Clinical, Obstetrical, and Neonatal Outcomes after Single Euploid Frozen-Thawed Blastocyst Transfer with Subcutaneous Versus Intramuscular Progesterone Administration

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ABSTRACT

OBJECTIVE: To assess whether subcutaneous progesterone (SC-P) is similar to intramuscular progesterone (IM-P) regarding live birth rates and perinatal and obstetrical outcomes following single euploid embryo transfer.

STUDY DESIGN: This retrospective cohort study involved 350 participants who underwent single euploid blastocyst frozen embryo transfer (FET), comprising 219 recipients of intramuscular progesterone (IM-P) and 131 recipients of subcutaneous progesterone (SC-P). All embryo transfers were conducted within a hormone replacement therapy (HRT) protocol utilizing incremental oral estradiol valerate. Upon reaching an endometrial thickness exceeding 7mm, patients received either intramuscular progesterone (50 mg) or subcutaneous progesterone (50 mg) ten to thirteen days after priming-the primary outcome measures included live birth rates, as well as obstetrical and perinatal outcomes.

RESULTS: The clinical pregnancy rates were comparable between the intramuscular progesterone (IM-P) group (61.6%; 135/219) and the subcutaneous progesterone (SC-P) group (69.5%; 91/131, p=0.139). The live birth rates exhibited no significant difference between the IM-P group (49.8%; 109/219) and the SC-P group (58%; 76/131, p=0.135). Miscarriage rates were also comparable, with 6.3% (22/135) in the IM-P group and 15.4% (14/91) in the SC-P group (p=0.854). Furthermore, there were no statistically significant variances observed between the IM-P and SC-P groups regarding the median gestational age of all live-born neonates (38 weeks in both groups; p=0.183), birthweights (median of 3205g in the IM-P group versus 3335g in the SC-P group; p=0.073), and the incidence of pregnancy-induced hypertension (4.6% in the IM-P group versus 5.3% in the SC-P group; p=0.833).

CONCLUSION: The study results offer clinical evidence indicating that subcutaneous progesterone (SC-P) demonstrates comparable efficacy in achieving live birth rates when compared to intramuscular progesterone (IM-P). Moreover, the findings suggest that SC-P does not pose an elevated risk for adverse obstetrical and perinatal outcomes.

Keywords: Endometrial preparation; Frozen embryo transfer; Intramuscular progesterone; Luteal phase support; Subcutaneous progesterone

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Introduction

In frozen embryo transfer (FET) protocols, three major types of protocols are commonly used to prepare endometrium: hormone replacement therapy (HRT), the natural cycle (NC), and mild ovarian stimulation, which is less frequently used. In clinical practice, this results in the ability to perform a programmed embryo transfer during HRT-based FET cycles with minimal monitoring required (1). In HRT cycles, the corpus luteum is absent; therefore, estradiol (E2) and progesterone (P) must be administered externally. Debates continue regarding the optimal timing, dosage, and duration of treatment before embryo transfer (ET).

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The endometrium before FET depends on progesterone to build a receptive environment for implantation and support the pregnancy. Progesterone can be administered by various routes in hormone replacement therapy for frozen embryo transfer (HRT-FET) cycles, and the most used forms are vaginal micronized progesterone (V-P), intramuscular (IM) injections, and more recently, subcutaneous (SC) injections. ESHRE guidelines suggest that in fresh embryo transfers, IM, SC, or vaginal progesterone are equivalent options (2). Smaller trials in women randomized to IM-P during FET vs. V-P have yielded conflicting results: two randomized controlled trials (RCTs) (3,4) showed no difference in clinical pregnancy rates between the different progesterone preparations, whereas a more recently published study has reported significantly higher live birth with the use of IM-P (5).

RCTs have shown that subcutaneous progesterone (SC-P) is not inferior to vaginal progesterone (P) for fresh embryo transfers (6,7), but so far only limited and contradictory data are available on the use of this route for frozen embryo transfer in HRT-FET. However, three retrospective studies suggest that SC-P may result in pregnancy outcomes similar to vaginal or IM progesterone when used for HRT-FET cycles (8-10).

The primary cause of implantation failure and miscarriages is embryo aneuploidy (11,12). Preimplantation genetic testing for aneuploidies (PGT-A) increases the likelihood of live birth per ET(13). We assessed the clinical efficacy of SC-P for endometrial priming before ET and luteal phase support previously by comparing IM-P (9). This study focused on FET cycles involving the transfer of euploid embryos assessed by PGT-A using Next Generation Sequencing (NGS). Using single euploid FET cycles minimizes multiple potential sources of bias and may more accurately assess the efficacy of SC-P compared to IM-P. The study aimed to investigate the possible differences in pregnancy outcomes in IVF patients who get luteal phase support with SC-P or IM-P.

Material and Method

Study Framework, Setting, and Patient Criteria: We retrospectively reviewed women who had a single euploid FET at a private IVF center between June 2016 and August 2018. The study was approved by the institutional review board on July 14, 2021 (registration number, 101). The study group encompassed a population of cycles performed from November 2016 to March 2018, consisting of women aged between 20 to 45 years who underwent PGT-A with the use of NGS. Patients who met the study criteria during the study period and had complete data available throughout the treatment process were included in the analysis. The exclusion criteria were endocrine or systemic pathologies, uterine anomalies or pathologies, unilateral or bilateral hydrosalpinx, endometrial thickness on P administration day <7 mm, more than one embryo transfer, progesterone daily dosage other than 50 mg, and cases with missing data (Figure 1).

Ovarian stimulation (OS): The ovarian stimulation was started in the GnRH antagonist protocol on day 2 or 3 of the menstrual cycle. A physician determined the dose for gonadotropins, including recombinant FSH (rFSH) and/or purified hMG (p-hMG, Menopur), according to analyses proposed by her age, body mass index (BMI), antral follicle count, and previous outcomes of OS. Final oocyte maturation was performed using 250 µg of human chorionic gonadotropin (hCG; Ovitrelle, Serono) or 0.2 mg triptorelin (Gonapeptyl, Ferring) when two or more follicles had a diameter of 18 mm. This was succeeded by transvaginal ultrasonographic-guided follicle aspiration to retrieve the oocytes 35 h after hCG administration (14).

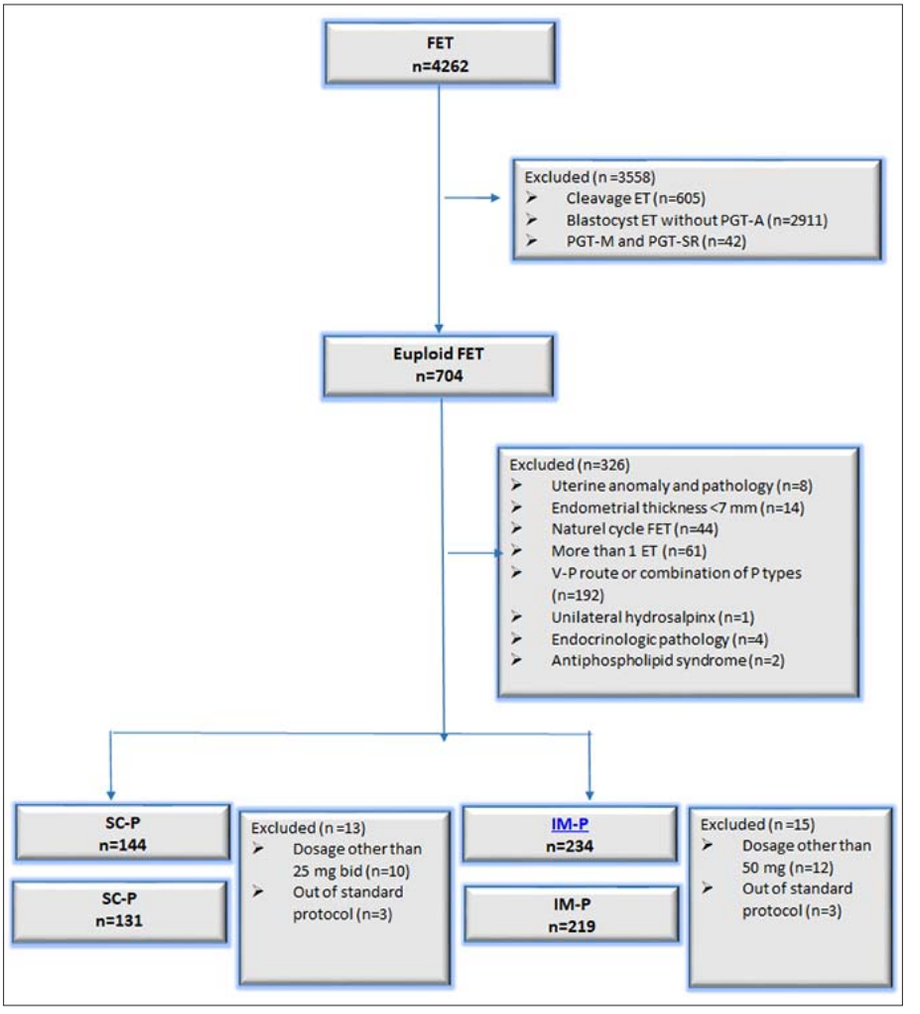

Figure 1: *Flowchart of the study*

Aneuploidy Assessment Through Preimplantation Genetic Testing (PGT-A): This study uses the NGS platform, Reproseq PGS Kit from Life-Thermofisher, USA. It has been validated and its results published in several other studies (14, 15). Embryos were defined as euploid (correct number of chromosomes), aneuploid absent or extra numbers of chromosomes) and chaotic abnormal by the analysis.

Endometrial priming and transfer of embryos: Endometrial preparation for ET involved HRT. Oral estrogen (Estrofem, Novo Nordisk, Türkiye) administration was used according to a step-up regimen: 4 mg/day on days 1-4, 6 mg/day on days 5–8, and 8 mg/day on days 9-12. Endometrial thickness was measured by TV-USG on days 10-13, and if the endometrial thickness was <7 mm and serum P concentrations were >1.5 ng/ml, the cycle was canceled. According to the patient's accessibility to the hospital and patient's preference, P was initiated either with 50 mg IM-P injection (Progestan, Kocak Farma, Türkiye) once per day or with 25 mg SC-P (Prolutex, IBSA, Switzerland) injections twice daily. The first doses of SC-P and IM-P were injected at 4 p.m., and subsequent doses were repeated every 24 hours at the same time interval for IM-P and continued twice daily at 8:00 am/8:00 pm for SC-P. All transfers were performed between 3 pm and 6 pm under ultrasonographic guidance. Serum β-hCG levels were measured 12 days after FET, and levels ≥5 IU were accepted as positive. Oral estrogen replacement was stopped at the 6th week of pregnancy, whereas P was continued until 10 weeks in both arms. A similar study examined the potential effect of estrogen on spontaneous abortion by recruiting women at 6 weeks of gestational age (16).

Pregnancy outcome measurements: The presence of an intrauterine gestational sac detected by TV-USG was considered a confirmation of clinical pregnancy. For this study, miscarriage was defined as the loss of a clinical pregnancy before the 12th gestational week. Live birth was defined as the delivery of a live infant at or beyond 24 gestational weeks. Preterm birth was considered as delivery before 37 weeks of gestation in this study. Very preterm birth was defined as <32 weeks gestation, and extremely preterm birth as <28 weeks.

Statistical Analysis

Continuous variables were tested for normality using the Kolmogorov-Smirnov test, and none were normally distributed. Consequently, continuous parameters are presented as median (Quartile 1-Quartile 3). The independent median test was carried out to compare the median values of continuous variables between the IM-P and SC-P arms. Statistical comparisons for patient and embryological characteristics were performed using the chi-squared test. Binary logistic regression models were used to evaluate and report factors influencing live birth outcomes. Model results were categorized based on whether a live birth was predicted for an individual.

Results

A total of 350 single euploid FET, including 219 in the IM-P group and 131 in the SC-P group, were included in the study. Baseline characteristics, such as female and male age, body mass index (BMI), number of previous attempts, number of previous miscarriages, number of previous live births, and indication for treatment, were similar between the groups. Ovarian stimulation parameters, endocrinological parameters in the FET cycle, endometrial thickness in FET on P administration day, and embryological parameters of transferred euploid embryo were also comparable in each group. These findings are presented in Table I.

Clinical pregnancy rates (CPRs) were 61.6% (135/219) versus 69.5% (91/131) (p=0.139), and live birth rates (LBRs) were 49.8% (109/219) versus 58% (76/131) (p=0.135) in the IM-P and SC-P groups, respectively. Miscarriage rates (MRs) per clinical pregnancy were similar [16.3% (22/135) vs. 15.4% (14/91) (p=0.854) IM-P and SC-P, respectively] (Table II).

Among 185 live-born neonates, 109 were from the FET with IM-P group and 76 from the FET with SC-P group. No stillbirths or multiple pregnancies were observed in either group. Table III presents obstetrical and neonatal characteristics of live-born neonates. The median gestational age of all live-born neonates was the same, IM-P vs. SC-P group (38 [39-38] vs. 38 [39-38] p=0.183). There was also no significant difference in birth weight between the treatment groups [3205 (3535-3000) g with IM-P vs. 3335 (3697.5-2992.5) with SCP p=0.073]. Furthermore, the incidence of pregnancy-induced hypertension was similar in both IM-P and SC-P groups [4.6% (5/109) vs. 5.3% (4/76), p=0.833].

Live birth was used as the dependent factor, while female age, BMI, previous live births, previous attempts, previous miscarriages, day of vitrification (day 5 or 6), embryo quality, and P administration route (SC-P+IM-P) were selected as independent factors. ET day (day 5 vs. day 6) was identified as a significant independent prognostic factor [OR: 1.582; CI: 0.956-2.618)) (Table IV). Binary logistic regression showed that live birth was not associated with a P administration route (SC-P vs. IM-P).

Discussion

Our retrospective analysis revealed comparable pregnancy outcomes between patients receiving SC-P and those receiving IM-P in FET cycles using euploid embryos identified through PGT-A. This equivalence extended to both maternal and neonatal outcomes, suggesting that SC-P could serve as an effective alternative for luteal support in hormone replacement therapy FET (HRT-FET) cycles.

The selection of the optimal progesterone delivery method for endometrial preparation in FET cycles remains a topic of

Table I: *Comparison of patient, cycle, and embryologic characteristics of the IM-P and SC-P groups*

	IM-P	SC-P	p
Female age (years)			
Median(Q3-Q1)	37 (40-33)	36 (40-33)	0.481
Male age (years)			
Median(Q1-Q3)	39 (43-35)	38 (42-34)	0.431
BMI (kg/m2)			
Median(Q1-Q3)	24.06 (27.55-21.83)	23.69 (26.84-21.20)	0.412
No. of previous live birth			
Median(Q1-Q3)	0 (0-0)	0 (0-0)	0.47
No of previous miscarriage			
Median(Q1-Q3)	0 (1-0)	0 (1-0)	0.247
No of previous attempt			
Median(Q1-Q3)	3 (4-1)	2 (4-0)	0.256
Diagnosis			
Combined	15/219 (6.8)	3/131 (2.3)	0.062
DOR	38/219 (17.4)	21/131 (16)	
Endometriosis	26/219 (11.9)	12/131 (9.2)	
Male Factor	61/219 (27.9)	31/131 (23.7)	
PCOS	15/219 (6.8)	19/131 (14.5)	
Tubal	16/219 (7.3)	7/131 (5.3)	
Unexplained	48/219 (21.9)	38/131 (29)	
Total gonadotrophin dosage IU			
Median(Q1-Q3)	2625 (3131.25-2025)	2550 (3187.50-2025)	0.973
No.of oocytes retrieved			
Median(Q1-Q3)	13 (16-6)	13 (17-7)	0.901
Fertilization rate (2PN/MII)			
Median(Q1-Q3)	80 (94-67)	81.82 (93.33-72)	0.761
Endometrial thickness on P administration day (mm)			
Median(Q1-Q3)	9 (10.30-8)	9.10 (10.18-8)	0.814
E2 levels on P administration day(pg/ml)			
Median(Q1-Q3)	276.60 (336-188)	290.50 (385.50-211.73)	0.054
P levels on P administration day (ng/ml)			
Median(Q1-Q3)	0.73 (0.93-0.40)	0.81 (1.32-0.52)	0.08
Trophectoderm Score			
A + B	195/219 (89)	120/131 (91.6)	0.439
C	24/219 (11)	11/131 (8.4)	
ICM Score			
A + B	93/219 (42.5)	63/131 (48.1)	0.305
C	126/219 (57.5)	68/131 (51.9)	
Day of Embryo Transfer			
Day 5	150/219 (68.5)	98/131 (74.8)	0.208

Table II: *Comparison of pregnancy outcomes between IM-P and SC-P groups*

	IM-P	SC-P	p
Clinical pregnancy rate (%)	135/219 (61.6)	91/131 (69.5)	0.139
Live birth rate (%)	109/219 (49.8)	76/131 (58)	0.135
Miscarriage rate (%)	22/135 (16.3)	14/91 (15.4)	0.854
Ectopic pregnancy rate (%)	4/135 (2.9)	1/91 (1.1)	0.356

Table III: *Comparison of obstetrical and neonatal outcomes between IM-P and SC-P groups*

	IM-P	SC-P	p
Duration of pregnancy (weeks)			
Median(Q3-Q1)	38 (39-38)	38 (39-38)	0.183
Cesarean delivery rate	103/109 (94.5)	67/76 (88.2)	0.12
Spontaneous vaginal delivery	6/109 (5.5)	9/76 (11.8)	0.12
Term birth (%)	94/109 (86.2)	68/76 (89.5)	0.512
Preterm birth (%)	15/109 (13.8)	8/76 (10.5)	0.512
Very preterm birth (<32 weeks)	4/109 (3.7)	3/76(3.9)	0.922
Very very preterm birth (<28 weeks)	1/109 (0.9)	1/76(1.3)	0.797
Birth weight			
Median(Q3-Q1)	3205 (3535-3000)	3335 (3697.5-2992.5)	0.073
Low birth weight (<2500 gr)(%)	5/109 (4.6)	6/76(7.9)	0.379
Very low birth weight (<1500gr)	1/109 (0.9)	1/76(1.3)	0.797
Pregnancy induced hypertension (%)	5/109 (4.6)	4/76(5.3)	0.833

Table IV: *Binary logistic regression analysis for live birth*

	B	S.E.	Wald	df	p	OR	95% CI for OR	
							Lower	Upper
ET_day_group (Day 5) Reference group: Day 6	0.459	0.257	3.191	1	0.074	1.582	0.956	2.618
Constant	-1.724	0.643	7.190	1	0.007	0.178		

clinical discussion. HRT cycles are commonly employed for endometrial preparation in FET procedures (17). Since there is no endogenous P production in HRT cycles, supplementation with exogenous progesterone is essential to support implantation. Initially, endometrial preparation cycles in the absence of ovaries relied on IM-P (18). However, IM injections are painful and require administration by healthcare providers, prompting the exploration of alternatives.

Contemporary progesterone formulations offer various administration routes, including oral, vaginal, rectal, intramuscular, and subcutaneous applications. While intramuscular administration has historically been the standard in many regions, its drawbacks include significant injection-related discomfort and reliance on healthcare providers. In contrast, the subcutaneous route addresses these limitations by enabling self-administration while maintaining therapeutic efficacy. The physiological response to SC-P administration, particularly its ability to support endometrial transformation and sustain pregnancy, is a key consideration. Recent research has demonstrated equivalent outcomes between SC-P and IM-P in fresh embryo transfer cycles. Our findings align with these observations, showing similar clinical outcomes, including live birth rates. Oral natural progesterone administration has poor bioavailability due to rapid metabolism and clearance (19). Vaginal progesterone administration results in low serum progesterone levels, but the uterine first-pass effect produces high tissue concentrations at the target site (20). Although existing literature extensively examines SC-P versus IM-P outcomes in fresh embryo transfers, research specifically focused on HRT-FET cycles remains limited. Our study contributes novel evidence supporting SC-P's efficacy in this context. However, our findings should be interpreted in light of the study's methodological limitations, including its retrospective design and limited cohort size.

Previous studies, including underpowered prospective analyses (21,22) and a retrospective study (16), reported similar pregnancy outcomes between vaginal progesterone and IM-P. Three RCTs compared IM-P and vaginal progesterone (3-5), though each had limitations. The first RCT randomized 354 patients and reported similar clinical pregnancy rates, evaluating cleavage-stage thawed ETs (3). Statistical analysis relied on the Student's t-test and χ^2 test without regression modeling. The second RCT, which aimed to compare IM-P and vaginal progesterone, administered oral dydrogesterone to all patients during the luteal phase, potentially confounding the results (4). This study also found similar pregnancy outcomes. The third RCT reported significantly lower ongoing pregnancy rates in the vaginal progesterone-only group, but its limitations included heterogeneity in FET timing across groups and lower-than-standard vaginal progesterone dosages (5).

The impact of tissue and serum progesterone levels on implantation, pregnancy, and obstetrical outcomes warrants further exploration. Current data suggest that tissue-level pelvic concentrations of progesterone are sufficient for embryo implantation and pregnancy, while systemic progesterone influences immune tolerance, a critical factor for pregnancy development. Recent findings indicate that serum progesterone levels below 10 ng/mL after vaginal progesterone administration are associated with worse pregnancy outcomes, including higher miscarriage rates (23-26).

The development of aqueous progesterone formulations, such as SC-P, has expanded parenteral administration options beyond IM-P. Pharmacokinetic and clinical trials have demonstrated that 25 mg SC-P induces pre-decidual transformation (27). Two RCTs confirm this finding for luteal phase support after fresh embryo transfer (6,7). However, a randomized controlled trial comparing SC-P with other progesterone routes in HRT-FET cycles is lacking. Retrospective and prospective studies comparing SC-P and IM-P in HRT-FET cycles reported similar ongoing pregnancy rates (9,28). One retrospective study investigating live birth rates (LBRs) after single euploid transfers within HRT-FET cycles found no significant differences between SC-P and IM-P groups, with LBRs of 49.8% (109/219) for IM-P and 58% (76/131) for SCP (p=0.135).

The rise of extended culture media and vitrification technology in IVF/ICSI treatments has heightened concerns about obstetrical and perinatal outcomes in FET cycles. Meta-analyses have reported lower risks of small-for-gestational-age (SGA) and low birth weight (LBW) but increased risks of large-for-gestational-age (LGA) infants (29,30). FET protocols are also associated with higher rates of pregnancy-induced hypertension, postpartum hemorrhage, and cesarean sections (31,32). Recent meta-analyses link HRT-FET cycles to higher rates of postpartum hemorrhage, pregnancy-induced hypertension, and preterm birth compared to natural FET cycles (33).

Despite its limitations, our study evaluated the safety of SC-P for obstetrical and neonatal outcomes in HRT-FET cycles. Unfortunately, due to data constraints, we relied on phone interviews to gather obstetrical and neonatal outcomes. In our country, IVF/ICSI pregnancies are traditionally classified as high-risk, often leading to cesarean deliveries. The median gestational age, birth weight, and rates of preterm and very preterm births were similar between the SC-P and IM-P groups. Pregnancy-induced hypertension rates were also comparable and consistent with those of spontaneous pregnancies (4.6% for IM-P vs. 5.3% for SC-P).

HRT-FET cycles are increasingly favored due to their scheduling flexibility and reduced monitoring requirements. These cycles typically involve administering 200 nM estradiol from day 2 of the menstrual cycle for 10-13 days, followed by progesterone to simulate the mid-cycle transition and prepare the endometrium for implantation. SC-P offers a well-tolerated, self-administered alternative to IM-P. A patient preference study comparing vaginal progesterone and SC-P found that SC-P was comfortable, easy to administer, and highly satisfactory, consistent with our clinical experience.

Limitations: This study has several limitations that must be acknowledged. The retrospective design inherently subjects it to biases such as selection bias, recall bias, and potential confounding variables. Retrospective studies rely on existing records, which may lack uniformity and comprehensiveness. Additionally, patients who have experienced negative events, such as early pregnancy loss, may be hesitant to share information about the process or may avoid communication altogether, potentially resulting in missing data for this group.

The relatively small sample size, particularly concerning obstetrical and neonatal outcomes, limits the robustness of our conclusions regarding the safety and efficacy of SC-P compared to IM-P. Larger, prospective studies are necessary to validate our findings and provide a more comprehensive assessment of these outcomes.

The study did not explore the detailed pharmacokinetics and pharmacodynamics of SC-P versus IM-P, which could provide deeper insights into their clinical effects. Understanding the precise mechanisms through which these different routes of progesterone administration impact pregnancy outcomes would be valuable for optimizing treatment protocols.

Overall, while our study provides preliminary evidence supporting the use of SC-P as a viable alternative to IM-P for luteal phase support in HRT-FET cycles, the highlighted limitations necessitate a cautious interpretation of the findings. Well-designed randomized controlled trials are essential to confirm our results and develop more patient-friendly and effective progesterone supplementation protocols in assisted reproductive technologies.

Conclusion

Our study provides preliminary clinical evidence that SC-P is as effective as IM-P in achieving live birth rates during HRT-FET cycles. This finding is significant, as SC-P offers practical advantages, including less pain, self-administration, and reduced clinical visits, enhancing patient comfort and compliance.

No significant differences were observed in obstetrical or neonatal outcomes between SC-P and IM-P groups, supporting SC-P as a viable alternative for luteal phase support. Clinicians may consider SC-P as a first-line option, particularly for patients who prefer self-administration or struggle with IM injections.

Future research with larger sample sizes is needed to confirm these findings and further explore perinatal and obstetrical outcomes. Studies should also investigate the pharmacokinetics and pharmacodynamics of SC-P versus IM-P, as well as the impact of progesterone route, dosage, and duration on maternal and neonatal health.

In conclusion, while our study highlights SC-P as an effective alternative to IM-P, further validation through prospective, randomized controlled trials is necessary. This research will help optimize FET protocols and improve the safety and success of assisted reproductive technologies.

Declarations

Ethics approval: The study was approved by the institutional review board (Approval number: 101). The study was conducted in compliance with the guidelines outlined in the Declaration of Helsinki.

Availability of data and materials: The data supporting this study is available through the corresponding author upon reasonable request.

Conflict of interest: The authors declare that they have no competing interests.

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Authors' contributions: ZY, FKB, MG, OC, and MG raised the presented idea. ZY, FKB, MG, MB, and OC designed the study. ZY conducted the analyses. ZY, FKB, MG, and OC developed the first draft of the manuscript. ZY, FKB, MG, and OC participated in data collection and result interpretation. ZY, FKB, MG, and OC assisted with data collection and analysis. ZY, FKB, and OC critically revised the manuscript. All authors contributed to the writing of the article and read and approved the final version of the article.

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