

DOI: <https://dx.doi.org/10.18203/2320-1770.ijrcog20252322>

Original Research Article

A retrospective cohort study on optimal serum progesterone levels before FET cycles using vaginal and parenteral progesterone supplementation and its pregnancy outcomes

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Received: 06 May 2025

Revised: 09 July 2025

Accepted: 10 July 2025

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ABSTRACT

Background: Hormone replacement therapy (HRT) allows the day of embryo transfer to be scheduled and reduces the need for monitoring. We want to evaluate the relationship between serum progesterone (P) levels on the day of embryo transfer and pregnancy outcomes determining an ideal level could enable frozen-thawed embryo transfer (FET) personalization.

Methods: This study included 212 women who underwent FET-ICSI cycles. Women were grouped into two groups of administration (vaginal and parenteral) based on the route of progesterone administration. We investigated the correlations between route of progesterone administration and pregnancy outcomes, route of progesterone administration and serum P4 levels and route of progesterone administration and endometrial thickness.

Results: Pregnancy outcome in vaginal group was 67.65% and parenteral group was 60.53%.

p value was 0.2969, which is statistically insignificant. Median value for vaginal group was 11.60 and for parenteral group, it was 32.90. p value was <0.001, which is statistically significant of serum progesterone. Median value for vaginal group was 8.550 and for parenteral group, it was 8.660, p value was <0.001, which is statistically significant of endometrial thickness.

Conclusions: Higher clinical pregnancies are obtained from vaginal group when compared to parenteral group. There was a statistically significant difference while correlating serum P4 value with route of progesterone and there was no statistical difference in the endometrial thickness between the vaginal and parenteral groups.

Keywords: Frozen-thawed embryo transfer, Hormonal replacement therapy, Route of progesterone, Serum progesterone

INTRODUCTION

According to the World Health Organization, infertility is the inability to conceive after 12 months or more of consistent, unprotected sexual intercourse. It is a major worldwide social and demographic issue.¹ In July 1978, Louise Joy Brown, the first “test-tube baby,” was born through IVF, marking a breakthrough in reproductive

medicine.² The use of routine blastocyst-stage embryo transfers instead of cleavage-stage embryo transfer, replacing fresh embryo transfer with embryo cryopreservation (“freezing”) and subsequent frozen-thawed embryo transfer, preimplantation genetic screening (PGS), single embryo transfer (SET) instead of double embryo transfer (DET) and minimal stimulation protocols are some of the new ART practice regimens that have been

integrated differently worldwide.³ The use of frozen-thawed embryo transfer (FET) procedures has significantly expanded globally over the past ten years. The primary factors are the rise in elective single embryo transfers, "freeze-all" strategies to prevent ovarian hyperstimulation syndrome and the detrimental effects of premature progesterone (P) elevation and supraphysiologic estradiol levels on embryo implantation during the fresh embryo transfer cycle. A popular endometrial preparation method is hormone replacement therapy (HRT), which allows the day of embryo transfer to be scheduled and reduces the need for monitoring. Given the widespread use of HRT-FET cycles, there is considerable discussion about the relationship between serum P levels on the day of embryo transfer and pregnancy outcomes, as determining an ideal level could enable FET personalization in HRT.⁴

Since the endometrial window of implantation (WOI) is confined to the stenosis interval in the luteal phase, progesterone seems to be a significant determinant of the WOI, in contrast to estrogen.⁵ Both low and high serum P levels on the day of ET were associated with lower pregnancy rates, according to data from two different studies.⁶ According to Coroleu and Gaggiotti-Marre, to create a suitable immunological environment that enables implantation and decreases pregnancy loss in FET cycles, a specific serum P value should be attained.⁷ To obtain high-quality embryos for transfer, ovarian stimulation aims to extract multiple mature oocytes for fertilization.⁸ Gonadotropins and gonadotropin-releasing hormone (GnRH) analogs are typically administered together as part of OS; the two most popular protocols are the multiple-dose GnRH-antagonist (GnRH-ant) and the long GnRH-agonist (GnRH-ag) suppressive protocol.⁹

It can be used for two purposes: timed insemination or timed intercourse and obtaining multiple oocytes at follicular aspiration for assisted reproduction.¹⁰ The last and most important step in ART is embryo transfer. The ET procedure is a precise technique that involves expelling the embryos after inserting the ET catheter into the endometrial cavity. There are two methods for embryo transfer: fresh transfer and frozen-thawed embryo transfer. The typical timing for fresh embryo transfer is 48–72 hours following oocyte insemination. Frozen-thawed embryo transfer involves thawing and transferring a frozen embryo into a woman's uterus. Since the first frozen embryo transfer (FET) baby was born in 1984, the procedure has grown in significance on a global scale.¹¹ Therefore, since COS may have negative effects on endometrial receptivity and embryo cryopreservation has become a usual procedure in ART centers, the "freeze-all" policy has evolved as an alternative to fresh ET to improve IVF outcomes.¹²

Indications for frozen-thawed embryo transfer include the freeze-all strategy involves cryopreservation of the embryo following biopsy and pre-implantation genetic testing (PGS) of day 5 and day 6 embryos. However, when

transferring embryos on day 5 of a fresh autologous cycle, controlled ovarian stimulation has a detrimental effect on embryo endometrium synchrony. This results from the day 5 blastocysts' inadequate implantation in fresh transfers. The freeze-all strategy is likely to be more beneficial in this state since it gives the clinician time to receive the PGT-A results and a euploid embryo would be transferred in a later cycle. Detrimental outcomes of elevated levels of supraphysiologic estradiol. A life-threatening side effect of ovarian stimulation during IVF cycles is ovarian hyperstimulation syndrome (OHSS). To reduce the risk of OHSS, all embryos may be electively cryopreserved and then transferred in non-stimulated cycles to avoid the endogenous rise in hCG in fresh transfer cycles. Increased progesterone impairs endometrial receptivity, which in turn affects implantation.¹³

Effective endometrial preparation is essential for FET success. The best procedure to prepare the endometrium is still up for debate.¹³ For frozen-thawed embryo transfer cycles, there are four typical endometrial preparation procedures. In Hormone replacement therapy, Exogenous estrogen and progesterone are administered as part of HRT, for FET to promote endometrial growth and suppress follicular growth. Progesterone is administered to prepare the endometrium for embryo transfer after estrogen is administered to promote endometrial growth. There are several ways to administer progesterone, including vaginal, oral, rectal, intramuscular and subcutaneous.

To prime the endometrium, estrogen medication is typically initiated in HRT during the first three days of a menstrual cycle. For embryo implantation and growth, the endometrial transition from the proliferative to the secretory phase is facilitated by progesterone at the appropriate level.¹⁴ The best time to administer progesterone in HRT for FET is essential to ensure appropriate endometrial preparation and synchronization with the embryo transfer. According to transvaginal ultrasonography, endometrial thickness (>7 mm), endometrial pattern (the triple line pattern) and endometrial blood flow (presence of endometrial blood flow) are the most often observed markers of excellent endometrial receptivity in HRT FET.¹⁵

METHODS

Study design

This retrospective cohort study included 212 women who underwent frozen-thawed embryo transfers at Malar Fertility and Research Centre, Tambaram, Chennai, Tamil Nadu, between 2022 and 2024. A total of 212 cases subjected to the ICSI-FET protocol and its outcome were recruited.

Information about the demographics of the patient, like age, day 2-E2, own/donor oocyte, type of stimulation protocol, semen parameters, total number of oocytes

collected and their stages, fertilization check, embryo stages of development (D1-D5/6), day of freezing, number of embryos frozen, FET-progesterone level on day 0, route of P administration, progesterone level before ET, endometrial thickness measurement before ET, number of embryos thawed and pregnancy outcome. Females aged ≤ 50 years, women undergoing the FET cycle using their own oocytes or donor oocytes, the presence of at least one good-quality embryo for transfer and women with a history of recurrent pregnancy loss were included. The presence of any uterine anomaly that may affect implantation was excluded.

Controlled ovarian stimulation

Cetrotide™ was used in a flexible gonadotropin antagonist protocol. Recombinant follicle-stimulating hormone (FSH) Gonal-f™ and highly-purified human menopausal gonadotropin (hMG) Menopur™, which had an activity of 75 IU per ampule for both FSH and luteinizing hormone (LH), were used to stimulate the ovaries.

Depending on the patient's age, baseline FSH, anti-mullerian hormone (AMH) and body mass index (BMI), the initial gonadotropin dosage varied between 150 and 300 IU. Accordingly, the number and size of follicles and the serum levels of progesterone (P4), LH and estradiol (E2) were tracked. The stimulation protocols began on day 2/3 of the menstrual cycle and concluded on the day that hCG was administered for the final oocyte maturation stage. When the leading follicle's diameter reached 18–22 mm, the dual trigger (agonist+injection hCG) was given.

Pituitary down-regulation was initiated in the mid-luteal phase of the menstrual cycle with (Lupride Depot 3.75 mg) as part of the long GnRHa regimen. After that, an ultrasound confirmed the down-regulation by measuring the thickness of the endometrium and the serum estradiol. Once down-regulation was established, gonadotropin injections (recombinant FSH, hMG or urinary follitropin) were administered at a dose of 300–450 IU/day to initiate ovarian stimulation. When the follicle reaches final maturation, an hCG trigger of 10,000 IU or 6,500 IU is given.

Oocyte retrieval

When two or more follicles reached 18–22 mm in diameter, hCG (Ovidrel™) was administered. The dosage ranged from 6500 to 13,000 IU, depending on the patient's age, baseline FSH and BMI. 34–36 hours after the hCG trigger, oocytes were retrieved. The intracytoplasmic sperm injection done on retrieved oocytes.

Luteal phase support

To trigger final oocyte maturation, the hCG injection (Ovitrelle 6,500 IU/day) was given with the aim of at least two to three follicles reaching ≥ 18 mm in diameter. The daily administration of progesterone (C-Hop® 400 mg)

and (Duphaston® 10 mg) was used to support the luteal phase in both groups until a clinical pregnancy was obtained.

Embryo culture and freezing

Intracytoplasmic sperm injection was used to inseminate freshly collected oocytes. 16–18 hours after insemination, the fertilization check was done and zygotes with two pronuclei were cultured with Invitro One-step Media™ media overlaid with V-OIL. On Days 3 and 5, the quality of the embryos was assessed. After incubation, the embryos were frozen after their quality was assessed. Gardner and Schoolcraft's blastocyst classification system was used to grade the embryo quality. Expansion, inner cell mass and trophectoderm were assessed after evaluation and the best morphologically graded embryos were chosen for embryo transfer. Using a Kitazato vitrification kit, the embryos were frozen in liquid nitrogen until the frozen-thawed embryo transfer process began.

Endometrial preparation methods before frozen-thawed embryo transfer

Beginning on the 2nd day of their menstrual cycle, patients receiving the HRT-FET protocol, where the menstrual flow should be good, had blood tests taken to evaluate E2, where E2 should be < 50 ng/ml; if > 50 ng/ml, leuprolide acetate 0.1-1 CC was given and a transvaginal ultrasound scan was done to evaluate the ET, which should not be > 4 mm and thickened and the cyst should not be present. They received 2 daily doses of estradiol (Estrin™ 2 mg), 1 daily dose of folic acid (5 mg), Ecospirin® (75 mg) and Invitro-f-sachet™ (5 mg).

Following a 10-day course of estradiol treatment, patients underwent transvaginal ultrasound scanning on Days 8, 10, 12 and 14 to measure the endometrial thickness. The dose was increased and the medication was continued if the endometrial thickness did not reach 8 mm on the day of the scan. On day 12 (which is the starting day of P, considered as P0), blood tests were taken to evaluate serum progesterone levels. If the progesterone is < 1 ng/ml, it is considered normal to continue with a prescription of C-Hop 400 and if the progesterone is > 1 ng/ml, the cycle is cancelled. Patients were advised to start taking vaginal progesterone twice daily for five days after the endometrium's thickness had increased to between 8 and 10 mm. On the day of ET, blood tests were taken to evaluate serum progesterone level, which should be at least 10 ng/ml and endometrial thickness was measured with the guidance of a transvaginal ultrasound scan. The endometrium should be echogenic and have a thickness of greater than 7 mm. If the serum progesterone < 10 ng/ml, 100 mg of progesterone injection is given before the transfer day. If the patient has a history of previously failed implantation or miscarriage, parenteral progesterone is supplemented instead of vaginal supplementation to support the body's natural progesterone production. The

following day, after progesterone supplementation was completed, the embryo was thawed and transferred.

Pregnancy determination

Pregnancy was defined as a positive β -hCG measurement 15 days following embryo transfer. A sonographic evaluation shows fetal cardiac motion, which led to the diagnosis of clinical pregnancy.

Statistical analysis

For the analysis, the subjects were divided into two groups depending on the route of progesterone administration: group 1: vaginal and group 2: parenteral. The collected data were analyzed using IBM SPSS Statistics for

Windows, version 23.0 (Armonk, NY: IBM Corp). The unpaired sample t-test was used to compare the means of two independent groups to determine if there was a significant difference between them. When the data was not normally distributed, the two independent groups were compared using the Mann-Whitney test. The chi-square test was used to explore the relationship between the two categorical variables. The probability value (p value) of 0.05 was considered a statistically significant value in all of the statistical methods mentioned above.

RESULTS

This study included 212 women who underwent ICSI-FET cycles in which (n=138) tested positive during clinical pregnancy tests.

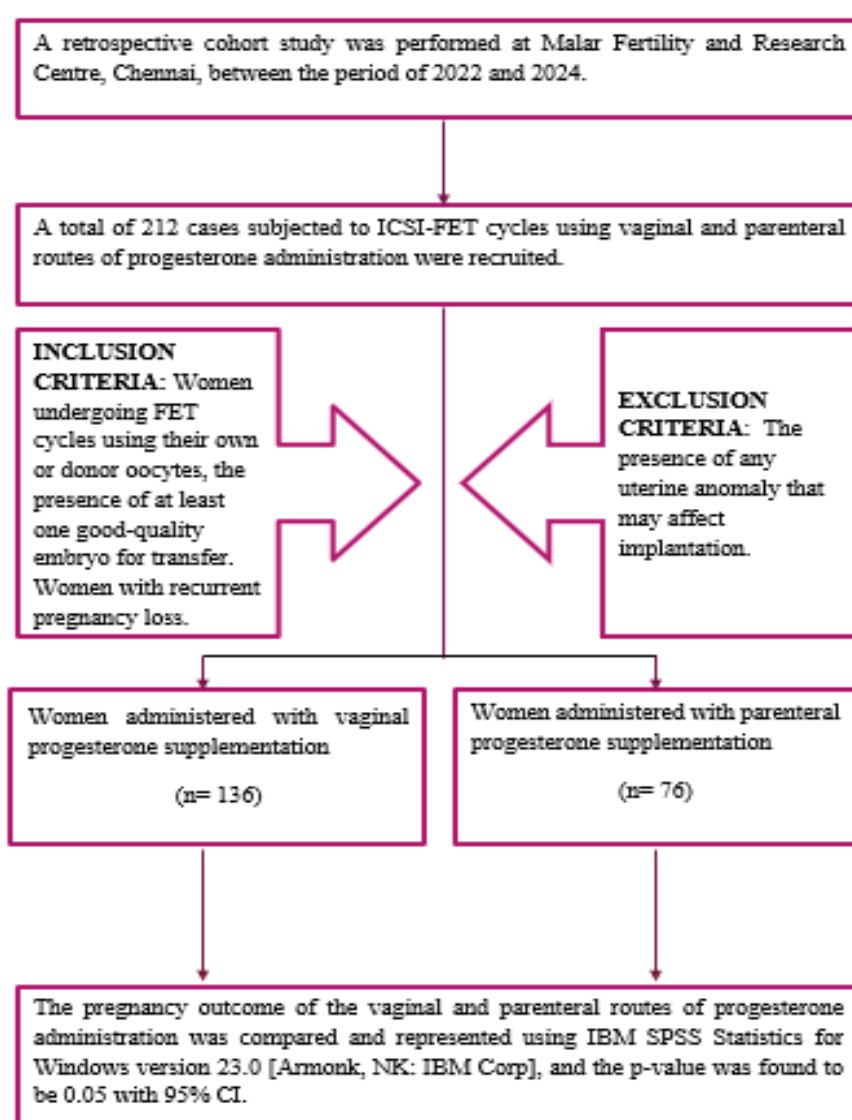


Figure 1: Flowchart explaining the workflow for methodology.

Women were grouped into two groups based on the route of progesterone administration (vaginal and parenteral), where the vaginal group (n=136), in which (n=92) tested

positive during a clinical pregnancy test and the parenteral group (n=76) in which (n=46) tested positive during a clinical pregnancy test. Table 1 and Figure 2 summarize

the correlation of the route of progesterone administration with pregnancy outcomes in patients who underwent ICSI-FET cycles, determined by the chi-square test. The pregnancy outcome in the vaginal group was 67.60% and the parenteral group was 60.53%. The p value was 0.2969 ($p>0.05$), which is statistically insignificant and it was a two-tailed p value.

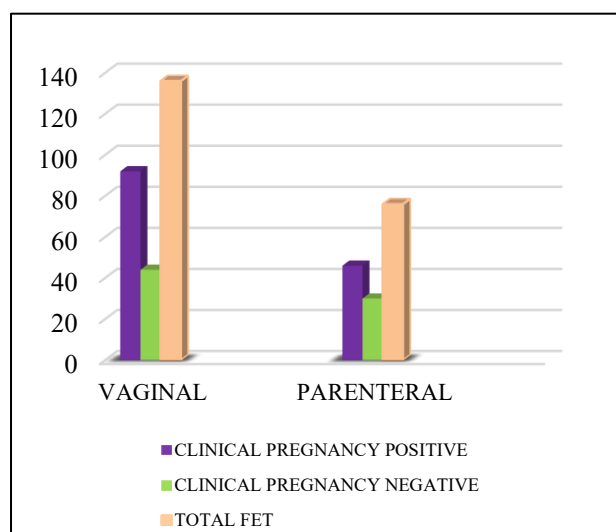


Figure 2: Correlation of route of progesterone administration with pregnancy outcomes in patients who underwent ICSI-FET cycles.

The table was analyzed using the Chi-square test. The p value was 0.2969 ($p>0.05$), which is statistically non-significant. Two-tailed P value.

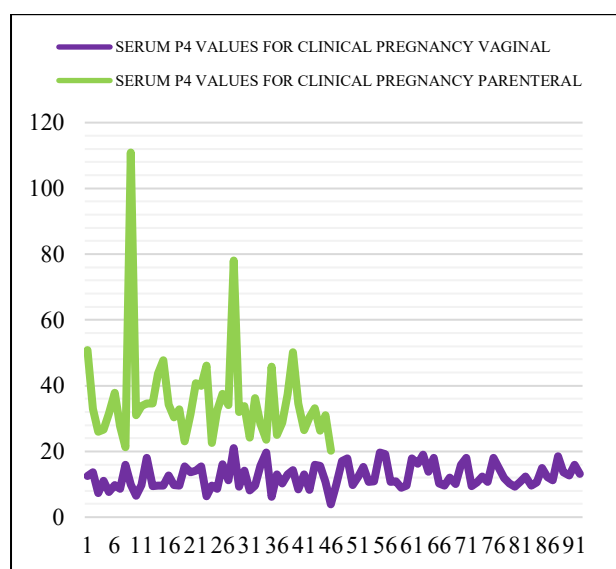


Figure 3: Correlation of the route of progesterone administration with Serum P4 value in patients who underwent ICSI-FET cycles.

The table was analyzed using the Mann-Whitney test, where Column B was compared with Column A. Column: Unpaired t-test. Column B-Parenteral. Column A-Vaginal. The p-value was <0.001 ($p<0.05$), which is statistically significant. Two-tailed P value. Actual median difference: 21.31.

Table 2 and Figure 3 summarize the correlation of serum P4 with the route of progesterone administration in patients who underwent ICSI-FET cycles determined by the Mann-Whitney test, where Column B was compared with Column A, where Column A (vaginal ($n=92$)) and Column B (parenteral ($n=46$)) were compared using the unpaired t-test.

The sum of Column A was 4279 and the sum of Column B was 5312. The median value for Column A was 11.60 and for Column B, it was 32.90. The actual median difference was 21.31. The Hodges-Lehmann difference was 20.63. The p value was <0.001 ($p<0.05$), which is statistically significant and it was a two-tailed p value.

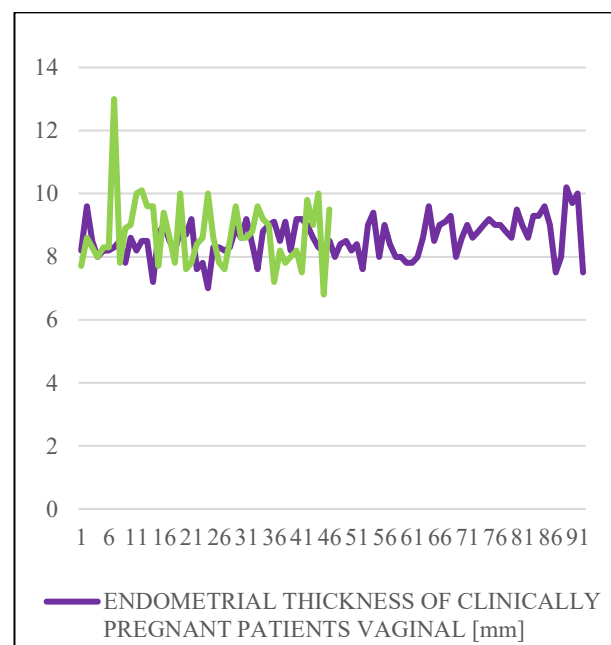


Figure 4: Correlation of the route of progesterone administration and endometrial thickness in patients who underwent ICSI-FET cycles.

The table was analyzed using the Mann-Whitney test, where Column B was compared with Column A. Column: Unpaired t-test. Column B-Parenteral. Column A-Vaginal. The p-value was 0.7997 ($p>0.05$), which is statistically non-significant. Two-tailed P value. Actual median difference: 0.05000.

Table 3 and Figure 4 summarize the correlation of endometrial thickness with the route of progesterone administration in patients who underwent ICSI-FET cycles determined by the Mann-Whitney test, where Column B was compared with Column A, where Column A (vaginal ($n=92$)) and Column B (parenteral ($n=46$)) were compared using the unpaired t-test.

The sum of Column A was 6338 and the sum of Column B was 3254. The median value for Column A was 8.550 and for Column B, it was 8.660. The actual median difference was 0.05000. The Hodges-Lehmann difference was 0.000. The p value was <0.001 ($p<0.05$), which is statistically significant and it is a two-tailed p value.

Table 1: Correlation of route of progesterone administration with pregnancy outcomes in patients who underwent ICSI-FET cycles.

Route of P	Pregnant	Non-pregnant	Total
Vaginal	92	44	136
Parenteral	46	30	76
Total	138	74	212

Table 2: Correlation of the route of progesterone administration with serum P4 value in patients who underwent ICSI-FET cycles.

Column	N	Median of serum P4
A (Vaginal)	92	11.60
B (Parenteral)	46	32.90

Table 3: Correlation of the route of progesterone administration with endometrial thickness in patients who underwent ICSI-FET cycles.

Column	N	Median of endometrial thickness
A (Vaginal)	92	8.550
B (Parenteral)	46	8.600

DISCUSSION

This retrospective cohort study aimed to investigate the relationship between the route of progesterone administration and pregnancy outcomes in frozen embryo transfer (FET) cycles using vaginal and parenteral progesterone supplementation. The results indicated that the pregnancy outcome in the vaginal supplementation group is higher when compared with the parenteral supplementation group. Wang et al, investigated the effects of vaginal progesterone gel versus intramuscular progesterone injection for luteal phase support. They discovered that both approaches produced comparable pregnancy outcomes, indicating that vaginal progesterone gel supplementation is an effective replacement for intramuscular injection.

The rate of clinical pregnancy or live birth is higher when high-dose vaginal progesterone supplements are taken. The investigation of Enatsu et al, has demonstrated this. In addition to having a lower serum level but a higher progesterone level in the uterine endometrium, the vaginal route of progesterone supplementation has several benefits over the intramuscular approach, such as fewer side effects, less pain and improved compliance, which benefits the pregnancy rate.

Transvaginal and intramuscular progesterone supplementation does not significantly differ in the incidence of clinical pregnancy, continuous pregnancy, ectopic pregnancy, abortion and live birth according to various reports. In comparison to the group of patients receiving intramuscular progesterone injections, a large study showed that vaginal progesterone supplementation in the sustained release had significantly higher implantation, delivery and live birth rates but a considerably lower abortion rate.

Chi et al, discovered that the vaginal gel progesterone supplementation group had a significantly higher rate of implantation, clinical intrauterine pregnancy or live birth than the intramuscular progesterone group.

In their investigation of the effects of vaginal progesterone gel versus intramuscular progesterone injection for support during the luteal phase of external fertilization, Silverberg et al, discovered that women who received vaginal progesterone gel had significantly higher rates of pregnancy and delivery than those who received intramuscular progesterone injection.

Additionally, Ho et al discovered that progesterone supplementation via vaginal gel can dramatically raise the rate of implantation and pregnancy.¹⁶ Similar findings were also shown in Table 1 and Figure 2, which examined the relationship between the route of progesterone administration and pregnancy outcomes. Vaginal progesterone was associated with a considerably higher rate of clinical pregnancy (67.65%) than parenteral progesterone (60.53%).

The correlation between the route of progesterone administration and serum P4 is displayed in Table 2 and Figure 3. The findings showed that the incidence of clinical pregnancy varies significantly. According to Brady et al, in donor-recipient cycles, p values below 20 ng/ml on the day of embryo transfer are linked to reduced rates of live birth and clinical pregnancy. According to Kofin et al, a lower OPR/LBR was associated with P levels of more than 20 ng/ml on the day of the transfer (during frozen single euploid embryo transfer cycles). When oral dydrogesterone and vaginal progesterone were used to treat LPS, a study found no correlation between blood P levels on the day of ET and the live birth rate in HRT cycles.

According to Volovsky et al, the route of delivery influences both serum and intraendometrial P4 levels. More research is required to determine the best HRT-FET protocol and how to tailor luteal progesterone medication based on serum P4 levels. The ideal serum P4 cut-off level may vary based on the type, dosage and mode of administration.¹⁷

The pharmacokinetic variations likely result in significant differences in the time it takes for serum P to reach its peak value, which could partially account for the differences in the effectiveness of various serum P levels.⁴ Remarkably, in this investigation, the median value for the vaginal route was 11.60 and for the parenteral route, it was 32.90. The Mann-Whitney test revealed a statistically significant difference between the two routes ($p < 0.001$).

The route of progesterone administration and endometrial thickness were correlated, as seen in Table 3 and figure 4. The findings showed that the vaginal and parenteral groups' endometrial thicknesses did not differ significantly according to some research that investigated endometrial thickness as a determinant of improved clinical pregnancy rates. Endometrial thicknesses between 7 and 12 mm may produce the most promising outcome results in IVF-FET. It is also advised that endometrial thicknesses greater than 7 mm are required to obtain the best clinical pregnancy outcomes.¹⁸

According to Zhao et al, a triple-line endometrial pattern only suggested a successful pregnancy outcome when the triple-line pattern had improved pregnancy outcomes and the endometrial pattern only significantly affected the pregnancy rate in patients receiving the extended agonist treatment plan.¹⁹ Although there is no endometrial thickness at which there is no probability of pregnancy, it appears that the outcome of IVF is adversely influenced when the endometrial thickness drops to less than 7 mm.

However, there might be a range of ideal thicknesses that increase the likelihood of getting pregnant. According to Ata et al, having an endometrium that was 101.2 mm in diameter led to a greater LBR, although the difference was not statistically significant. Only 1.4% of reported instances had a thin endometrium < 7 mm, according to a comprehensive review and meta-analysis of 22 research papers.

According to the conclusion, this meta-analysis does not appear to support the use of endometrial thickness as a criterion for determining whether to freeze all embryos, cancel cycles or postpone additional IVF attempts.²⁰ In this study, the median values for the vaginal route and the parenteral route were 8.550 and 8.660, respectively.

CONCLUSION

This study presents that high clinical pregnancies were obtained from the vaginal supplementation group when compared with the parenteral supplementation group.

While correlating serum P4 value with the route of progesterone administration, there was a statistically significant difference between vaginal and parenteral groups. Furthermore, the endometrial thickness in the parenteral and vaginal groups was not significantly significant. Similar research has shown that progesterone injected intramuscularly had substantially lower rates of pregnancy or delivery than progesterone supplemented vaginally for luteal phase support.

There is a dispute over the best way to administer P. On the day of embryo transfer, there are significant differences in the serum P level even when the precise dosage and protocol are implemented. Therefore, while assessing serum P levels during the luteal phase of HRT cycles, it is crucial to take into consideration the P route of administration (vaginal, parenteral or oral), dose and time of P measure. Further investigations and study could help to choose the noble way to administer P in order to increase the implantation rates.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Panneerselvam P, Yuvashree K, Prakash S, Sritharan M, Elanchezhian M. A retrospective cohort study on optimal serum progesterone levels before FET cycles using vaginal and parenteral progesterone supplementation and its pregnancy outcomes. *Int J Reprod Contracept Obstet Gynecol* 2025;14:2544-51.