ACCSCIENCE PUBLISHING

Innovative Medicines & Omics

ORIGINAL RESEARCH ARTICLE

# Intramuscular progesterone and frozen embryo transfer outcomes: A multicenter prospective evaluation of the clinical relevance of serum progesterone monitoring

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**Citation:** Chandra V, Roque M, Murdia K, *et al.* Intramuscular progesterone and frozen embryo transfer outcomes: A multicenter prospective evaluation of the clinical relevance of serum progesterone monitoring. *Innov Med Omics*. 2025;2(4):93-101. doi: 10.36922/IMO025310037

**Received:** August 1, 2025

**Revised:** September 2, 2025

**Accepted:** October 10, 2025

**Published online:** November 14, 2025



## Abstract

The progesterone concentration in endometrial tissue after using vaginal progesterone supplementation is significantly higher than that with IM progesterone administration, whereas; serum progesterone levels are approximately four times higher with IM progesterone compared with vaginal administration. Therefore, serum progesterone cut-off points will differ according to the route of progesterone administration. This study aims to assess the association between serum progesterone levels on the day of frozen embryo transfer (FET) and pregnancy outcomes in artificial FET cycles. This multicenter prospective cohort study, conducted at different centers of Indira IVF Hospitals across India, included 353 women aged 21–40 years who underwent hormone replacement therapy-based FET cycles with intramuscular (IM) progesterone administered up to the day of embryo transfer. The mean serum progesterone level was 31.36 ± 13.78 ng/mL. Participants were categorized into quartiles based on serum progesterone levels: Q1 (<21.7 ng/mL), Q2 (21.7–28.1 ng/mL), Q3 (28.2–40.0 ng/mL), and Q4 (≥40.0 ng/mL). No significant differences in ongoing pregnancy rate (OPR), clinical pregnancy rate (CPR), first-trimester miscarriage rates, or live birth rate (LBR) were observed across the quartiles. Binary logistic regression revealed no statistically significant differences in OPR among the

quartiles. Our findings suggest that serum progesterone levels do not significantly influence clinical outcomes, including OPR, CPR, and LBR, in patients undergoing artificial FET cycles with IM progesterone support.

***Keywords:*** Serum progesterone; Frozen embryo transfer; *In vitro* fertilization; Ongoing pregnancy; Live birth rate

## 1. Introduction

Assisted reproductive technology (ART) has significantly evolved over the past decades, with frozen-thawed embryo transfer (FET) emerging as a key technique to enhance pregnancy outcomes. Increasing evidence highlights a concerning association between *in vitro* fertilization (IVF)/embryo transfer (ET) and adverse obstetric outcomes, including ectopic pregnancy, twin gestation, and placenta accreta spectrum (PAS) disorders.

Ectopic pregnancy, defined as the implantation of an embryo outside the uterine cavity, occurs in approximately 1.4–5.4% of IVF cycles, significantly higher than the 1–2% rate observed in spontaneous conceptions.[1] This elevated risk is influenced by factors such as tubal damage, multiple ETs, and embryo quality. Moreover, the incidence of twin pregnancies in IVF cycles remains disproportionately high—up to 32.1% compared to 1.5% in natural conceptions—primarily due to the transfer of multiple embryos.[2] Twin gestations are associated with increased maternal and neonatal complications, including preterm birth, hypertensive disorders, and cesarean delivery. Another critical concern is the rising prevalence of PAS disorders in IVF pregnancies. PAS encompasses a range of conditions characterized by abnormal placental adherence and invasion into the uterine wall, often leading to massive postpartum hemorrhage, organ injury, and hysterectomy. These findings underscore the importance of individualized risk assessment, judicious frozen ET practices, and multidisciplinary prenatal care in ART-conceived pregnancies. As fertility preservation and ART become more integrated into oncologic and reproductive medicine, understanding and mitigating these risks is essential for optimizing maternal and neonatal outcomes.

The success of FET largely depends on optimal endometrial receptivity, which is intricately regulated by the hormonal environment, particularly progesterone.[3] Progesterone plays a critical role in facilitating implantation by preparing the endometrium to support the embryo, thereby creating a conducive environment for implantation and pregnancy progression.[4,5]

Recent advancements have emphasized the importance of monitoring serum progesterone levels to predict the success of ART cycles. Studies have shown that suboptimal progesterone levels on the day of ET can negatively impact pregnancy outcomes.[6-8] Specifically, in cycles utilizing vaginal micronized progesterone, serum progesterone levels below a certain threshold have been linked to reduced clinical pregnancy rates (CPR) and ongoing pregnancy rates (OPR).[9,10]

Intramuscular (IM) progesterone has been used as an alternative to vaginal administration because it can achieve higher serum progesterone levels. The pharmacokinetics of IM progesterone differs from those of the vaginal route, potentially influencing its efficacy and clinical outcomes. While vaginal progesterone directly affects the endometrial tissue with lower systemic levels, IM progesterone results in higher systemic levels; however, its impact on endometrial receptivity and subsequent pregnancy outcomes remains unclear.[11,12]

Despite widespread use of IM progesterone in FET cycles, there is limited data on the association between luteal serum progesterone levels and FET outcomes when IM progesterone is used for luteal phase support. Existing literature primarily focuses on vaginal routes, leaving a knowledge gap regarding the predictive value of serum progesterone levels in cycles utilizing IM administration. This gap underscores the need for studies that specifically address whether serum progesterone levels on the day of FET correlate with clinical outcomes when using IM progesterone.[13-18]

This prospective cohort study aims to address this knowledge gap by investigating the relationship between serum progesterone levels on the day of ET and pregnancy outcomes in FET cycles utilizing IM progesterone. By categorizing participants based on their serum progesterone levels, this study seeks to identify if there is a threshold or optimal range that predicts better clinical outcomes. The findings could significantly impact clinical practice, potentially simplifying protocols and improving patient management in ART.

## 2. Materials and methods

### 2.1. Study design and participants

This prospective multicenter cohort study included 353 women aged 21–40 years undergoing FET under hormone

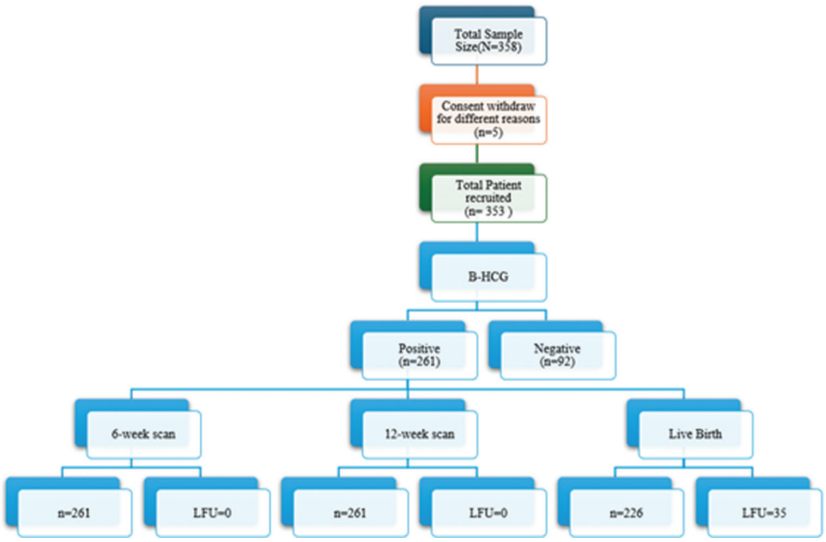


**Figure 1.** Patient flowchart from screening to the end of follow-up
Abbreviations: B_HCG: Beta-human chorionic gonadotropin; LFU: Luteinized unruptured follicles.

replacement therapy (HRT) cycles between February and December 2022. Participants were considered eligible if they had a triple-layer endometrium >7 mm and at least two high-quality embryos (Gardner's grade 3/4/5/AA/AB/BA/BB) available for transfer. Exclusion criteria included significant comorbidities such as uncontrolled diabetes, hypertension, heart disease, autoimmune disorders, uterine infertility factors, severe male factor infertility (total sperm count <5 million/mL), and cases requiring surgically retrieved sperm for intracytoplasmic sperm injection. All participants provided written informed consent before participating in the study. The patient flowchart from screening to the end of study follow-up is presented in Figure 1.

### 2.2. Ethical approval

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethics Committee at Indira IVF Hospital Private Limited under approval number ECR/1614/lns/RJ/2021, and the trial registration number is CTRI/2022/11/046995. All participants were informed of the study's objectives, procedures, potential risks, and benefits, and written consent was obtained before their participation. Data confidentiality was strictly maintained, with personal identifiers removed and data stored securely in compliance with data protection regulations.

### 2.3. Endometrial preparation and hormone administration

Endometrial preparation commenced with oral estradiol valerate (2 mg, twice or thrice daily) starting either on the 2nd day of the menstrual cycle or within 21 days of gonadotropin-releasing hormone agonist depot administration for downregulated cycles. Endometrial thickness was evaluated after 14 days of estradiol treatment. Progesterone supplementation was initiated if the endometrial thickness was ≥7 mm and the serum progesterone level was <1 ng/mL. IM progesterone was administered at a dose of 100 mg daily for 6 days before ET. Luteal phase support included oral estradiol valerate (6 mg daily), vaginal progesterone gel (Crinone 8%®) twice daily, and oral dydrogesterone (10 mg, twice daily) starting from the day of ET. A serum pregnancy test was performed 14 days after ET to confirm pregnancy.[19-21]

### 2.4. Embryo transfer procedure

Embryo transfer was conducted on the 6th day of progesterone administration. On the day of transfer, embryos were assessed for viability, expansion, and degeneration. Viable day-5 or day-6 blastocysts with good expansion were selected for transfer. The decision to perform a single or double ET was based on patient characteristics and clinical discretion. ETs were performed under ultrasound guidance using a soft catheter to ensure precision and reduce variability.[23]

### 2.5. Serum hormonal measurements

Serum progesterone levels were measured on the day of ET, approximately 20 h after the previous progesterone dose. Blood samples were processed within 8 h, and serum progesterone was quantified using a quantitative electrochemiluminescence immunoassay. The assay's reportable range for progesterone was 0.030–60.00 ng/mL.

Participants were stratified into quartiles based on their serum progesterone levels for subsequent analysis. Serum beta-human chorionic gonadotropin levels were determined by quantitative electrochemiluminescence immunoassay on day 14 post-ET; values ≥100 IU were considered positive for biochemical pregnancy.

### 2.6. Outcome measures

The primary outcome measure was the OPR. Secondary outcomes included CPR, miscarriage rate, and live birth rate (LBR). Clinical pregnancy was defined as the presence of one or more gestational sacs on ultrasound at 7 weeks of gestation or definitive clinical signs of pregnancy with fetal heart activity. Ongoing pregnancy was defined as a viable pregnancy beyond 12 weeks of gestation. First-trimester miscarriage was classified as a pregnancy loss occurring within the first 12 weeks.[23]

### 2.7. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 28.0. Continuous variables were presented as mean ± standard deviation (SD) or median (range), and categorical variables as frequencies and percentages. Serum progesterone levels were categorized into quartiles based on the 25$^{th}$, 50$^{th}$, and 75$^{th}$ percentiles. Comparisons between groups were performed using the unpaired *t*-test or Mann–Whitney U-test for continuous variables and the Chi-square test for categorical variables. Binary logistic regression analysis was conducted to evaluate the impact of serum progesterone levels on clinical pregnancy and ongoing pregnancy outcomes. $p<0.05$ was considered statistically significant.

## 3. Results

The mean age of the study population was 32.09 ± 4.19 years, and the mean body mass index was 24.70 ± 3.07 kg/m$^2$. The mean endometrial thickness was 8.87 ± 1.06 mm. The mean serum progesterone level on the day of ET was 31.36 ± 13.78 ng/mL (Table 1).

Participants were categorized into four groups based on serum progesterone levels as follows: Q1 (<21.7 ng/mL), Q2 (21.7–28.1 ng/mL), Q3 (28.2–40.0 ng/mL), and Q4 (≥40.0 ng/mL) (Table 2). The CPR, OPR, miscarriage rates, and LBR showed no significant differences across quartiles (Table 3 and Figure 2). To adjust for confounders, a binary logistic regression analysis was performed to compare IVF outcomes across different progesterone levels (ng/mL) on the day of ET, with Q1 as the reference (Table 4).

The OPRs by serum progesterone quartiles were as follows: Q1: 57.5%; Q2: 62.6%; Q3: 59.6%; and Q4: 62.8%. There were no statistically significant differences among these groups. Binary logistic regression analysis revealed no significant associations between progesterone quartiles and clinical outcomes.

**Table 1. Baseline patient characteristics**

| Characteristic (*n*=353) | Mean | SD | Median | Range | Minimum | Maximum |
|---|---|---|---|---|---|---|
| Age (years) | 32.09 | 4.19 | 32.00 | 19.00 | 21.00 | 40.00 |
| BMI (kg/m$^2$) | 24.70 | 3.07 | 24.80 | 18.50 | 18.50 | 30.00 |
| Endometrial thickness (mm) | 8.87 | 1.06 | 8.80 | 5.00 | 7.00 | 12.00 |
| P4 level on the day of ET (ng/mL) | 31.36 | 13.78 | 28.10 | 81.39 | 4.21 | 85.60 |

Abbreviations: BMI: Body mass index; ET: Embryo transfer; P4: Serum progesterone.

**Table 2. Quartile-wise distribution of serum progesterone among patients receiving embryo transfers**

| Variables | Categories | Number of patients | Percentage |
|---|---|---|---|
| Serum progesterone level by quartile (ng/dL) | Q1 (<21.7) | 87 | 24.6 |
| | Q2 (21.7–28.1) | 91 | 25.8 |
| | Q3 (28.2–40.0) | 89 | 25.2 |
| | Q4 (>40) | 86 | 24.4 |
| Number of embryos transferred | Single embryo transfer | 61 | 17.3 |
| | Double embryo transfer | 292 | 82.7 |

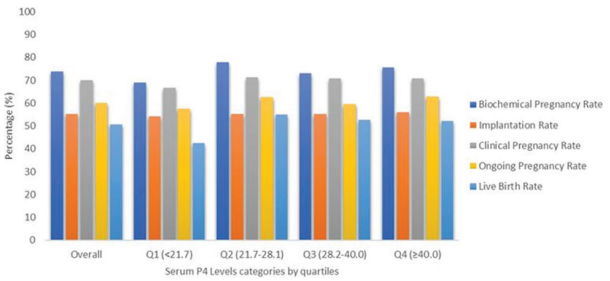


**Figure 2.** The *in vitro* fertilization outcomes among different quartiles

## 4. Discussion

Current evidence suggests that a minimum clinically significant serum luteal progesterone level is necessary to achieve optimal IVF clinical outcomes, and that lower progesterone levels on the day of ET may adversely affect these outcomes.[24] However, most of this data come from studies using vaginal progesterone for luteal phase support. There is limited evidence regarding patients undergoing artificial endometrial preparation

**Table 3. Pregnancy and perinatal outcomes across serum progesterone quartiles**

| Characteristics | Total, *n* (%) | Q1 | Q2 | Q3 | Q4 | *p*-value |
|---|---|---|---|---|---|---|
| Biochemical pregnancy rate | 261/353 (73.9%) | 60/87 (69.0%) | 71/91 (78.0%) | 65/89 (73.0%) | 65/86 (75.6%) | 0.565 |
| Biochemical loss rate | 14/261 (5.4%) | 2/60 (3.3%) | 6/71 (8.5%) | 2/65 (3.1%) | 4/65 (6.1%) | 0.382 |
| Implantation rate | 356/645 (55.2%) | 85/157 (54.1%) | 93/168 (55.3%) | 89/161 (55.3%) | 89/159 (55.9%) | 0.402 |
| Clinical pregnancy rate | 247/353 (70.0%) | 58/87 (66.7%) | 65/91 (71.4%) | 63/89 (70.8%) | 61/86 (70.9%) | 0.895 |
| 1st-trimester miscarriage rate | 33/247 (13.4%) | 8/58 (13.7%) | 8/65 (12.3%) | 10/63 (15.9%) | 7/61 (11.5%) | 0.908 |
| Ongoing pregnancy rate | 214/353 (60.6%) | 50/87 (57.5%) | 57/91 (62.6%) | 53/89 (59.6%) | 54/86 (62.8%) | 0.869 |
| 2nd-trimester and perinatal loss rate | 19/247 (7.7%) | 6/58 (10.3%) | 4/65 (6.2%) | 3/63 (4.8%) | 6/61 (9.8%) | 0.613 |
| Live birth rate | 179/353 (50.7%) | 37/87 (42.5%) | 50/91 (54.9%) | 47/89 (52.8%) | 45/86 (52.3%) | 0.551 |
| Loss of follow-up | 16/353 (4.53%) | 7/87 (8.04%) | 3/91 (3.29%) | 3/89 (3.37%) | 3/86 (3.48%) | NA |

**Table 4. Binary logistic regression for *in vitro* fertilization outcomes according to different progesterone levels on the day of embryo transfer**

| *In vitro* fertilization outcomes | Serum progesterone levels (ng/mL) | Odds ratios | 95% confidence interval | *p*-values |
|---|---|---|---|---|
| Clinical pregnancy | 21.7–28.1 | 1.25 | (0.66, 2.36) | 0.49 |
| | 28.2–40.0 | 1.21 | (0.64, 2.29) | 0.55 |
| | >40 | 1.22 | (0.64, 2.32) | 0.54 |
| Ongoing pregnancy | 21.7–28.1 | 1.24 | (0.68, 2.26) | 0.48 |
| | 28.2–40.0 | 0.99 | (0.54, 1.81) | 0.98 |
| | >40 | 1.18 | (0.65, 2.18) | 0.58 |
| Live birth | 21.7–28.1 | 1.52 | (0.83, 2.81) | 0.17 |
| | 28.2–40.0 | 1.40 | (0.76, 2.58) | 0.28 |
| | >40 | 1.38 | (0.74, 2.54) | 0.31 |

using IM progesterone. In this prospective study, we found that when using IM progesterone until the day of ET in HRT-FET cycles, neither low nor high serum progesterone levels adversely impacted clinical outcomes. To the best of our knowledge, this is the first study to propose a protocol of just 6 days of IM progesterone until the day of ET, followed by vaginal gel progesterone and oral dydrogesterone.[25]

The evolving landscape of reproductive medicine and gynecologic oncology has brought forth significant advancements in fertility preservation, particularly for women diagnosed with early-stage endometrial and cervical cancers. A multidisciplinary approach integrating molecular diagnostics, minimally invasive techniques, and ARTs is reshaping clinical decision-making and patient outcomes. The debate between open and closed vitrification systems centers on biosafety and clinical outcomes. Closed systems, such as the Rapid-i device, offer superior protection against contamination from liquid nitrogen, addressing concerns about disease transmission.[26] While survival rates of oocytes and embryos are comparable across systems, the closed system is increasingly favored for its aseptic handling and reduced risk of cross-contamination.

Recent systematic reviews have expanded the scope of fertility-sparing treatment to include Grade 2 Stage IA endometrioid endometrial cancer, traditionally excluded from conservative protocols.[27] Hormonal therapies—particularly progestins such as medroxyprogesterone acetate and levonorgestrel intrauterine devices—combined with hysteroscopic resection, have shown promising oncologic and reproductive outcomes.[28] However, recurrence remains a concern, necessitating rigorous follow-up and molecular profiling.

The integration of molecular classification (e.g., *POLE*-mutated, *MMR*-deficient, and p53-abnormal) into fertility-sparing strategies marks a shift toward precision medicine. Biomarkers such as *PTEN*, *POLE*, and *SPAG9* have demonstrated predictive value for treatment response, enabling personalized therapeutic plans.[29] This approach not only improves oncologic safety but also enhances reproductive success rates.

Assisted reproductive technology, including IVF and intracytoplasmic sperm injection, has revolutionized fertility preservation but raises concerns about long-term child health. A critical appraisal of neuro-psycho-motor outcomes found no significant correlation between ART and cognitive or developmental disorders. However, data on cerebral palsy remain inconclusive due to confounding factors such as prematurity and multiple gestations.[30] In addition, sentinel lymph node biopsy is emerging as a less invasive alternative to pelvic lymphadenectomy in early-stage cervical cancer. Sentinel lymph node biopsy offers comparable diagnostic accuracy with reduced morbidity, although its long-term oncologic safety is still under investigation.[31] Its adoption could facilitate fertility-sparing surgeries by minimizing surgical trauma.

Children born from FET exhibit higher birth weights and increased rates of macrosomia and large-for-gestational-age status, with reduced incidence of low birth weight and small-for-gestational-age outcomes. While these findings suggest favorable perinatal outcomes, long-term developmental data remain limited, warranting further research. Circulating microRNAs are gaining traction as non-invasive biomarkers for early detection of endometrial cancer. Panels of microRNAs can potentially guide both diagnosis and fertility-sparing eligibility, offering a dual benefit of early intervention and personalized care.[32] Their integration into clinical practice could revolutionize screening protocols.

Transcriptomic tools like the microenvironment cell population-counter enable quantification of immune and stromal cell populations within the tumor microenvironment, providing insights into prognosis and therapeutic response.[33] Understanding the tumor microenvironment composition is critical for tailoring immunotherapies and predicting fertility-sparing treatment outcomes. Cell-free fetal DNA testing has become a cornerstone of non-invasive prenatal diagnosis, particularly for chromosomal aneuploidies and monogenic diseases.[34] Techniques such as next-generation sequencing and haplotype dosage analysis offer high accuracy, though ethical and legal considerations remain pivotal in clinical implementation.[1]

It is estimated that over 8 million newborns worldwide have been born as a result of IVF. Despite significant refinements and advancements in reproductive treatments over the past 20 years, including improved ovarian stimulation protocols and laboratory techniques, the LBR per initiated cycle remains relatively low, ranging between 25% and 30%.[35] These low success rates have a considerable psychological impact on couples struggling with infertility, often being the primary reason for discontinuing IVF treatments. After an initial failure, up to two-thirds of infertile couples do not seek further treatment, with many citing the intense psychological stress of IVF treatments as the main deterrent.

The optimal route of progesterone administration in FET cycles remains uncertain. A randomized controlled trial, involving 1125 women undergoing FET, randomly assigned participants to endometrial preparation using daily vaginal micronized progesterone, daily IM progesterone, or a combination of vaginal and IM routes. The study was prematurely terminated after an interim analysis showed lower LBRs in the vaginal route group. However, biases such as the relatively low dose of vaginal progesterone (200 mg twice daily) and the differing intervals between the start of progesterone supplementation and ET timing between groups might have influenced the results.[9]

Concerns have recently been raised about the impact of low serum progesterone levels on clinical outcomes in FET cycles, even in natural cycles[11] or those using IM progesterone. However, the detrimental threshold levels can vary depending on the method of luteal phase support administration. Recent studies, both prospective[14,16] and retrospective,[4,8,11,12,22] have shown that low progesterone levels on the day of or the day before ET, in both HRT and natural cycles, are negatively correlated with clinical outcomes. However, these studies mainly focused on cycles with vaginal progesterone. Conversely, a recent retrospective study found no association between progesterone levels and clinical outcomes,[24] although patients with progesterone levels below 8 ng/mL received additional doses, potentially influencing the results.

In a multicenter prospective cohort study, the association between serum progesterone levels on the day of FET and the probability of live birth was evaluated among different progesterone administration regimens. The study found that the relationship between serum progesterone levels and IVF clinical outcomes varied depending on the FET cycle regimen and the route of progesterone administration. For women undergoing HRT cycles with vaginal progesterone, the mean probability of live birth increased approximately linearly as serum progesterone levels rose. In contrast, when combining vaginal and subcutaneous progesterone, there was a slight variation in the likelihood of live birth according to different serum progesterone levels.[21] The study evaluated 402 FET cycles, with only 111 patients (27.6%) receiving luteal phase support with injectable progesterone. This distribution aligns with clinical practice, where vaginal progesterone is used alone as a single agent in approximately 77% of IVF cycles worldwide.[25]

This study adds to this body of knowledge by providing data supporting the notion that neither low nor high serum progesterone levels affects clinical outcomes when using IM progesterone in FET cycles.

This study has several strengths, including its prospective cohort design, standardized FET protocol, and consistent hormonal analysis timing. In addition, the same immunoassay was used for progesterone measurements, reducing variability. However, some limitations must be acknowledged. Despite being the largest prospective study evaluating the impact of IM progesterone on FET outcomes to date, the findings may be subject to bias. Future research with larger sample sizes and randomized clinical trials is warranted to confirm these results and explore the complex interactions between progesterone levels, administration routes, and patient characteristics.

## 5. Conclusion

The results of this study have important implications for clinical practice. They indicate that serum progesterone testing before ET in FET cycles using IM progesterone may not be necessary, potentially simplifying treatment protocols and reducing patient burden. Given that the success of ART largely hinges on individualized patient care, our findings could help refine FET protocols by avoiding unnecessary interventions and focusing on factors that truly influence reproductive outcomes. In conclusion, our study provides evidence that serum progesterone levels on the day of FET do not correlate with the likelihood of achieving pregnancy in artificial FET cycles using IM progesterone. These findings can inform clinical decision-making and streamline FET protocols, ultimately improving patient experiences and ART outcomes. Further research is warranted to continue optimizing treatment approaches for individuals undergoing assisted reproduction.

## Acknowledgments

None.

## Funding

None.

## Conflict of interest

The authors declare that they have no competing interests.

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## Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee at Indira IVF Hospital Private Limited (IIHPL-UDR/P/005_2021). This study was conducted with the patient's informed consent and in accordance with all ethical guidelines (ECR\1614\Inst\RJ\2021).

## Consent for publication

Patients provided written consent for their data to be published.

## Availability of data

Data from the study will be made available on request to the corresponding authors.

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