

Effect of Intrauterine Autologous Platelet-Rich Plasma Instillation in Infertile Women With Thin Endometrium Undergoing Frozen-Thawed Embryo Transfer: A Randomized Controlled Trial

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Abstract

Background: Endometrial thickness is a key factor in determining the suitability for embryo transfer (ET) and influences the success of assisted reproductive technology (ART) outcomes. The aim of this study is to compare endometrial thickness in patients undergoing frozen-thawed embryo transfer (FET) who received platelet-rich plasma (PRP) injections alongside standard estrogen therapy with those receiving standard estrogen therapy alone. **Methods:** In this randomized controlled trial (RCT), a total of 30 infertile women from Siriraj Infertility Clinic undergoing FET were enrolled and randomly assigned to three groups. Group 1 (PRP-1 group) received a single intrauterine PRP instillation on day 8 (D8) of the cycle prior to ET; Group 2 (PRP-2 group) received two intrauterine PRP instillations on D8 and D10 before ET; and Group 3 (Control) received standard estrogen therapy alone. Endometrial thickness, chemical pregnancy rate, clinical pregnancy rate, abortion rate, and cycle cancellation rate were recorded. **Results:** The mean differences in endometrial thickness on D8 and D12 were compared. The PRP-1 group showed the greatest increase in endometrial thickness compared to the other groups; however, the difference was not statistically significant (1.52 ± 1.10 in PRP-1, 0.72 ± 0.72 in PRP-2, 1.43 ± 0.88 in the Control group; $p = 0.153$). Chemical pregnancy rates were comparable across groups, with 33.34% in PRP-1 group and 42.85% in the control group. Similarly, clinical pregnancy rates were 16.67% in PRP-1 group and 42.85% in the control group; ($p = 0.790$ and $p = 0.585$, respectively). However, the cancellation rate was significantly higher in the PRP-2 group (77.78%, $p = 0.015$). **Conclusions:** Single intrauterine PRP instillation on D8 prior to ET in a frozen-thawed cycle may improve endometrial thickness, although without statistical significance. In contrast, repeated PRP administration was associated with a higher cycle cancellation rate. **Clinical Trial Registration:** The study has been registered on <https://clinicaltrials.gov/> (registration number: NCT06234540; registration link: <https://clinicaltrials.gov/study/NCT06234540?cond=NCT06234540&rank=1>).

Keywords: PRP; platelet rich plasma; endometrial thickness; thin endometrium; refractory endometrium; IVF frozen thaw cycles

1. Introduction

The global total fertility rate (TFR) has been declining, reaching 2.2 live births per woman in 2024, down from 3.3 in the 1990s, and is projected to fall to 1 by 2050 [1]. This decline poses a significant challenge globally, prompting public health organizations to actively promote assisted reproductive technologies (ART) as a viable solution.

Among the various ART options, *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the most widely used techniques, with success rates ranging from 35% to 40%, depending on the treatment center. These success rates are influenced by multiple factors, including the physiological condition of the patient and the clinical practices employed [2]. One critical factor affecting outcomes is endometrial thickness, which plays a significant role in the implantation process and overall outcomes of IVF/ICSI treatments [2,3]. A deeper understanding of these variables is essential for improving IVF/ICSI success rates and addressing infertility worldwide.

A thin endometrium (less than 7 mm during the mid-cycle or anovulatory phase) is associated with poor pregnancy outcomes [4], including reduced implantation rates, lower pregnancy rates, and increased miscarriage rates [5]. While an endometrial thickness of at least 8 mm is generally considered optimal for embryo transfer (ET), several studies suggest that a thickness of ≥ 7 mm also significantly improves pregnancy rates [6,7].

Although women diagnosed with a thin endometrium often receive hormone therapy to increase endometrial thickness according to standard protocols, alternative approaches also exist. These include increased estrogen doses, low-dose anticoagulants, vitamin E supplementation, sildenafil to enhance uterine blood flow, and treatments involving granulocyte-colony stimulating factor (G-CSF) injections. However, thin endometrial conditions may persist, resulting in the cancellation of ET during IVF cycles [5,8]. This can cause increased financial and psychological stress for couples experiencing infertility [9].



Platelet-rich plasma (PRP) has emerged as a promising therapeutic approach, with research indicating its potential to enhance endometrial thickness and improve blood supply in women with a thin endometrium [9]. A 2015 study by Chang *et al.* [10] found that PRP not only increased endometrial thickness but also showed a trend toward improved pregnancy rates. This effect is likely due to PRP's ability to increase cell proliferation, promote cell regeneration, and repair damaged cellular components [8]. These mechanisms are driven by growth factors and cytokines present in PRP, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), and insulin-like growth factor-1 (IGF-1) [4,11]. Western blot analyses have shown that VEGF levels in PRP are up to 2.4 times higher than in plasma, while PDGF levels are up to 2.8 times higher, both differences being statistically significant ($p = 0.030$ and $p = 0.001$, respectively) [12].

Additionally, studies on both human [12] and murine [13] endometrial tissues have shown that PRP injection into the uterine cavity increases the expression of Homeobox A10 (HOXA10), a key marker of endometrial receptivity for embryo implantation. These studies also reported elevated Ki67 expression, indicating enhanced proliferation of glands and stroma in the endometrial tissue. Such histopathological findings further support PRP's potential to improve endometrial receptivity. Moreover, the PRP preparation process is straightforward, and neither the plasma nor platelet components have been found to cause adverse effects [9,14]. Due to its safety and efficacy, PRP has been widely applied across various medical fields, including the treatment of hair loss (alopecia), the reduction of inflammation and joint pain, and cosmetic procedures for skin rejuvenation [8].

In reproductive medicine, several studies have explored the application and optimization of PRP for infertility treatment, investigating its effect on both the endometrium and ovaries. However, results remain inconsistent, and a standardized protocol to optimize ART outcomes has yet to be established. For example, a 2022 study by Dogra *et al.* [8] measured endometrial thickness every 48 hours, administering PRP as needed until the thickness reached 7 mm. Their findings showed a significant increase in endometrial thickness following PRP administration in women with a history of cycle cancellation, with an average increase of 1.07 mm in fresh IVF cycles and 0.83 mm in frozen embryo transfer (FET) cycles [8]. In contrast, a 2020 study by Aghajanzadeh *et al.* [9] found no significant changes in endometrial thickness, implantation rate, clinical pregnancy rate, ongoing pregnancy rate, or miscarriage rate among women with recurrent implantation failure (RIF) submitted who received PRP 48 hours before ET. However, no prior studies have conducted a direct comparative analysis of the effects of a single PRP injection versus repeated PRP injections on endometrial thickness.

This study aims to evaluate the potential effects of PRP in enhancing endometrial thickness in women with a thin endometrium. Specifically, we compare endometrial thickness in patients undergoing FET across three groups: those receiving a single PRP injection with standard estrogen therapy, those receiving repeated PRP injections with standard estrogen, and those receiving standard estrogen therapy alone. As a secondary objective, we compare chemical pregnancy rates, clinical pregnancy rates, ongoing pregnancy rates, abortion rates, and cycle cancellation rates among the groups.

2. Materials and Methods

2.1 Study Design and Participants

A single-blind, randomized controlled trial (RCT) was conducted. Inclusion criteria were Thai women aged 18 years and older undergoing ET in a frozen-thawed cycle with a history of ET cancellation due to thin endometrium (<7 mm). Participants were excluded if they had a platelet disorder (e.g., von Willebrand disease), platelet counts below 100,000 cells/mm³, were using antiplatelet medications, had pathological uterine conditions (e.g., submucous fibroids, endometrial polyps), or had untreated uterine synechiae (Asherman syndrome). All 30 eligible women provided written informed consent and completed the record form prior to enrollment. Participants were randomly to one of three groups, assigned via computer-generated randomization (block of 3) to ensure equal allocation: single PRP (PRP-1 group), repeated PRP (PRP-2 group), or the control group. Sealed envelopes were used to prevent selection bias. This study was approved by the Siriraj Institutional Review Board (Protocol No. 853/2566, IRB4; (COA No. Si 987/2023). Additionally, the clinical trial was registered in <https://clinicaltrials.gov/> (registration number: NCT06234540; registration link: <https://clinicaltrials.gov/study/NCT06234540?cond=NCT06234540&rank=1>).

2.2 Endometrial Preparation

Endometrial thickness was measured using transvaginal ultrasonography on days 3, 8, 12, and 14 (D3, D8, D12, and D14) of the menstrual cycle. Endometrial thickness was measured with a 5 MHz vaginal transducer with the TOSHIBA Xario 100 TUS-X100 (Toshiba Medical Systems Co., Ltd., Otawara, Tochigi, Japan). All measurements were performed by the same experienced operator (intra-observer variation of $<5\%$). At each time point, three measurements were obtained 1.5 centimeters below the upper edge of the endometrium and averaged.

On D3, participants underwent a baseline ultrasound and were prescribed Estradiol hemihydrate (Estrofem®, Novo Nordisk Limited, Basel, Switzerland) at 2 mg orally, three times daily (6 mg/day) for five days.

On D8, participants with an endometrial thickness ≥ 7 mm continued with standard treatment. Those with a thickness <7 mm received interventions based on their

group assignment: the PRP-1 and PRP-2 groups underwent PRP preparation and instillation that day, combined with an increased estrogen dosage of 8 mg/day. The control group received only the increased estrogen dose of 8 mg/day.

On D12, participants with an endometrial thickness ≥ 7 mm were scheduled for ET. If the thickness remained < 7 mm, the estrogen dosage was increased to 12 mg/day (2 tablets orally, three times daily), with a follow-up ultrasound scheduled two days later. On D14, participants with an endometrial thickness ≥ 7 mm proceeded to ET, while those with a thickness < 7 mm were counseled by their physician regarding treatment options, including cycle cancellation or further estrogen dose adjustment with continued monitoring.

2.3 PRP Preparation

For PRP preparation, 15 mL of peripheral blood was collected into Anticoagulant Citrate Dextrose Solution, Solution A (ACD-A) tubes (Lot No.: MFG 20230526; Shandong Hygeia Medical Packing Technology Co., Ltd., Rizhao, Shandong, China). The blood was processed using a Thermo Scientific™ Labofuge™ 400 Centrifuge (Thermo Electron Corporation, Karlsruhe, Germany) with a two-step centrifugation protocol: an initial spin at 1800 rpm for 12 minutes to separate red blood cells, followed by a second spin at 3400 rpm for 7 minutes to isolate PRP. Approximately two-thirds of the supernatant were discarded, and the remaining one-third (about 1 mL) was carefully mixed using a sterile pipette before being loaded into a syringe for intrauterine instillation. The PRP was activated and instilled within one hour of activation [8]. According to Dogra *et al.* [8], PRP preparations should contain platelet concentration exceeding 1 million/ μ L, providing an abundant source of growth factors [13]. To ensure quality, a hemocytometer (BOECO™ Neubauer Improved, Hamburg, Germany) was used to analyze two to three drops of the PRP product, confirming that platelet counts were 4–5 times higher in the serum [4,5]. A complete blood count (CBC) was also performed to verify PRP concentration.

2.4 PRP Instillation

The final PRP preparation (1 mL) was instilled into the uterine cavity using an intrauterine insemination catheter (Gynetics, Gynetics Medical Products NV, Lommel, Belgium) under transabdominal ultrasound guidance and sterile conditions. All instillations were performed by a single operator.

2.5 ET Procedure

ET in the frozen-thawed cycle followed standard protocols and lasted approximately 15–20 minutes. Participants were advised to empty their bladder before the procedure and were positioned in the lithotomy position under sterile conditions. A physician inserted a sterile speculum and an ET catheter. The embryo, either at the cleavage stage (D5) or cleavage stage (D3), was transferred into the

uterine cavity under transabdominal ultrasound guidance, approximately 1–2 centimeters below the uterine fundus. After the procedure, participants rested in bed for 30 minutes before resuming normal activities.

2.6 Sample Size Calculations

Based on the RCT by Nazari *et al.* [15], average endometrial thickness was compared among three groups: the control group, the single-intervention group, and the double-intervention group, with mean values of 5.76 ± 0.97 mm, 5.99 ± 0.70 mm, and 7.21 ± 0.18 mm, respectively. Sample size calculations were performed using G*power version 3.1.9.7 (Heinrich Heine University Düsseldorf, North Rhine-Westphalia, Germany), with a significance level of 0.05 ($\alpha = 0.05$) and a power of $1 - \beta$ of 80% ($\beta = 0.2$). The calculated sample size was 9 participants per group, and accounting for a 10% dropout rate, the final sample size for the project was adjusted to 10 participants per group.

2.7 Statistical Analysis

Baseline characteristics, including demographic data, reproductive history, and underlying conditions, were analyzed using the Student's *t*-test. Continuous data was reported as mean $\pm$ standard deviation (SD) or median (interquartile range, IQR), with normality assessed using the Kolmogorov-Smirnov test. Categorical data were compared among the three groups using the Chi-square test or Fisher's exact test, as appropriate. Continuous variables were analyzed using analysis of variance (ANOVA) when assumptions were met. A *p*-value < 0.05 was considered statistically significant. Statistical analyses were conducted using SPSS version 26 for Windows (IBM SPSS Inc., Armonk, NY, USA).

3. Results

3.1 Population Characteristics

A total of 30 participants with thin endometrium were enrolled after meeting the eligibility criteria. 3 individuals were withdrawn due to endometrial thickness ≥ 7 mm after 5 days of standard hormone treatment, leaving 27 participants for analysis. These included 10 who received a single intrauterine PRP instillation before ET in frozen-thawed cycles, 9 who received repeated PRP instillations, and 8 in the control group. Of the 27 participants, 12 had their FET cycle canceled due to inadequate endometrial thickness, absence of viable embryos, or the presence of persistent growing follicles. ET was ultimately performed in 15 patients (Fig. 1). The average age of the study population was 40.70 ± 3.82 years, with a mean body mass index (BMI) of 22.95 ± 3.81 kg/m² and a median duration of infertility of 36 (IQR: 18, 59) months. Baseline demographic or clinical parameters among the groups are shown in Table 1.

Baseline platelet concentration in the PRP-1 and PRP-2 groups were $0.30 \pm 0.086 \times 10^6$ platelets/ μ L and $0.33 \pm$

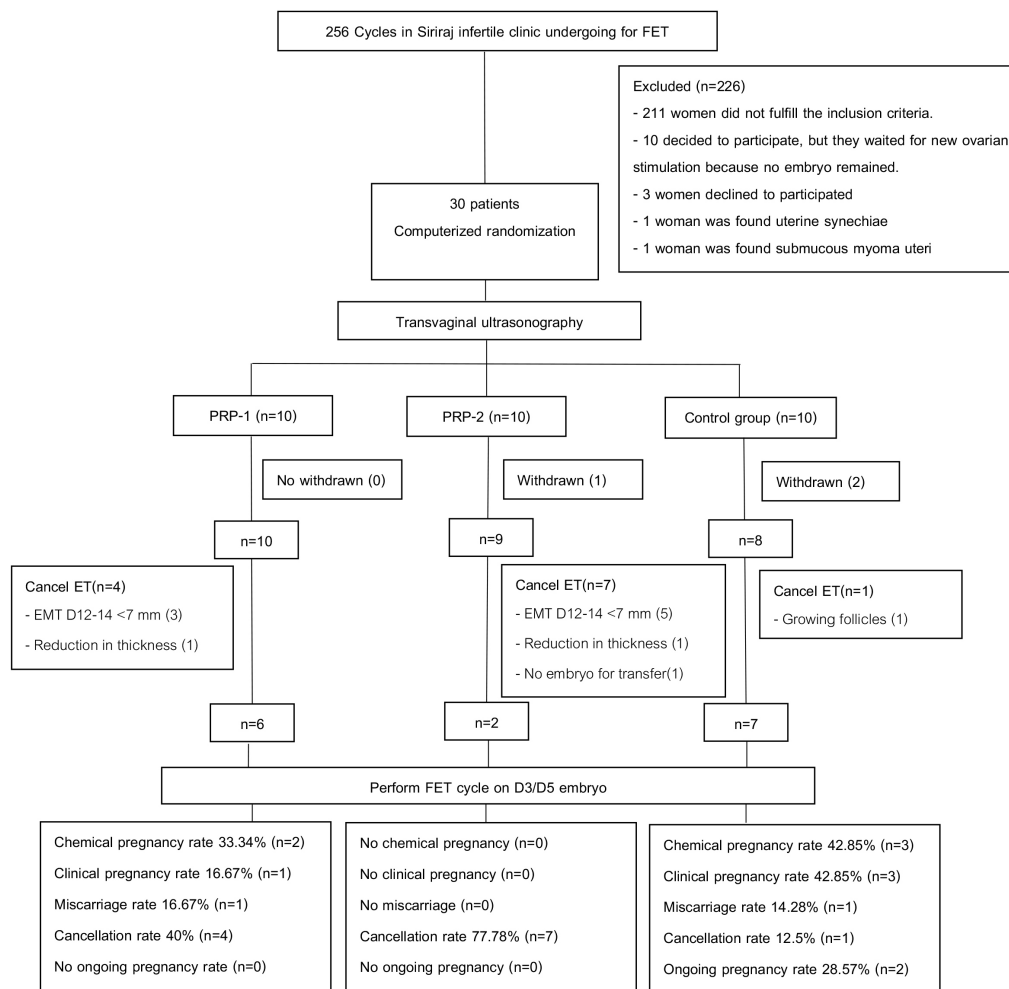


Fig. 1. Study flowchart. FET, frozen embryo transfer; PRP, platelet-rich plasma; ET, embryo transfer; EMT, endometrial thickness; D, day of menstruation cycle.

0.084×10^6 platelets/ μL , respectively. In contrast, the median platelet concentration in the prepared PRP was 5.8×10^6 (IQR: 5, 6.2) platelets/ μL . Thus, the platelet concentration in PRP preparation used in this study exceeded one million cells/ μL , representing a four- to five-fold increase compared to baseline levels, which aligns with the threshold associated with enhanced PRP efficacy [8]. Table 1 summarizes the PRP properties.

A total of 28 PRP attempts were performed, with endometrial thickness and ART outcomes evaluated. All participants undergoing FET cycles followed the same hormone replacement therapy (HRT) protocol. The median number of embryos transferred was 1 (range: 0, 1), with 66.67% transferred on D5 (10/15), and 33.33% transferred on D3 (5/15). According to the Istanbul Consensus 2011 (Supplementary Material), and 26.67% were classified as good-quality embryos (2 embryos in PRP-1, 1 embryo in PRP-2, and 1 embryo in Control), 53.55% as fair quality embryos (3 embryos in PRP-1, 1 embryo in PRP-2, and 4 embryos in Control), and 20% as poor quality embryos (1 embryo in PRP-1 and 2 embryos in Control).

3.2 Endometrial Thickness

To evaluate whether PRP improved reproductive outcomes in patients with thin endometrium, we analyzed changes in endometrial thickness and pregnancy outcomes. The greatest increase in endometrial thickness was observed in the PRP-1 group (1.52 ± 1.10 mm) after a single PRP instillation, followed by the control group (1.43 ± 0.88 mm) and the PRP-2 group (0.72 ± 0.72 mm). However, these differences were not statistically significant ($p = 0.153$) (Table 2).

3.3 Pregnancy Outcomes

Secondary outcome analyses is demonstrated in Table 3. The chemical pregnancy rates were 33.34%, 0%, and 42.85% ($p = 0.790$) in the PRP-1, PRP-2, and control groups, respectively. Clinical pregnancy rates were 16.67%, 0% and 42.85% ($p = 0.585$), respectively; ongoing pregnancy rates were 0%, 0%, and 28.57% ($p = 0.600$), respectively; and the miscarriage rates were 16.67%, 0%, and 14.28% ($p = 1.000$), respectively. Notably, the cycle cancellation rate was significantly higher in the repeated PRP group (77.78%) than in the control group 12.5% ($p = 0.015$).

Table 1. Population characteristics.

Patient characteristics	PRP-1 group (n = 10)	PRP-2 group (n = 9)	Control group (n = 8)	All group (n = 27)
Age (yr.)*	43.22 ± 2.69	36.98 ± 2.56	41.71 ± 3.07	40.70 ± 3.82
AMH (ng/mL)#	0.68 (0.08, 1.71)	2.02 (1.37, 3.69)	1.32 (0.37, 2.59)	1.32 (0.60, 2.59)
BMI*	22.35 ± 3.24	23.98 ± 4.93	22.49 ± 3.16	22.95 ± 3.81
P#	0	0	0	0
A#	1 (0.00, 2.00)	1 (0.50, 2.00)	0.5 (0.00, 1.75)	1 (0.00, 2.00)
Infertile period (mo.)#	49 (28.50, 81.50)	26 (15.25, 45.50)	31 (12.25, 47.00)	36 (18.00, 59.00)
Previous FET#	0 (0.00, 2.25)	0 (0.00, 2.00)	2 (0.00, 3.75)	0 (0.00, 3.00)
Previous cancellation (times)#	2 (1.00, 2.25)	1 (1.00, 2.00)	1 (1.00, 2.00)	1 (1.00, 2.00)
Previous uterine curettage#	0 (0.00, 2.00)	1 (0.00, 1.50)	0 (0.00, 0.75)	0 (0.00, 1.00)
Number of embryos#	1 (0.00, 2.00)	0 (0.00, 0.50)	1 (1.00, 1.75)	1 (0.00, 1.00)
Serum platelet (platelets /μL)*	307,500.00 ± 86,447.48	337,111.00 ± 84,610.05	-	321,526.00 ± 84,552.00
PRP platelet (Million)				
D8#	5.80 (5.00, 6.20)	5.40 (5.20, 6.40)	-	5.80 (5.00, 6.20)
D10#	-	3.17 (1.63, 5.39)	-	
Embryo morphology (n)				
D5				
Good quality	1	1	1	
Fair quality	2	1	5	
Poor quality	1	0	0	
D3				
Good quality	1	0	0	
Fair quality	0	0	1	
Poor quality	1	0	0	
Infertility factors, n (%)				
Male	3 (30.00)	1 (11.11)	4 (50.00)	
Female				
Uterine				
Myoma uteri	2 (20.00)	4 (44.44)	3 (37.50)	
Adenomyosis	0	1 (11.11)	0	
Non-uterine	8 (80.00)	5 (55.60)	6 (75.00)	
RIF	2 (20.00)	4 (44.44)	5 (62.50)	
RPL	4 (40.00)	2 (22.22)	2 (25.00)	
APS	0	1 (11.11)	1 (12.50)	
Unexplained infertility	0	2 (22.22)	1 (12.50)	

Abbreviations: AMH, antimullerian hormone; BMI, body mass index; P, parity; A, abortion; FET, frozen-thawed embryo transfer; PRP, platelet-rich plasma; D, day of menstruation cycle; RIF, recurrent implantation failure; RPL, recurrent pregnancy loss; APS, antiphospholipid syndrome; yr., year; mo., month.

Data presented as Mean ± SD or Median (IQR) as appropriate.

*Mean ± SD; #Median (IQR).

3.4 Adverse Outcomes

Among the 19 women who underwent 28 PRP attempts, no serious adverse events such as pain, irritation, or infections related to the PRP procedure were reported (70.37%). This suggests that PRP instillation is well-tolerated and safe.

4. Discussion

This study investigated the effects of intrauterine instillation of autologous PRP on endometrial thickness and pregnancy outcomes in infertile women undergoing FET cycles. Our findings indicate that a single PRP instilla-

tion resulted in higher endometrial thickness compared to the control group; however, this difference was not statistically significant. In contrast, repeated PRP instillations showed limited benefit in increasing endometrial thickness and were associated with a higher cycle cancellation rate.

The observed increase in endometrial thickness in the PRP-1 group aligns with previous studies [16–19], which suggest that PRP promotes angiogenesis and tissue regeneration through growth factors such as VEGF, PDGF, and IGF-1 [4,11,12]. Similarly, a 2024 meta-analysis [20] and a recent 2025 Turkish study [21] showed that PRP can increase endometrial thickness. However, the lack of statisti-

Table 2. Comparison of primary outcomes among the three groups.

Outcomes	PRP-1 group (n = 10)	PRP-2 group (n = 9)	Control group (n = 8)	p-value
Preparation duration (days)	18.80 ± 4.39	15.89 ± 3.95	18.13 ± 3.44	0.278
Endometrial thickness (mm)				
D3	3.85 ± 1.4	4.03 ± 0.84	4.31 ± 1.29	0.726
D8	5.04 ± 0.98	5.76 ± 0.75	6.18 ± 0.76	0.027†
D12	6.56 ± 1.33	6.48 ± 0.61	7.60 ± 0.59	0.040†
Endometrial thickness difference between D8 to D12	1.52 ± 1.10	0.72 ± 0.72	1.43 ± 0.88	0.153

Data presented as Mean ± SD.

† Statistically significant.

Endometrial thickness shows significant difference among the groups on D8 and D12, $p = 0.027$ (Control vs. PRP-1 $p = 0.028†$) and $p = 0.040$, respectively (Control vs. PRP-2 $p = 0.004†$).

Table 3. Comparison of secondary outcomes among the three groups: chemical pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate, abortion rate, and cancellation rate.

Secondary outcomes	PRP-1 group (n = 6)	PRP-2 group (n = 2)	Control group (n = 7)	p-value
Chemical pregnancy, n (%)	2 (33.34)	0	3 (42.85)	0.790
Clinical pregnancy, n (%)	1 (16.67)	0	3 (42.85)	0.585
Ongoing pregnancy, n (%)	0	0	2 (28.57)	0.600
Abortion rate, n (%)	1 (16.67)	0	1 (14.28)	1.000
Cancellation rate, n (%)	4 (40.00)	7 (77.78)	1 (12.50)	0.027†

† Statistically significant.

Cancellation rate shows significant differences among the groups ($p = 0.027$).

Pairwise comparisons showed no significant difference between the Control vs. PRP-1 ($p = 0.314$), or between PRP-1 and PRP-2 ($p = 0.170$), but a significant difference was observed between the Control vs. PRP-2 ($p = 0.015†$).

cal significance in our study, compared to previous reports, may be explained by the control group starting with a higher endometrial thickness after 5 days of standard HRT (D8; Control vs. PRP-1). Despite this, following the full effect of PRP instillation, the PRP-1 group demonstrated a greater increase in thickness compared to the control group (1.52 ± 1.1 mm vs. 1.43 ± 0.88 mm). This may be one of the confounding factors in our results. Despite starting with the same baseline endometrial thickness, their responses to standard HRT differed. Another possible reason is the variation in definitions of endometrial thickness across studies; for instance, the meta-analysis included patients with endometrial thickness <8 mm and combined protocols that included single and repeated PRP injections, which likely contributed to the high heterogeneity observed ($I^2 = 84\%$).

Table 2 shows that the difference in mean endometrial thickness among the three groups reached only marginal statistical significance. Although the 1.52 mm gain observed in the PRP-1 group did not translate into higher pregnancy or live-birth rates, it may still increase the chance of proceeding to ET in women with a history of repeated cycle cancellations.

Although the PRP-1 group exhibited improved endometrial thickness, no significant differences were observed among the groups in terms of clinical pregnancy rates, chemical pregnancy rates, ongoing pregnancy rates, or miscarriage rates. These results are consistent with previous studies. Allahveisi *et al.* (2020) [22] reported no significant differences between PRP and control groups in

chemical pregnancy rates (28% vs. 36%) and clinical pregnancy rates (28% vs. 24%). Similarly, Aghajanzadeh *et al.* (2020) [9] found no significant differences in chemical pregnancy, clinical pregnancy, and ongoing pregnancy rates. One potential explanation for these findings is the baseline characteristics of our study population, which included a high proportion of patients with unfavorable prognostic factors, such as refractory thin endometrium and RIF. The absence of significant differences in pregnancy rates highlights the need for further investigation into the effects of PRP on additional implantation-related factors and pregnancy outcomes, including the potential impact on embryonic chromosomal abnormalities. Notably, this study did not include embryos screened by Preimplantation Genetic Testing for Aneuploidy (PGT-A).

In contrast, the PRP-2 group did not show improvements in endometrial thickness and had a significantly higher cycle cancellation rate, which differs from the findings by Nazari *et al.* (2019) [15]. Although the exact causal relationship of repeated PRP treatment is not fully understood, it may be influenced by various factors, including the accumulation of excessive endometrial fluid inside the uterine cavity following repeated PRP administration [23,24]. This may lead to endometrial dysfunction, potentially disrupting the endometrial microenvironment, similar to conditions like hydrosalpinx or uterine isthmocele. Excess intrauterine fluid may also increase uterine peristalsis, potentially leading to embryo expulsion [25]. In future research, we recommend more precise diagnostic evaluations

to detect intrauterine fluid or hydrosalpinx fluid (e.g. 3D-ultrasonography, office hysteroscopy) in cases where PRP was instilled into the uterine cavity and the cycle was cancelled, in order to confirm this hypothesis.

Furthermore, the PRP-2 group exhibited a significantly higher cycle cancellation rate (77.78%) compared to the PRP-1 and control groups. The underlying mechanism for this increased cancellation rate remains unclear but may be associated with excessive exposure to PRP, growth factors, or cytokines beyond an optimal therapeutic range.

To our knowledge, this is the first RCT study to compare the effects of single PRP instillation, repeated PRP instillation, and standard hormone replacement therapy on endometrial thickness and ART outcomes. Additionally, this study examined both baseline serum platelet concentrations and platelet concentrations in PRP preparations to ensure that each sample contained more than 1 million platelets per preparation, to maximizing PRP quality [8]. Another key finding is that repeated PRP instillations do not appear to provide additional benefits for endometrial thickness and may, in fact, contribute to higher cycle cancellation rates. Therefore, a single PRP instillation should be considered as an alternative treatment option for patients with thin endometrium who fail to achieve the target thickness after standard hormonal treatment.

This study has some limitations. First, the inclusion of non-next generation sequencing (NGS) embryos may have influenced ART success rates, particularly given the average participant age of 40 years. However, pregnancy rates were not the primary focus of this study, and the absence of NGS did not impact the primary outcome of interest (endometrial thickness). Second, the study population included patients with inherently poor prognoses, such as those with refractory thin endometrium or RIF. While a single PRP instillation increased endometrial thickness, the difference was not statistically significant in this study. Third, our study excluded women with intrauterine adhesions, who may represent one of the targets that could benefit from PRP treatment. A recent study [26] and meta-analysis [27] have shown that PRP can increase other aspects of reproductive function, such as increase menstrual blood flow, improving menopausal hormonal profile, or increasing the Anti-Mullerian Hormone (AMH) level.

Finally, although the data were sufficient for analysis, the small sample size warrants cautious interpretation of the results. Therefore, future RCT studies should include larger sample sizes to increase statistical power and aim to elucidate the comparative efficacy of single versus repeated PRP injections. Furthermore, future studies should include euploid embryos to better assess PRP's effects on clinical pregnancy rates. Additionally, investigating endometrial receptivity through molecular markers (e.g., HOXA10, leukemia inhibitory factor [LIF]) assessing microbiome changes, before and after PRP administration, may provide valuable insights, particularly for patients with thin endometrium or RIF. Future research should also ex-

plore other reproductive aspects like anti-adhesive efficacy of PRP in postoperative intrauterine surgery, to decrease risk of adhesion recurrence or prevent adhesion formation, which represents an important area for further research.

5. Conclusions

The study found that a single intrauterine PRP instillation prior to a FET cycle improved endometrial thickness, although the difference was not statistically significant. However, repeated PRP instillations did not provide additional benefits in endometrial thickness or ART outcomes and were associated with a higher cycle cancellation rate. These findings suggest that a single PRP injection may be sufficient to treat and improve thin endometrium in infertile women, potentially eliminating the need for repeated or multiple injections. Additionally, PRP instillation is a safe and straightforward method for enhancing endometrial thickness, but it does not appear to significantly improve ART outcomes. Further research is warranted to explore the long-term effects of PRP on pregnancy outcomes and to refine its clinical application in assisted reproductive technology.

Availability of Data and Materials

All data is available upon request as a supplementary file. Please contact the corresponding authors for further information.

Author Contributions

SPO, NP and SPE designed the project. SPO and NP performed the research and wrote the manuscript. SPO, NP, PL, SPE, IT, JP, PH and PT collected the data. SPO and NP analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

According to the guidelines of the Declaration of Helsinki, this study was approved by the Siriraj Institutional Review Board (Protocol No. 853/2566, IRB4; (COA No. Si 987/2023). The study has been registered on <https://clinicaltrials.gov/> registration number: NCT06234540. All participants provided written informed consent.

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Conflict of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/CEOG39260>.

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