstrual cycle through oral estradiol valerate administration (6 mg/day, Aburaihan Pharmaceutical Co., Tehran, Iran). Transvaginal ultrasonography (TV-USG) (Philips Healthcare, Netherlands, Model: Affiniti 70) was performed periodically by an expert infertility fellowship to measure endometrial thickness and to confirm a triple layer pattern. Once the endometrial thickness reached ≥8 mm, 50 mg of IM progesterone (Fertigest, Aburaihan Pharmaceutical Co., Tehran, Iran) was administered daily for LPS.

Embryo transfers were conducted at two distinct time points following the initiation of progesterone (P4) administration: at day 4 for embryos at the cleavage stage and at day 6 for those that had reached the blastocyst stage. Blood sampling occurred between 7:00 and 10:00 AM on the ET day to measure serum P4 concentrations. The biochemical analysis was performed using chemiluminescent microparticle immunoassay (CMIA) (ARCHITECT Progesterone, Abbott Laboratories, Illinois, USA). For patients receiving routine LPS and having serum P4 levels <10.0 ng/ml on the FET day, we added oral DYD (20 mg/day, Duphaston, Abbott Healthcare Co., Netherlands) to salvage the FET cycles.

E2 and P4 supplementation were continued until the 10th week of pregnancy in patients with confirmed pregnancies. Participants were followed up until the 12th week to monitor miscarriage or ongoing pregnancy status.

Outcome measures: Serum β-HCG levels were measured two weeks after ET day. Patients who demonstrated positive β-HCG tests (>50 IU/L) were scheduled for their initial TV-USG two weeks later to verify the presence of a gestational sac. Chemical pregnancy (positive β-HCG test), clinical pregnancy (visualization of a gestational sac with fetal heartbeat on ultrasound), miscarriage (pregnancy loss before 12 weeks), and ongoing pregnancy (presence of fetal heartbeat beyond 12 weeks) were examined.

Recognizing the optimum range of serum P4 levels on ET day (10-32.5 ng/mL) to improve pregnancy outcomes based on prior research, the primary objective of the study was to determine the efficacy of daily 50 mg intramuscular P4 administration in achieving these optimal serum P4 levels, and the second objective was to comprehensively evaluate pregnancy outcomes between participants whose serum P4 levels fall within this target range and those whose levels remain outside it.

Statistical analysis: P4 levels above 10 ng/mL (including women who received only IM P4) were stratified into four quartiles to facilitate more analysis. Descriptive statistics were used to summarize patient demographic characteristics, including age, BMI, infertility type (primary or secondary), and duration of infertility. Quantitative variables were reported as mean ± standard deviation (SD), and categorical variables were presented as frequency and percentage. Quartiles of serum P4 levels were compared using analysis of variance (ANOVA) and chi-square tests for continuous and categorical variables, respectively. The relationships between serum P4 levels and pregnancy outcomes were evaluated using logistic regression analysis. A p-value <0.05 was considered statistically significant. All statistical analyses were conducted using SPSS version 20 (IBM Corp., Armonk, NY, USA).

Results

Participants: A total of 121 eligible women were included in the final analysis. The mean age of participants was 35.6 ± 6.1 years (range: 22-45), and the mean duration of infertility was 6.0 ± 4.0 years. Most participants (85 women (70.3%)) experienced primary infertility, while 36 women (29.7%) had secondary infertility. Male factor infertility accounted for 27 cases (22.3%), with the remainder attributed to ovulatory disorders, tubal factor, unexplained, or multifactorial causes (Table 1).

A total of 235 embryos were transferred, with single embryo transfer performed in 7 cases (5.8%) and double embryo transfer in 114 cases (94.2%). Regarding embryo developmental stage at transfer, 67 patients (55.4%) received day 3 embryos (cleavage stage), while 54 patients (44.6%) received day 5 embryos (blastocyst stage).

The mean serum progesterone (P4) level on the day of embryo transfer (ET) was 22.8 ± 10.1 ng/mL, ranging from 7.5 to 60.8 ng/mL (Table 1). Following the administration of 50 mg/day IM P4, a significant proportion of participants (95 women (78.5%)) achieved the target optimal serum P4 concentration. Specifically, these patients demonstrated P4 levels

within the clinically favorable range of 10-32.5 ng/mL. Conversely, five cases (4.1%) exhibited persistently low P4 concentrations, with serum levels remaining< 10 ng/mL threshold on the ET day and 21 patients (17.4%) experienced P4 levels> 32.5 ng/mL.

Table 1. Baseline characteristics of the study participants[a]

Continuous Variables[b]	Mean ± SD	Min, Max
Age (year)	35.6 ± 6.1	22, 45
BMI (kg/m^2)	26.3 ± 4.6	17.1, 45
Duration of infertility (year)	6.0 ± 4.0	1, 20
Endometrial thickness (mm)	8.9 ± 1.7	7, 19.2
Duration of E2 consumption (day)	14.6 ± 2.1	11, 24
P4 level on ET day (ng/mL)	22.8 ± 10.1	7.5, 60.8
Continuous Variables[c]	**Frequency**	**Percentage**
Type of infertility		
Primary	85	70.3
Secondary	36	29.7
Cause of infertility		
Ovulatory	41	33.9
Tubal	8	6.6
Male factor	27	22.3
Un-explained	5	4.1
Multifactorial	40	33.1
Embryo transfer cycle number		
First	67	55.4
Second	40	33.1
Third	14	11.5
Number of transferred embryos		
One	7	5.8
Two	114	94.2
Embryo age at transfer		
Day 3	67	55.4
Day 5	54	44.6

Min: Minimum, Max: Maximum, BMI: Body mass index, E2: Estradiol, P4: Progesterone
[a]Total number of participants = 121, [b]Values are presented as mean ± standard deviation (SD) and range for continuous variables. [c]Categorical variables are presented as number and percentage.

Serum progesterone quartiles and baseline characteristics: Participants receiving IM P4 for luteal phase support (LPS) were stratified into quartiles based on serum P4 levels on ET day: Q1 (10.0–16.2 ng/mL), Q2 (16.3–20.9 ng/mL), Q3 (21.0–27.8 ng/mL), and Q4 (>27.8 ng/mL). No statistically significant differences in demographic or clinical characteristics—including age (p = 0.94), BMI (p = 0.16), duration of infertility (p = 0.92), endometrial thickness (p = 0.63), number of transferred embryos (p = 0.053), and age of transferred embryos (p = 0.31)—were observed across quartiles (Table 2).

Pregnancy outcomes: The overall chemical pregnancy rate was 23.1%, the clinical pregnancy rate was 18.2%, and the ongoing pregnancy rate was 14.1%, with a miscarriage rate of 5.0%. Pregnancy outcomes did not significantly differ between P4 administration protocols (IM P4 vs. IM P4 + DYD; p = 0.36 for chemical pregnancy, p = 0.31 for clinical pregnancy, p = 0.75 for ongoing pregnancy) (Table 3). Although higher chemical and clinical pregnancy rates were observed in Q4 compared to other quartiles, these differences did not reach statistical significance among quartile groups (p = 0.15 for chemical pregnancy, p = 0.75 for clinical pregnancy) (Table 2).

Multivariate analysis: Multivariate logistic regression analysis revealed that higher serum P4 levels on ET day (Q4: >27.8 ng/mL) were significantly associated with reduced odds of chemical pregnancy compared to Q1 (OR = 0.20; 95% CI: 0.05–0.86; p = 0.03) (Table 4). Other variables, including age (p = 0.09), BMI (p = 0.32), duration of infertility (p = 0.79), endometrial thickness (p = 0.23), duration of E2 consumption (p = 0.31), number of transferred embryos (p = 0.66), and embryo age (developmental stage) at transfer (p = 0.42) were not significantly associated with pregnancy outcomes (Table 4).

Combined quartile analysis: To further assess the relationship between serum P4 levels and pregnancy outcomes, quartiles were grouped into two categories: Q1 & Q2 (10.0–20.9 ng/mL) and Q3 & Q4 (> 21.0 ng/mL). Figure 1 illustrates the comparison of pregnancy outcomes between these groups.

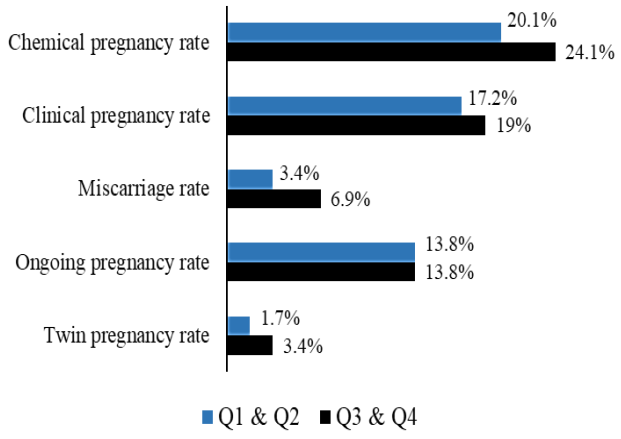

Figure 1: Pregnancy outcomes according to serum P4 levels on the embryo transfer day split in Q1 & Q2 vs. Q3 & Q4

Table 2. Comparison of variables according to serum progesterone quartiles[a,b]

Variable	Serum P4 levels[c] (ng/mL)					p-value between quartiles[d]
	IM P4 + DYD	IM P4				
	P4 <10 (n=5)	Q1 (10.0-16.2) (n=29)	Q2 (16.3-20.9) (n=29)	Q3 (21.0-27.8) (n=29)	Q4 (>27.8) (n=29)	
Age (year)	37.9 ± 5.1	35.5 ± 5.7	35.3 ± 5.3	36.1 ± 6.9	35.2 ± 6.9	0.94
BMI (kg/m^2)	26.8 ± 4.4	27.6 ± 4.2	26.6 ± 4.4	25.8 ± 5.4	25.0 ± 3.9	0.16
Duration of infertility (year)	8.0 ± 5.1	6.9 ± 5.5	5.9 ± 3.7	5.6 ± 2.8	5.3 ± 3.1	0.92
Endometrial thickness (mm)	9.0 ± 1.9	9.2 ± 1.5	8.9 ± 2.2	8.6 ± 1.3	9.1 ± 1.8	0.63
Duration of E2 consumption (day)	13.4 ± 1.3	14.2 ± 2.2	14.4 ± 2.3	14.9 ± 2.1	14.8 ± 1.7	0.58
Number of transferred embryos n (%)						0.053
One	0 (0)	0 (0)	0 (0)	4 (13.8)	3 (10.3)	
Two	5 (100)	29 (100)	29 (100)	25 (86.2)	26 (89.7)	
Embryo quality n (%)						0.12
Excellent	2 (40)	10 (34.5)	15 (51.7)	8 (27.6)	9 (31.1)	
Good	3 (60)	18 (62.1)	11 (38)	17 (58.6)	17 (58.6)	
Average	0 (0)	1 (3.4)	3 (10.3)	4 (13.8)	3 (10.3)	
Embryo age at transfer n (%)						0.31
Day 3	3 (60)	20 (69)	13 (44.8)	16 (55.2)	15 (51.7)	
Day 5	2 (40)	9 (31)	16 (55.2)	13 (44.8)	14 (48.3)	
Chemical pregnancy n (%)	2 (40)	4 (13.8)	8 (27.6)	4 (13.8)	10 (34.5)	0.15
Clinical pregnancy n (%)	1 (20)	4 (13.8)	6 (20.6)	3 (10.3)	8 (27.6)	0.75
Miscarriage n (%)	0 (0)	1 (3.4)	1 (3.4)	1 (3.4)	3 (10.3)	0.90
Ongoing pregnancy n (%)	1 (20)	3 (10.3)	5 (17.2)	2 (6.9)	6 (20.6)	0.91
Twin pregnancy n (%)	1 (20)	1 (3.4)	0 (0)	0 (0)	2 (6.9)	0.39

Q: Quartile, IM P4: Intramuscular progesterone, DYD: Dydrogesterone, BMI: Body mass index, E2: Estradiol
[a]Continuous variables are presented as mean ± standard deviation, [b]Categorical variables are presented as number (percentage). [c]Progesterone levels were measured on the day of embryo transfer. [d]p-values were calculated using ANOVA for continuous variables and chi-square test for categorical variables to represent the comparison between different serum progesterone levels (categorized into quartiles: Q1 (10.0-16.2 ng/mL), Q2 (16.3-20.9 ng/mL), Q3 (21.0-27.8 ng/mL), Q4 (>27.8 ng/mL)).

Patients in Q3 & Q4 demonstrated numerically higher rates of pregnancy outcomes, but these findings lacked statistical significance (p = 0.66 for chemical pregnancy, p = 0.76 for clinical pregnancy, p = 0.77 for miscarriage, p = 0.62 for ongoing pregnancy, and 0.64 for twin pregnancy).

Discussion

This prospective cohort study aimed to investigate the potency of IM P4 administration to reach serum P4 levels on the day of ET to the favorable range in the HRT-FET cycles. The study also explored pregnancy outcomes across varying serum P4 levels and evaluated whether the addition of oral DYD in patients with suboptimal P4 levels (<10 ng/mL) could influence outcomes. All patients initially received IM P4 (50 mg/day) according to the standard clinical protocol.

Table 3. Pregnancy outcomes based on serum progesterone levels on embryo transfer day[a]

Pregnancy outcome	Total (%)	Serum P4 levels[b] (ng/mL)		p-value[c]
		>10 (n=116)	<10 (n=5)	
Chemical pregnancy rate	28 (23.1%)	2 (40%)	26 (22.4%)	0.36
Clinical pregnancy rate	22 (18.2%)	1 (20%)	21 (18.1%)	0.31
Miscarriage rate	6 (5.0%)	0 (0%)	6 (5.2%)	0.54
Ongoing pregnancy rate	17 (14.1%)	1 (20%)	16 (13.8%)	0.75
Twin pregnancy rate	4 (3.3%)	1 (20%)	3 (2.6%)	0.13

[a]Values are presented as number (percentage). [b]Progesterone (P4) levels were measured on the day of embryo transfer. [c]p-values were calculated using chi-square test or Fisher's exact test as appropriate to compare pregnancy outcomes between participants with serum P4 levels >10 ng/mL (n=116) and those with P4 levels <10 ng/mL (n=5).

Table 4. Multivariate logistic regression analysis of factors associated with chemical pregnancy

Variable	β Coefficient	Odds Ratio[a] (95% CI)	p-value[b]
Age	0.07	1.07 (0.99-1.16)	0.09
BMI	-0.06	0.95 (0.85-1.06)	0.32
Duration of infertility	0.02	1.02 (0.89-1.16)	0.79
Endometrial thickness	-0.16	0.85 (0.66-1.11)	0.23
Duration of E2 consumption	0.14	1.15 (0.88-1.50)	0.31
Number of transferred embryos	-0.55	0.58 (0.05-6.52)	0.66
Embryo age at transfer			
Day 3		Reference group	
Day 5	0.42	1.53 (0.54-4.23)	0.42
Serum P4 levels[c] (ng/mL)			
Q1 (10.0-16.2)		Reference group	
Q2 (16.3-20.9)	-1.14	0.32 (0.08-1.33)	0.12
Q3 (21.0-27.8)	-0.47	0.63 (0.13-3.14)	0.57
Q4 (>27.8)	-1.61	0.20 (0.05-0.86)	0.03

CI: Confidence interval, BMI: Body mass index, E2: Estradiol, P4: Progesterone, Q: Quartile
[a]Odds ratios were adjusted for all variables included in the model. [b]Values in bold represent statistically significant associations ($p<0.05$). [c]Quartile 1 serves as the reference group for serum progesterone level comparisons.

In a limited number of cases with low serum P4 levels (<10 ng/mL), oral DYD (20 mg/day) was added to the protocol as an ethical and clinical measure to prevent potential treatment failure. This subgroup was analyzed secondarily to observe outcomes in patients requiring supplementation.

Notably, despite lower P4 levels, there was no statistically significant difference in clinical pregnancy rate (CPR), ongoing pregnancy rate (OPR), or miscarriage rate (MR) between patients receiving IM P4 alone and those receiving additional DYD. Administration of 50 mg/day IM P4 resulted in achieving serum P4 levels >10 ng/mL in approximately 96% of participants. Among them, 78.5% had serum P4 levels within the predefined optimal range of 10–32.5 ng/mL, supporting its role as an effective and reliable treatment strategy.

The CPR, OPR, and MR in our study were reported as 18.2%, 14.1%, and 5.0%, respectively. In a prior study, Alyasin and colleagues estimated a CPR of 35.6% in the group that received IM P4. They demonstrated a significant correlation between serum P4 levels and CPR as well as the LBR (10). Gaggiotti-Marre et al. demonstrated that women with lower quartile P4 levels have a higher MR compared to women with higher quartile levels (13). The findings of Álvarez et al.'s study indicated that individualized LPS led to higher OPR in patients receiving HRT one day before ET (25). Results from several previous studies also showed that serum P4 level is a significant factor in predicting independent LBR (26, 27), which contradicts our study's results. Possible reasons for this difference include the variations in P4 route and dose of administration, timing of measurement, the baseline P4 levels, and individual metabolic characteristics of participants. Nevertheless, some studies support our findings. In line with our results, Volovsky et al. in their study showed that P4 levels >10 ng/mL on the day of FET are not a significant factor in predicting CPR and LBR (12). Alyasin et al. did not find any correlation between serum P4 levels and miscarriage (10). Polat and colleagues, in a retrospective cohort study, demonstrated that while examining the impact of VP4 compared to IM P4 on OPR, the prescription of P4 is not an independent predictor of OPR (14). The findings of the study by Cédrin-Durnerin et al. indicated that doubling the dose of VP4 on the ET day has no effect on fertility outcomes (27). In the study by Chen and colleagues, serum P4 levels on ET day showed no significant association with LBR or CPR in artificial cycles with IM P4, while D5 blastocysts and good-quality embryos were independently linked to higher LBR (28).

The findings of this study indicated that there is no significant difference in serum P4 levels across age and BMI quartiles. However, González-Foruria et al. found that a woman's age can influence the absorption of P4 (26), which contrasts with our study's results. The potential reason for this

difference may be the variation in the method of VP4 administration compared to IM P4. The researchers in that study also attributed the potential increase in drug absorption to vaginal atrophy and thin vaginal mucosa in older women.

The optimal timing of P4 testing and the ideal P4 blood concentrations have not been definitively established, and treatment strategies based on serum P4 are still under investigation. Findings from Kofinas et al.'s study indicate that maintaining P4 levels within the range of 10-20 ng/ml is essential (6). Furthermore, the results of this study showed that P4 levels higher than 30 ng/ml have an adverse effect on the cycle. In interpreting the findings of this study, it can be said that low P4 levels hinder endometrial growth. On the other hand, significantly higher P4 levels accelerate endometrial growth, potentially delaying the window of implantation.

The main source of P4 in the early stages of NC is the corpus luteum, which persists until approximately the first 8 weeks of pregnancy. After that, placental P4 synthesis takes over. After the luteal phase declines, infertility treatment necessitates P4 supplementation (29). In our study, we examined P4 levels during cycles controlled by IM exogenous hormone therapy. While the IM method presented notable challenges in patient tolerance, its potential clinical advantages warrant careful consideration in assisted reproductive techniques. The results of a study by Devine et al. demonstrated that women who received only VP4 for LPS, without any IM administration of P4, had significantly poorer OPR (18). Kaser et al. found that women who received Crinone vaginal gel, had 44% lower odds of CPR and 49% lower odds of LBR, compared with women who received IM P4 (19). In a randomized clinical trial by Tehraninejad et al., IM P4 administration for LPS demonstrated superior chemical pregnancy rates compared to vaginal and subcutaneous routes, despite patients reporting higher discomfort related to pain and swelling (17). However, some other research appeared that endometrial P4 levels play a more significant role in the success of FET cycles compared to serum levels (16, 30). In total, due to the lack of strong randomized controlled trials, a consensus on the best P4 administration method during FET cycles has not been reached.

There is a prevailing belief among a proportion of Iranian reproductive endocrinology and infertility specialists that administering 50 mg/day of IM P4 for endometrial preparation in FET cycles is considered insufficient. Consequently, they routinely prescribe 100 mg/day of IM P4 (Fertigest, Aburaihan Pharmaceutical Co., Tehran, Iran) (22, 31-33), 800 mg/day of VP4 (Cyclogest®, Actoverco, Iran) (22, 34-37), or a combination of IM P4 and VP4 (10, 24), despite increased patient discomfort. In the present study, administration of 50 mg/day IM P4 alone not only resulted in achieving substantial serum P4 levels (>10 ng/mL) on the ET day in about 96% of women but also yielded comparable pregnancy outcomes to other similar studies that utilized higher P4 doses.

In prior studies by Arabian et al. and Lorillon et al., oral DYD was found to be as effective as IM P4 (24) and VP4 (21) in maintaining pregnancy, as measured by CPR, OPR, and MR. Also, Rashidi et al. evaluated the pregnancy outcomes of oral, IM, and vaginal P4 administration for LPS during HRT-FET (22). According to their results, the pregnancy rates, abortion, and LBR were not significantly different among groups. Consequently, these findings positioned DYD as a compelling hormonal support protocol, characterized by reduced local complications, lower treatment costs, and enhanced patient satisfaction. In our current study, among 5 women receiving DYD combined with IM P4 according to the specified protocol, the OPR and MR were 20% and 0%. Interestingly, despite observing lower serum P4 levels on the ET day in this cohort, the pregnancy outcomes were superior compared to the group receiving IM P4 alone, although this difference did not reach statistical significance. However, it is crucial to acknowledge the limitation of our study's small sample size, which substantially constrains the potential for robust statistical analysis and broader result generalization.

This study had several limitations. One limitation is the relatively short follow-up period, which only extended to the first 12 weeks of pregnancy. Additionally, the study did not investigate the outcomes of IM P4 administration after childbirth and the LBR. Given the limited sample size and the potential variability in individual responses to P4, further research is necessary to explore the potential benefits of individualized LPS. Future studies should aim to include larger, multicenter cohorts, assess the impact of different P4 administration routes and dosages, and evaluate outcomes, especially LBR and neonatal health rate during FET cycles.

Conclusion

Daily administration of 50 mg IM P4 remains a

promising approach for endometrial preparation in FET cycles, offering accessibility and effective elevation of serum P4 levels to optimal ranges. However, monitoring serum P4 levels on ET day is recommended to ensure adequate LPS. The addition of DYD can serve as a supplementary measure in these cases. This study identified a significant negative association between elevated serum P4 levels (>27.8 ng/mL) on the ET day and chemical pregnancy rates. However, the absence of significant correlations with other pregnancy outcomes highlights the multifactorial nature of embryo implantation success. These findings emphasize the need for further research to refine P4 thresholds and identify additional predictive factors influencing pregnancy outcomes in FET cycles.

Conflict of Interests

Authors declare no conflict of interests.

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