Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2025**; 52(12): 43161
https://doi.org/10.31083/CEOG43161

Original Research

Effect of Autologous Platelet-Rich Plasma Intrauterine Infusion on the Clinical Outcomes of Freeze-Thawed Embryo Transfer Cycles in Patients With Atypical Recurrent Implantation Failure

Jie Song[1], Yuanjiao Liang[1,*], Jinchun Lu[1], Fang Yang[1], Li Ling[1]

[1]Reproductive Medicine Department, Zhongda Hospital, Southeast University, 210009 Nanjing, Jiangsu, China
*Correspondence: yuanjiao1965@126.com (Yuanjiao Liang)
Academic Editor: Michael H. Dahan
Submitted: 8 June 2025 Revised: 7 September 2025 Accepted: 19 September 2025 Published: 23 December 2025

Abstract

Background: Atypical recurrent implantation failure (RIF) poses a challenge for freeze-thawed embryo transfer (FET) as current interventions showing limited efficacy. Autologous platelet-rich plasma (PRP) may improve the pregnancy outcomes, but its value before FET in such patients remains unclear. **Methods**: A retrospective analysis was conducted on the medical records of patients with atypical RIF (a history of one or two prior failed embryo transfers) who underwent another FET in Reproductive Medicine Center of Zhongda Hospital between January 1, 2022, and June 1, 2024. Patients who received autologous PRP intrauterine infusion before FET were designated as the PRP group (n = 59), while matched patients from the same period who did not receive PRP served as the control group (n = 79). The two groups were compared for endometrial thickness on the day of embryo transfer, biochemical pregnancy rate, embryo implantation rate, clinical pregnancy rate, and early miscarriage rate. **Results**: No statistically significant differences were observed in the baseline characteristics between the control and PRP groups (all $p > 0.05$). The PRP group had a significantly higher biochemical pregnancy rate (66.10% vs. 45.57%), embryo implantation rate (43.75% vs. 30.83%), and clinical pregnancy rate (57.63% vs. 39.24%) compared to the control group ($p < 0.05$). **Conclusions**: For patients with atypical RIF, intrauterine infusion with autologous PRP can increase the embryo implantation and clinical pregnancy rates in subsequent FET cycles.

Keywords: autologous platelet-rich plasma; freeze-thawed embryo transfer cycle; intrauterine infusion; atypical recurrent implantation failure; embryo implantation rate; clinical pregnancy rate

1. Introduction

Embryo implantation failure is an important factor leading to pregnancy failure in assisted reproductive technology (ART) [1]. Many factors can contribute to the failure of embryo implantation, such as embryo quality, endometrial receptivity, immune factors, and thrombophilia [2]. Among these, impaired endometrial receptivity accounts for approximately two-thirds of embryo implantation failures [3]. Recurrent implantation failure (RIF) refers to the failure of at least three embryo implantations, involving the transfer of a total of four high-quality embryos, and including both fresh embryo transfer and freeze-thawed embryo transfer (FET) [4]. However, previous research in ART has revealed that the proportion of patients with atypical RIF (history of one or two prior implantation failures) is much higher than that of patients with standard RIF [5]. Due to factors such as insufficient embryo quantity and the financial burden associated with multiple embryo transfers, in clinical practice these patients may not have the opportunity to attempt additional transfers after experiencing three implantation failures. Consequently, a key focus of our research has been to identify effective strategies in atypical RIF patients that enable intervention in endometrial receptivity at an early stage.

Endometrial receptivity has been regarded as a pivotal influencing factor in previous clinical studies on embryo implantation failure, garnering significant attention [6]. Approaches to enhance endometrial receptivity include testing for endometrial receptivity [7], endometrial scratching [8], and intrauterine infusion. Among these methods, intrauterine infusion is widely applied in clinical settings as an effective means of improving endometrial receptivity [9]. A study has demonstrated that intrauterine infusion of substances such as human chorionic gonadotropin (HCG) [10], autologous peripheral blood mononuclear cells (PBMC) [11], granulocyte colony-stimulating factor (G-CSF) [12], and autologous platelet-rich plasma (PRP) [13] can improve embryo implantation and pregnancy outcomes.

Notably, the beneficial effects of autologous PRP intrauterine infusion in RIF patients have been validated in a study [14]. Autologous PRP is derived from the patient's own peripheral blood and is a plasma product enriched with platelets, proteins, and growth factors. It can promote cell proliferation, tissue repair, and regeneration, thereby playing a vital role in the regeneration of various tissues [15]. In the context of the endometrial microenvironment, intrauterine infusion of autologous PRP can facilitate endometrial development [16]. For RIF patients complicated

by chronic endometritis, PRP can significantly improve clinical outcomes [17]. For RIF patients with a thin endometrium, PRP can effectively increase endometrial thickness and increase the rate of clinical pregnancy [18]. Meanwhile, a study has indicated that PRP is rich in a variety of growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF). These factors may improve pregnancy outcomes by promoting endometrial proliferation, increasing endometrial thickness, and enhancing endometrial receptivity [19]. Nevertheless, it remains unclear whether PRP can exert similar beneficial effects on the clinical outcomes of atypical RIF patients.

Therefore, in this study we conducted a retrospective cohort study on atypical RIF patients who received intrauterine infusion of autologous PRP at the Reproductive Center of Zhongda Hospital. Our aim was to investigate whether intrauterine infusion with autologous PRP can improve the clinical outcomes of atypical RIF patients undergoing FET.

2. Materials and Methods

2.1 Study Design and Patients

We retrospectively analyzed patients (n = 726) receiving FET at the Reproductive Medicine Center of Zhongda Hospital, Affiliated with Southeast University, from January 1, 2022 to June 1, 2024. Among them, 186 cycles received intrauterine infusion with autologous PRP before embryo transfer, and 540 cycles had no intrauterine intervention.

The inclusion criteria were: (1) a history of one or two failed embryo transfers; (2) age $\leq$40 years; and (3) normal ovarian function as indicated by anti-Müllerian hormone (AMH) $>$1.5 ng/mL. Exclusion criteria were: (1) abnormal body mass index (BMI $>$28 kg/m^2 or BMI $<$18.5 kg/m^2); (2) abnormal uterine anatomy, including partial uterine septum, uterus didelphys, or uterine scar diverticulum; (3) other systemic diseases, including genetic disorders (e.g., chromosomal abnormalities), endocrine diseases (e.g., hyperthyroidism, diabetes), or infectious diseases (HIV, syphilis or hepatitis); (4) use of medications within the past three months that are contraindicated or should be used with caution during the preconception period (e.g., antipsychotics such as chlorpromazine or perphenazine, antiepileptics such as valproate or phenobarbital, antituberculosis drugs such as rifampicin or isoniazid, and antineoplastic agents such as methotrexate or cyclophosphamide; (5) receipt of allogeneic blood transfusion, organ transplant, or stem cell therapy within the past 6 months; or (6) the presence of malignant tumor.

A total of 138 atypical RIF patients were included: 39 with a history of one failed embryo transfer, and 99 with a history of two failed embryo transfers. Among them, 59 patients had received PRP prior to FET (the PRP group), while 79 matched patients received no infusion (no placebo) prior to FET (control group). Additionally, we reviewed FET cycles of RIF patients who received autologous PRP infusion during the same period. After applying the above criteria and excluding patients with $\geq$5 prior implantation failures, only 9 such cycles remained. These were excluded from the comparative analysis due to their small number.

2.2 Endometrial Preparation Protocols and Embryo Quality Assessment for FET Cycles

Depending on the patient's clinical scenario, endometrial preparation for FET was performed via one of the following protocols:

- GnRHa (Gonadotropin-releasing hormone agonist) downregulation hormone replacement therapy (HRT) cycle: On days 2–4 of the menstrual cycle, 3.75 mg of leuprorelin acetate (Livzon, H20090299, Shanghai, China) was administered via subcutaneous injection for pituitary downregulation. After 28–30 days, and once downregulation was confirmed to be satisfactory, oral estradiol valerate (Femoston red tablets) was started at 4 mg/day (Abbott Healthcare Products BV, H20110208, Weesp, Netherlands). One week after estrogen administration, endometrial thickness was measured by ultrasound, and the estradiol dose was adjusted accordingly. The maximum duration of estrogen administration was 20 days. When ultrasound indicated the endometrial thickness was $\geq$8 mm, or if it was $<$8 mm but had reached the patient's maximal endometrial response, endometrial transformation was induced by progesterone applied vaginally at 90 mg/day (Merck Serono, H20140552, Geneva, Switzerland) and dydrogesterone (Femoston yellow tablets) taken orally at 40 mg/day. On the 4th day after progesterone exposure, one or two D3 embryos were thawed and transferred. Alternatively, on the 6th day after progesterone exposure, one or two blastocysts were thawed and transferred.
- HRT cycle (no downregulation): Femoston (red tablets, 4 mg/day) was started directly from D2–4 of the menstrual cycle. One week later, endometrial thickness was measured by ultrasound, and the dose of red tablets was adjusted according to the endometrial response. Estrogen administration continued for a maximum of 20 days. When ultrasound revealed an endometrial thickness of $\geq$8 mm (or $<$8 mm but with optimal thickness achieved), progesterone support was initiated to induce endometrial transformation (progesterone at 90 mg/day applied vaginally, and Femoston yellow tablets at 40 mg/day taken orally). On the 4th day after progesterone exposure, one or two D3 embryos were thawed and transferred. Alternatively, on the 6th day after progesterone exposure, one or two blastocysts were thawed and transferred.
- Controlled ovarian stimulation (COS) cycle: 2.5–5 mg of letrozole (Hengrui, H19991001, Lianyungang,

Jiangsu, China) was taken orally for 5 days from D4 of the menstrual cycle. Ultrasound was used to monitor the follicular size, and 75–150 U HMG (Livzon, H10940097, Shanghai, China) was administered to support follicle growth when necessary. When the serum estradiol level reached 200–300 pg/mL, or the follicle diameter was >18 mm, an ovulation trigger of 10,000 IU of HCG (Livzon, H44020672, Shanghai, China) was given. After ovulation was confirmed, endometrial transformation was induced by progesterone at 90 mg/day applied vaginally and Femoston yellow tablets taken orally at 40 mg/day. On the 3rd day after ovulation, one or two cleavage-stage embryos were thawed and transferred, or on the 5th day after ovulation, one or two blastocysts were transferred.

The quality of the embryo was assessed before transplantation. A high-quality, cleavage-stage embryo was defined as an embryo derived from a normal fertilized egg, with an embryonic cell count of 7–9 on the 3rd day after fertilization, and <10% fragmentation [20]. According to the Gardner scoring system, a high-quality blastocyst is at stage 3 or above, with neither the inner cell mass (ICM) score nor the trophectoderm (TE) score being grade C [21].

2.3 Preparation and Intrauterine Infusion of PRP

All patients signed the informed consent form for intrauterine infusion of autologous PRP before receiving treatment. A two-step centrifugation method was used for PRP preparation as described in a previous report [22]. One or two rounds of PRP intrauterine infusion were applied depending on the patient's individual condition. Two days before embryo transfer (or 4 days if possible), 15 mL of peripheral venous blood from the patient was drawn into a syringe preloaded with 5 mL of 3.2% sodium citrate anticoagulant solution (NIGALE, H20058913, Jianyang, Sichuan, China). This was immediately centrifuged at 300 $\times$g for 10 min at 18 °C. The upper two layers were then transferred to a new sterile tube and centrifuged for 15 min at 700 $\times$g and 18 °C. Approximately 75% of the resulting supernatant was discarded, and about 1 mL of PRP from below was aspirated. The PRP was then activated by adding thrombin powder (25 U/mL, HITECK, H42020042, Wuhan, Hubei, China) and calcium chloride (20 mmol/mL, Southwest Pharmaceutical Co., Ltd., H50020138, Chongqing, China). Following activation, the levels of platelet-derived growth factor (PDGF) and transforming growth factor β (TGF-β) in the sample were quantified to ensure quality control. Intrauterine infusion of the PRP was then performed. The patient was placed in the lithotomy position, the vulva was disinfected, a speculum was used to expose the cervix, and the vagina was sterilized. The PRP was gently infused into the uterine cavity through the cervical canal using a syringe attached to an intrauterine insemination catheter. After infusion, the patient remained in a supine position for 30 min.

2.4 Outcome Measures

Baseline characteristics for both groups were collected by review of medical records. These included baseline follicle-stimulating hormone (basal FSH), age, baseline luteinizing hormone (basal LH), years of infertility, baseline estradiol 2 (basal E2), BMI, baseline progesterone (basal P), AMH, and number of embryos transferred. The clinical outcomes were: endometrial thickness on the day of embryo transfer; embryo implantation rate = (number of implanted embryos/number of embryos transferred) $\times$ 100%; biochemical pregnancy rate = (number of HCG-positive cycles/number of transfer cycles) $\times$ 100%; clinical pregnancy rate = (number of clinical pregnancy cycles/number of transfer cycles) $\times$ 100%; miscarriage rate = (number of miscarriage cycles/number of clinical pregnancy cycles) $\times$ 100%; and live birth rate = (number of successful live births/number of transfer cycles) $\times$ 100%. Clinical pregnancy was defined as the presence of a gestational sac and fetal heartbeat on ultrasound 5 weeks after embryo transfer.

2.5 Statistical Analysis

SPSS (version 26.0, IBM, Armonk, NY, USA) was used for data analysis. Comparisons between two groups were made using Student's *t*-test for data with a normal distribution and homogeneous variance, or the nonparametric Wilcoxon rank-sum test for data that was not normally distributed. Data that did not show a normal distribution were represented by the median and interquartile range [M (P25, P75)]. Categorical data were analyzed using the Chi-square test or Fisher's exact test. Logistic regression analysis was performed with clinical pregnancy as the dependent variable, and potential influencing factors as covariates. This was used to calculate odds ratios (ORs) with 95% CIs and *p*-values. Considering interaction effects, the variables of PRP, duration of infertility, AMH, and endometrial thickness on transfer day were included in the final model using the forward selection method. To quantify the clinical impact of PRP on primary outcomes, the effect size (Cohen's d) was calculated using the arcsin transformation method to standardize and present the actual magnitude of differences between groups. To assess the limitations of sample size in subgroup analysis, a post-hoc power analysis was conducted on key outcome indicators (biochemical pregnancy rate and clinical pregnancy rate) using G*Power (version 3.1.9, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, North Rhine-Westphalia, Germany). A two-tailed p value < 0.05 was considered statistically significant.

3. Results

The total number of FET cycles at our center from January 1, 2022 to June 1, 2024 was 726. Of these, 186 cycles included autologous PRP intrauterine infusion before FET,

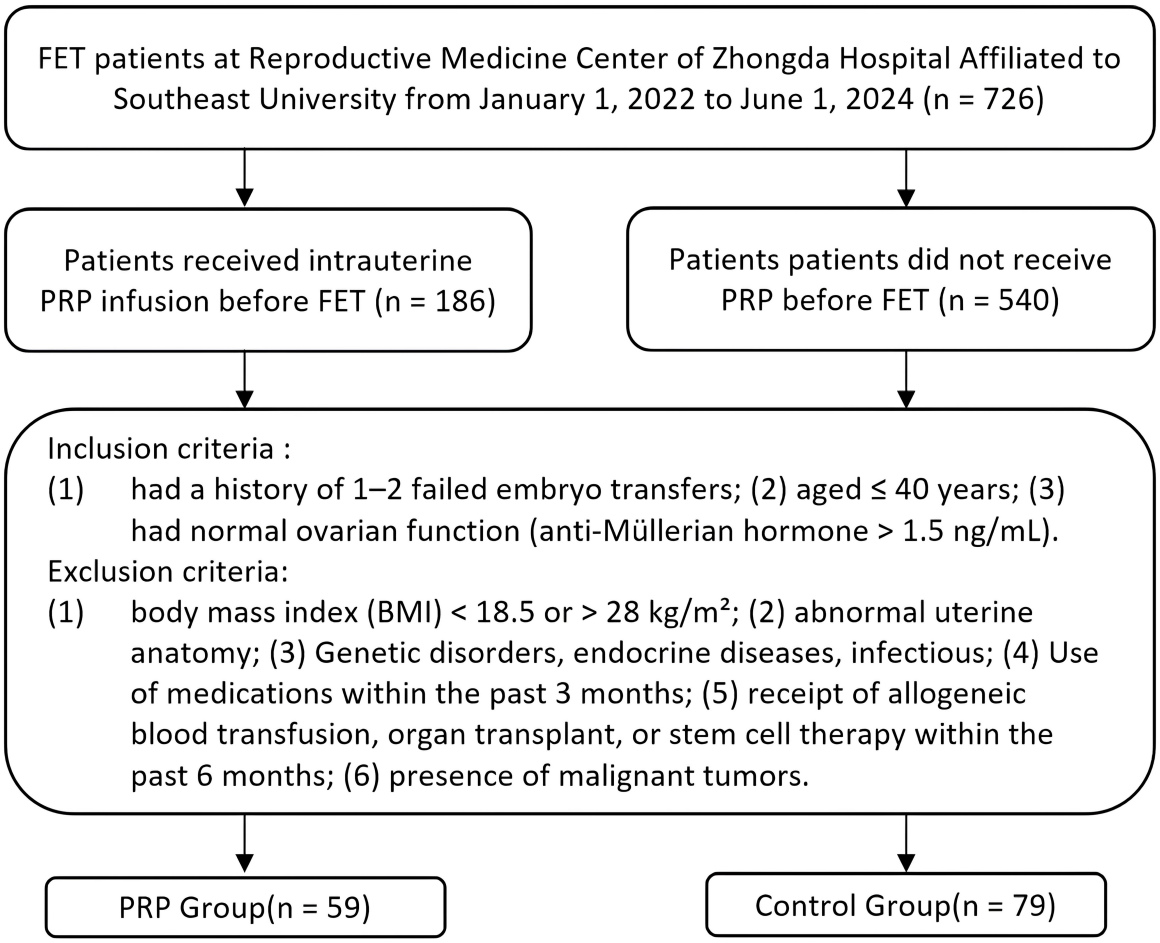

Fig. 1. Flowchart for screening and grouping of patients in freeze-thawed embryo transfer (FET) cycles. PRP, platelet-rich plasma.

and 540 cycles had no intrauterine intervention. After filtering based on the inclusion and exclusion criteria, 59 cycles remained in the PRP group, and 79 cycles in the control group (Fig. 1).

3.1 Baseline Characteristics

No significant differences were detected between the PRP and control groups in terms of age, duration of infertility, BMI, AMH, basal FSH, basal LH, basal E2, basal P, embryo transfer number, or high-quality embryo rate ($p > 0.05$), indicating the two groups were comparable. Detailed results are shown in Table 1.

3.2 Pregnancy Outcomes

As shown in Table 2, the biochemical pregnancy rate in the PRP group was significantly higher than in the control group (66.10% vs. 45.57%, $p < 0.05$). The PRP group also had a higher clinical pregnancy rate (57.63% vs. 39.24%, $p < 0.05$). The effect size (Cohen's d) for the biochemical pregnancy rate was 0.45, and for the clinical pregnancy rate it was 0.40. The embryo implantation rate in the PRP group was also higher than in the control group (43.75% vs. 30.83%, respectively). No significant differences were detected between the PRP and control groups in terms of the transfer day endometrial thickness, transfer day E2 level, transfer day P level, early miscarriage rate, or live birth rate ($p > 0.05$).

3.3 Logistic Regression Analysis of Factors Affecting Clinical Pregnancy

The results of logistic regression analysis are shown in Table 3. In univariate analysis, the use of PRP had a positive impact on clinical pregnancy outcomes (OR = 2.25, 95% CI: 1.11–4.60, $p = 0.025$). Other variables such as the number of embryos transferred and patient age showed no significant effect in the univariate analysis. In multivariate analysis, the OR for PRP showed very wide CIs and this factor was no longer significant for pregnancy outcomes (OR = 92.82, 95% CI: 0.04–243,922.60, $p = 0.259$). Most variables, such as BMI and basal FSH, were not significantly associated with pregnancy outcomes. In the final model, the adjusted OR for PRP was 2.76 (95% CI: 1.29–5.90, $p = 0.009$), thus indicating a significant impact.

Table 1. Comparison of general characteristics between the PRP and control groups.

Characteristic	PRP group (59 cycles)	Control group (79 cycles)	*p* value
Age (years)	32.19 ± 3.50	31.23 ± 4.11	0.151
Duration of infertility (years)	3.90 ± 2.98	3.95 ± 3.30	0.925
BMI (kg/m^2)	23.47 ± 2.42	23.49 ± 2.58	0.952
AMH (ng/mL)	5.2 (3.1, 7.5)	4.6 (2.6, 7.6)	0.637
Basal FSH (mIU/mL)	7.0 (5.8, 8.5)	6.9 (6.1, 8.3)	0.752
Basal LH (IU/L)	4.1 (3.2, 6.2)	4.0 (3.1, 6.0)	0.836
Basal E2 (pg/mL)	29.3 (23.0, 35.6)	32.2 (23.3, 37.3)	0.541
Basal P (ng/mL)	0.5 (0.3, 0.7)	0.5 (0.3, 0.7)	0.812
Average number of embryos transferred	1.63 ± 0.49	1.52 ± 0.50	0.207
High-quality embryos rate	57.29%	65.00%	0.247

AMH, anti-Müllerian hormone; basal FSH, baseline follicle-stimulating hormone; basal LH, baseline luteinizing hormone; basal E2, baseline estradiol 2; basal P, baseline progesterone; BMI, body mass index.

Table 2. Comparison of embryo transfer outcomes between the PRP and control groups.

Outcome measure	PRP group (59 cycles)	Control group (79 cycles)	*p* value
Endometrial thickness on transfer day (mm)	10.75 ± 2.49	11.44 ± 2.21	0.343
E2 on transfer day (pg/mL)	456.9 [206.0, 1117.3]	398.0 [190.3, 802.5]	0.416
P on transfer day (ng/mL)	8.5 [5.0, 10.8]	7.0 [3.4, 10.8]	0.332
Biochemical pregnancy rate (%)	66.10%	45.57%	0.017
Embryo implantation rate (%)	43.75%	30.83%	0.050
Clinical pregnancy rate (%)	57.63%	39.24%	0.032
Early miscarriage rate (%)	26.47%	16.13%	0.311
Live birth rate	42.37%	32.91%	0.255

Embryo implantation rate = (number of implanted embryos/number of embryos transferred) × 100%.
Biochemical pregnancy rate = (number of HCG-positive cycles/number of transfer cycles) × 100%.
Clinical pregnancy rate = (number of clinical pregnancy cycles/number of transfer cycles) × 100%.
Early miscarriage rate = (number of miscarriage cycles/number of clinical pregnancy cycles) × 100%, before 12 weeks.
Live birth rate = (number of successful live births/number of transfer cycles) × 100%.

3.4 Subgroup Analysis

To further analyze the effect of autologous PRP on pregnancy outcomes in patients with a history of embryo implantation failure, patients were subdivided into those with a history of one failed embryo transfer (subgroup 1), and those with a history of two failed embryo transfers (subgroup 2). We then compared subgroup 1 after PRP treatment (PRP-1) to subgroup 1 with no intervention (control-1), and subgroup 2 after PRP treatment (PRP-2) to subgroup 2 with no intervention (control-2). No significant differences were detected between subgroups 1 and 2 in terms of BMI, age, basal FSH, duration of infertility, basal LH, basal E2, AMH, basal P, or average number of embryos transferred ($p > 0.05$), indicating that each of the two pairs of subgroups were comparable (Table 4).

As shown in Table 5, the biochemical pregnancy rate in PRP-1 of subgroup 1 was significantly higher than in control-1 (68.97% vs. 47.14%, respectively, $p < 0.05$). The embryo implantation rate (43.90% vs. 25.83%) and clinical pregnancy rate (55.17% vs. 40.00%) also showed improvement, however these did not reach statistical significance. In subgroup 2, the biochemical pregnancy rate (63.33% vs. 33.33%), embryo implantation rate (53.33% vs. 25.83%) and clinical pregnancy rate (60.00% vs. 33.33%) of PRP-2 also showed improvement compared to control-2, but these differences did not reach statistical significance (Table 5). To assess the limitations imposed by the relatively small sample sizes in the subgroup analysis, a post-hoc power analysis was conducted of the key outcome indicators: the biochemical pregnancy rate and clinical pregnancy rate. For subgroup 1, the Achieved Power for biochemical pregnancy rate and clinical pregnancy rate were both 71%, while for subgroup 2 this was 62% and 58%, respectively.

4. Discussion

The development of ART has assisted many infertile patients. However, the failure of embryo transfer remains a major issue for medical professionals and patients. A previous study has mostly focused on improving the clinical outcomes of RIF patients. Nevertheless, previous data indicate that embryo implantation rates and clinical outcomes decrease significantly according to the number of previous

Table 3. Logistic regression analysis of variables associated with the rate of clinical pregnancy.

Variable	OR univariable	OR multivariable	OR final
No PRP			
PRP	2.25 (1.11–4.60, $p = 0.025$)	92.82 (0.04–243,922.60, $p = 0.259$)	2.76 (1.29–5.90, $p = 0.009$)
Number of embryos transferred	1.33 (0.61–2.88, $p = 0.469$)	0.88 (0.34–2.31, $p = 0.800$)	
Age	0.97 (0.88–1.06, $p = 0.444$)	1.04 (0.88–1.24, $p = 0.639$)	
Duration of infertility	0.91 (0.80–1.02, $p = 0.115$)	0.89 (0.77–1.03, $p = 0.120$)	0.90 (0.79–1.02, $p = 0.093$)
BMI	0.95 (0.82–1.09, $p = 0.462$)	0.95 (0.81–1.11, $p = 0.507$)	
Basal FSH	0.93 (0.78–1.11, $p = 0.403$)	0.96 (0.78–1.17, $p = 0.660$)	
Basal E2	1.01 (0.99–1.03, $p = 0.460$)	1.01 (0.99–1.03, $p = 0.525$)	
Basal P	1.06 (0.42–2.71, $p = 0.897$)	1.32 (0.44–3.95, $p = 0.626$)	
Basal LH	1.04 (0.93–1.16, $p = 0.517$)	1.02 (0.89–1.17, $p = 0.765$)	
AMH	1.06 (0.98–1.15, $p = 0.154$)	1.16 (0.66–2.06, $p = 0.601$)	1.07 (0.98–1.16, $p = 0.146$)
Endometrial thickness on transfer day	1.11 (0.95–1.30, $p = 0.188$)	1.26 (0.99–1.59, $p = 0.059$)	1.16 (0.99–1.38, $p = 0.074$)
E2 on transfer day	1.00 (1.00–1.00, $p = 0.843$)	1.00 (1.00–1.00, $p = 0.929$)	
P on transfer day	1.01 (0.97–1.05, $p = 0.628$)	1.01 (0.96–1.06, $p = 0.644$)	
No PRP: Age	0.94 (0.86–1.04, $p = 0.218$)		
PRP: Age	0.97 (0.88–1.06, $p = 0.484$)	0.95 (0.77–1.17, $p = 0.618$)	
No PRP: Endometrial thickness	1.12 (0.95–1.31, $p = 0.168$)		
PRP: Endometrial thickness	1.21 (1.01–1.44, $p = 0.036$)	0.85 (0.60–1.21, $p = 0.376$)	
Age: AMH	1.00 (1.00–1.00, $p = 0.192$)	1.00 (0.98–1.02, $p = 0.713$)	

Table 4. Comparison of general characteristics within subgroup 1 and 2.

Characteristic	Subgroup 1			Subgroup 2		
	PRP 1 (29 cycles)	Control 1 (70 cycles)	*p* value	PRP 2 (30 cycles)	Control 2 (9 cycles)	*p* value
Age (years)	32.45 ± 3.42	31.16 ± 4.17	0.144	31.93 ± 3.62	31.78 ± 3.80	0.912
Duration of infertility (years)	3.86 ± 3.17	3.91 ± 3.25	0.942	3.93 ± 2.83	4.22 ± 3.87	0.807
BMI (kg/m^2)	23.35 ± 2.76	23.61 ± 2.55	0.653	23.58 ± 2.08	22.57 ± 2.80	0.246
AMH (ng/mL)	5.0 (3.2, 6.6)	4.8 (2.5, 8.3)	0.848	5.3 (3.1, 9.2)	4.6 (2.8, 5.0)	0.243
Basal FSH (mIU/mL)	7.1 (5.7, 8.3)	7.0 (6.1, 8.2)	0.619	7.0 (5.9, 8.5)	6.2 (6.0, 8.6)	0.671
Basal LH (IU/L)	4.0 (3.2, 6.2)	4.1 (3.2, 6.6)	0.781	4.1 (3.2, 6.2)	3.2 (1.9, 3.5)	0.147
Basal E2 (pg/mL)	28.3 (22.8, 36.2)	32.5 (24.9, 37.8)	0.314	30.8 (23.5, 35.6)	23.2 (17.2, 34.1)	0.190
Basal P (ng/mL)	0.5 (0.3, 0.6)	0.5 (0.3, 0.7)	0.229	0.5 (0.4, 0.8)	0.5 (0.4, 0.6)	0.505
Number of embryos transferred	1.59 ± 0.50	1.50 ± 0.50	0.440	1.67 ± 0.48	1.67 ± 0.50	1.000

Subgroup 1: patients with a history of one failed embryo transfer cycle.

Subgroup 2: patients with a history of two failed embryo transfer cycles.

transfer failures [23]. Therefore, in the current study we implemented early intervention for atypical RIF patients to minimize the number of repeated cycles as much as possible. PRP has recently found several applications in the field of reproductive medicine. Due to its cost-effectiveness and easy accessibility, it is used to treat reproductive system disorders such as Diminished Ovarian Reserve (DOR), Premature Ovarian Insufficiency (POI), and thin endometrium [24]. When the number of previous implantation failures in patients is $\geq$3, the effectiveness of intrauterine PRP infusion is reported to be significantly lower. In other words, if intervention with intrauterine PRP infusion is delayed until after 3 implantation failures, its efficacy may be greatly reduced [25]. Consequently, in the current study we applied PRP to atypical RIF patients. This was found to reduce the time and financial cost of ART treatment, as well as some improvement in their clinical outcomes.

The embryo implantation rate, biochemical pregnancy rate and clinical pregnancy rate of atypical RIF patients in the PRP group were found to be significantly higher than in the control group. The effect size for biochemical pregnancy rate and clinical pregnancy rate both showed moderate impact (Cohen's d of 0.45 and 0.40, respectively), and both outcomes were significantly better with PRP (p = 0.024 and p = 0.032, respectively). These findings indicate that PRP can lead to clinically meaningful improvements in the positive rate of early pregnancy biochemical markers, suggesting potential value in the initial stage of embryo implantation. Additionally, we also observed a 10% absolute increase in the live birth rate in the PRP group (35% vs. 25%). Although this did not reach statistical significance due to the relatively small sample size, the magnitude of the improvement suggests that PRP has clinical value, especially since the 95% CI ranged up to 23%.

Table 5. Comparison of embryo transfer outcomes for subgroups 1 and 2.

Outcome Measure	Subgroup 1			Subgroup 2		
	PRP 1 (29 cycles)	Control 1 (70 cycles)	*p* value	PRP 2 (30 cycles)	Control 2 (9 cycles)	*p* value
Endometrial thickness on transfer day (mm)	10.66 ± 2.76	11.51 ± 2.33	0.123	10.85 ± 2.25	10.91 ± 0.77	0.934
E2 on transfer day (pg/mL)	353.5 (190.5, 846.0)	367.9 (190.2, 760.5)	0.717	367.9 (190.2, 760.5)	1233.4 (209.1, 1329.7)	0.484
P on transfer day (ng/mL)	7.3 (4.6, 10.8)	6.5 (3.0, 9.6)	0.329	6.5 (3.0, 9.6)	13.6 (7.4, 16.3)	0.129
Biochemical pregnancy rate (%)	68.97%	47.14%	0.048	63.33%	33.33%	0.142
Embryo implantation rate (%)	43.90%	25.83%	0.357	53.33%	25.83%	0.084
Clinical pregnancy rate (%)	55.17%	40.00%	0.167	60.00%	33.33%	0.255
Early miscarriage rate (%)	37.50%	14.29%	0.133	16.67%	33.33%	0.489

The biochemical pregnancy rate, embryo implantation rate and clinical pregnancy rate in subgroup 1 were analyzed using the chi-square test.

The biochemical pregnancy rate, embryo implantation rate and clinical pregnancy rate in subgroup 2, and the early miscarriage rate in both subgroups, were analyzed using Fisher's exact test.

We observed that early miscarriage rates in both the control and PRP groups were relatively high in this study, at 16.1% (5/31) and 26.5% (9/34), respectively. A previous study has shown that conditions such as chronic endometritis, adenomyosis, and endometriosis can lead to RIF and may increase the risk of early miscarriage [26]. A recent endometrial transcriptomic study revealed a large overlap in abnormally expressed gene profiles between women with RIF and those with recurrent early pregnancy loss [27]. In order to identify possible factors that influence the high rate of early miscarriage in our study, we first analyzed whether the three endometrial preparation protocols could potentially impact treatment outcomes. The use of the three pretreatment methods (GnRHa downregulation HRT, HRT, COS) between the two groups was 54.24%, 30.51%, and 15.25%, respectively, for the PRP group, and 50.63%, 36.71%, and 12.66%, respectively for the control group. There was no significant difference in the proportion of pretreatment methods used between the two groups. However, a study has shown that the natural cycle endometrial preparation protocol may be more effective at reducing the miscarriage rate in RIF patients compared with the HRT protocol [28]. This could be one of the factors contributing to the relatively high miscarriage rate. Second, we analyzed the potential impact of embryo quality. Since preimplantation genetic screening (PGS) was not performed on the embryos in this study, we cannot completely rule out the possibility of aneuploid embryos, which could be another contributing factor to the relatively high miscarriage rate. We next analyzed the data for the 14 miscarriage cycles. The overall PRP and control groups showed no significant differences in high-quality embryos (57% vs. 65%, respectively) and average number of transferred embryos (1.63 vs. 1.52). However, amongst the 14 miscarriage cycles, the PRP group had fewer high-quality embryos (54.55% vs. 87.5%) and average number of transferred embryos (1.22 vs. 1.60) compared to the control group. This result suggests that embryo quality factors may have been an important cause of early miscarriage in the current study. A recent study also found that the number of high-quality embryos is an independent prognostic factor for early miscarriage [29]. Finally, the potential risks of PRP cannot be ignored completely, with larger studies needed to properly evaluate the safety profile of PRP treatment.

Univariate logistic regression analysis revealed that PRP had some impact on clinical pregnancy outcomes. In multivariate analysis, the OR for PRP was very unstable, but the adjusted OR for PRP in the final model again showed a significant impact. A possible reason for this is that after variable screening and adjustment, some confounding factors were excluded, thus allowing a more accurate reflection of the effect of PRP on clinical pregnancy. Variables such as the duration of infertility and AMH also showed near-significant results in the final model, suggesting they could have some impact on clinical pregnancy outcomes when all relevant factors are comprehensively considered.

After further subgrouping of patients, the biochemical pregnancy rate of subgroup 1 (patients with a history of one failed embryo transfer) was found to be significantly higher than that of subgroup 2, with a history of two failed transfers (68.97% vs. 47.14%, respectively, $p = 0.048$). This result indicates that PRP can improve the clinical outcomes of ART even in women with a history of only one failed embryo transfer. Based on the results before subgrouping in the current study, and together with the use of PRP in RIF patients in previous studies, it appears that PRP not only benefits standard RIF patients, but may also have significant benefit for atypical RIF patients. These findings support early PRP intervention in patients with a history of only one failed embryo transfer in future clinical practice.

Despite not reaching statistical significance, the substantial improvements seen in subgroup 2 with PRP compared to controls (clinical pregnancy rate: 60.00% vs. 33.33%; embryo implantation rate: 53.33% vs. 25.83%) represent clinically meaningful differences. In order to clar-

ify whether the non-statistical significance was due to the small sample size of subgroup 2, we conducted a post-hoc power analysis of these key indicators. For the biochemical pregnancy rate, with Cohen's h = 0.48 and α = 0.05, the Achieved Power was 62% at <0.8. This indicates that due to the small sample size of the subgroup (n = 9 in the control-2 group), there was only a limited ability to detect true differences. For the clinical pregnancy rate, with Cohen's h = 0.45 and α = 0.05, the Achieved Power was 58% at <0.8. This provides further confirmation that the statistical power of our subgroup analysis was limited by sample size, which may have masked the true therapeutic effect of PRP. Clinical significance was also suggested in the different clinical pregnancy rates (55% vs. 40%) observed in subgroup 1 with and without PRP, respectively. However, the Achieved Power for the biochemical pregnancy rate in subgroup 1 was 71%. Combined with the effect size and the trend for the *p*-value, this result suggests that PRP has a potential positive effect on the biochemical pregnancy rate in subgroup 1. For the clinical pregnancy rate, the Achieved Power was also 71%, which is close to the ideal threshold of 0.8. Nevertheless, due to the relatively limited sample size of the PRP-1 group (n = 29), the "potential moderate effect" did not reach statistical significance. The results of post-hoc power analysis suggest that expansion of the sample size may further confirm the beneficial effect of PRP on the clinical pregnancy rate in subgroup 1. In conclusion, although this study failed to demonstrate a statistically significant effect of PRP in subgroup analysis, the clinically meaningful trend, the consistent direction of improvement across multiple indicators, and the limited statistical power all strongly suggest that PRP is a promising therapeutic option. We recommend that more rigorously designed, large-sample, multicenter randomized controlled trials be carried out to further clarify the clinical value of PRP.

Several limitations to this study should be acknowledged. Firstly, as a retrospective study, it relied on existing data records. Due to the incompleteness of data, some indicators that may have affected the conclusions (e.g., endometrial blood flow, PRP-related growth factor levels, and differences in chromosomal status) could not be included in the statistical analysis. In subsequent studies, we will incorporate more complete data derived from a larger sample size in order to increase the reliability of conclusions. Secondly, there may have been some uncontrollable factors, such as individual variations in ovarian stimulation protocols and endometrial preparation protocols. When designing subsequent studies, researchers should strive to ensure these factors remain consistent, thereby reducing their potential impact on the results and conclusions. Thirdly, long-term indicators such as obstetric outcomes or neonatal outcomes were not included in the study. This limitation prevented us from tracking the longer-term effects and safety of PRP treatment.

5. Conclusions

In summary, intrauterine infusion with autologous PRP during FET cycles in atypical RIF patients may help to increase the biochemical pregnancy rate, embryo implantation rate, and clinical pregnancy rate.

Availability of Data and Materials

The data analyzed during the current study are not publicly available due to the confidentiality factors of patient information, but are available from the corresponding author on reasonable request.

Author Contributions

YL designed this study, JS conducted the study, JL collected data for the study, and FY and LL 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

The study was carried out in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Zhongda Hospital Affiliated with Southeast University (2024ZDSYLL486-P01). Informed patient consent was waived, as the study was retrospective in nature and analyzed patient data anonymously.

Acknowledgment

We would like to express our gratitude to all those who helped us during the writing of this manuscript. Thanks to all the peer reviewers for their opinions and suggestions.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Ma J, Gao W, Li D. Recurrent implantation failure: A comprehensive summary from etiology to treatment. Frontiers in Endocrinology. 2023; 13: 1061766. https://doi.org/10.3389/fendo.2022.1061766.

[2] Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Human Reproduction Update. 2006; 12: 731–746. https://doi.org/10.1093/humupd/dml004.

[3] Craciunas L, Gallos I, Chu J, Bourne T, Quenby S, Brosens JJ, *et al*. Conventional and modern markers of endometrial receptivity: a systematic review and meta-analysis. Human Reproduc-

tion Update. 2019; 25: 202–223. https://doi.org/10.1093/humupd/dmy044.

[4] Cimadomo D, Craciunas L, Vermeulen N, Vomstein K, Toth B. Definition, diagnostic and therapeutic options in recurrent implantation failure: an international survey of clinicians and embryologists. Human Reproduction (Oxford, England). 2021; 36: 305–317. https://doi.org/10.1093/humrep/deaa317.

[5] Fang Y, Jingjing F, Tiantain C, Huanhuan X, Qiaohua H. Impact of the number of previous embryo implantation failures on IVF/ICSI-ET pregnancy outcomes in patients younger than 40 years: a retrospective cohort study. Frontiers in Endocrinology. 2023; 14: 1243402. https://doi.org/10.3389/fendo.2023.1243402.

[6] Kozyra O, Medvediev M, Tinelli A. Unique Implantation Window as a Possible Reason of Embryo Transfer Failure. Retrospective Analysis. Clinical and Experimental Obstetrics & Gynecology. 2023; 50: 108. https://doi.org/10.31083/j.ceog5005108.

[7] Zhao L, Yin F, Hu X, Li J, Wei C, Zhou F, *et al*. Pinopode versus endometrial receptivity analysis for personalized embryo transfer in recurrent implantation failure. Reproductive Biomedicine Online. 2025; 51: 104875. https://doi.org/10.1016/j.rbmo.2025.104875.

[8] Rahmati M, Lédée N. Targeted Endometrial Scratching: An Example of Endometrial Diagnosis Usage in Reproductive Medicine. Frontiers in Immunology. 2020; 11: 589677. https://doi.org/10.3389/fimmu.2020.589677.

[9] Kong X, Tang G, Liu Y, Zheng Z, Li Y, Yan F. Efficacy of intrauterine infusion therapy before embryo transfer in recurrent implantation failure: A systematic review and network meta-analysis. Journal of Reproductive Immunology. 2023; 156: 103819. https://doi.org/10.1016/j.jri.2023.103819.

[10] Xie H, Zeng H, He D, Liu N. Effect of intrauterine perfusion of human chorionic gonadotropin before embryo transfer after two or more implantation failures: A systematic review and meta-analysis. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2019; 243: 133–138. https://doi.org/10.1016/j.ejogrb.2019.10.039.

[11] Pourmoghadam Z, Abdolmohammadi-Vahid S, Pashazadeh F, Aghebati-Maleki L, Ansari F, Yousefi M. Efficacy of intrauterine administration of autologous peripheral blood mononuclear cells on the pregnancy outcomes in patients with recurrent implantation failure: A systematic review and meta-analysis. Journal of Reproductive Immunology. 2020; 137: 103077. https://doi.org/10.1016/j.jri.2019.103077.

[12] Zhang L, Xu WH, Fu XH, Huang QX, Guo XY, Zhang L, *et al*. Therapeutic role of granulocyte colony-stimulating factor (G-CSF) for infertile women under in vitro fertilization and embryo transfer (IVF-ET) treatment: a meta-analysis. Archives of Gynecology and Obstetrics. 2018; 298: 861–871. https://doi.org/10.1007/s00404-018-4892-4.

[13] Bakhsh AS, Maleki N, Sadeghi MR, SadeghiTabar A, Tavakoli M, Zafardoust S, *et al*. Effects of Autologous Platelet-Rich Plasma in women with repeated implantation failure undergoing assisted reproduction. JBRA Assisted Reproduction. 2022; 26: 84–87. https://doi.org/10.5935/1518-0557.20210046.

[14] Gurkan N, Alper T. The effect of endometrial PRP on fertility outcomes in women with implantation failure or thin endometrium. Archives of Gynecology and Obstetrics. 2025; 311: 1195–1204. https://doi.org/10.1007/s00404-025-07948-1.

[15] Solakoglu Ö, Heydecke G, Amiri N, Anitua E. The use of plasma rich in growth factors (PRGF) in guided tissue regeneration and guided bone regeneration. A review of histological, immunohistochemical, histomorphometrical, radiological and clinical results in humans. Annals of Anatomy = Anatomischer Anzeiger: Official Organ of the Anatomische Gesellschaft. 2020; 231: 151528. https://doi.org/10.1016/j.aanat.2020.151528.

[16] Karadbhajne P, Dzoagbe HY, More A. Platelet-Rich Plasma (PRP) for Endometrial Treatment Efficacy and Safety in Assisted Reproductive Technology: A Comprehensive Review. Cureus. 2024; 16: e59728. https://doi.org/10.7759/cureus.59728.

[17] Li J, Li X, Ding J, Zhao J, Chen J, Guan F, *et al*. Analysis of pregnancy outcomes in patients with recurrent implantation failure complicated with chronic endometritis. Frontiers in Cell and Developmental Biology. 2023; 11: 1088586. https://doi.org/10.3389/fcell.2023.1088586.

[18] Huang C, Ye X, Ye L, Lu L, Liu F. Platelet-Rich Plasma Intrauterine Infusion as Assisted Reproduction Technology (ART) to Combat Repeated Implantation Failure (RIF): A Systematic Review and Meta-Analysis. Iranian Journal of Public Health. 2023; 52: 1542–1554. https://doi.org/10.18502/ijph.v52i8.13394.

[19] Mouanness M, Ali-Bynom S, Jackman J, Seckin S, Merhi Z. Use of Intra-uterine Injection of Platelet-rich Plasma (PRP) for Endometrial Receptivity and Thickness: a Literature Review of the Mechanisms of Action. Reproductive Sciences (Thousand Oaks, Calif.). 2021; 28: 1659–1670. https://doi.org/10.1007/s43032-021-00579-2.

[20] Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Human Reproduction (Oxford, England). 2011; 26: 1270–1283. https://doi.org/10.1093/humrep/der037.

[21] Gardner DK, Vella P, Lane M, Wagley L, Schlenker T, Schoolcraft WB. Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. Fertility and Sterility. 1998; 69: 84–88. https://doi.org/10.1016/s0015-0282(97)00438-x.

[22] Mehrafza M, Pourseify G, Zare Yousefi T, Azadeh R, Saghati Jalali S, Hosseinzadeh E, *et al*. The Efficiency of Introducing Intrauterine Infusion of Autologous Platelet-Rich Plasma versus Granulocyte Colony-Stimulating Factor in Repeated Implantation Failure Patients: An Unblinded Randomised Clinical Trial. International Journal of Fertility & Sterility. 2024; 18: 30–34. https://doi.org/10.22074/ijfs.2024.2013900.1557.

[23] Wang Y, Tian Y, Liu L, Li TC, Tong X, Zhu H, *et al*. The number of previous failed embryo transfer cycles is an independent factor affecting implantation rate in women undergoing IVF/ICSI treatment: A retrospective cohort study. Medicine. 2021; 100: e25034. https://doi.org/10.1097/MD.0000000000025034.

[24] Sharara FI, Lelea LL, Rahman S, Klebanoff JS, Moawad GN. A narrative review of platelet-rich plasma (PRP) in reproductive medicine. Journal of Assisted Reproduction and Genetics. 2021; 38: 1003–1012. https://doi.org/10.1007/s10815-021-02146-9.

[25] Fujii S, Oguchi T. The number of previous implantation failures is a critical determinant of intrauterine autologous platelet-rich plasma infusion success in women with recurrent implantation failure. Reproductive Medicine and Biology. 2024; 23: e12565. https://doi.org/10.1002/rmb2.12565.

[26] Pirtea P, Cicinelli E, De Nola R, de Ziegler D, Ayoubi JM. Endometrial causes of recurrent pregnancy losses: endometriosis, adenomyosis, and chronic endometritis. Fertility and Sterility. 2021; 115: 546–560. https://doi.org/10.1016/j.fertnstert.2020.12.010.

[27] Liaqat Ali Khan N, Nafee T, Shao T, Hart AR, Elliott S, Ola B, *et al*. Dysregulation in Multiple Transcriptomic Endometrial Pathways Is Associated with Recurrent Implantation Failure and Recurrent Early Pregnancy Loss. International Journal of Molecular Sciences. 2022; 23: 16051. https://doi.org/10.3390/ijms232416051.

[28] Mu X, Liu X, Zhou H, Shi J. The natural cycle protocol of

endometrial preparation for frozen embryo transfer decreases the miscarriage rate in women with recurrent pregnancy loss. Gynecological Endocrinology: The Official Journal of the International Society of Gynecological Endocrinology. 2023; 39: 2269269. https://doi.org/10.1080/09513590.2023.2269269.

[29] Zhang M, Ji X, Hu X, Zhu Y, Ma H, Xu H, *et al*. Development and validation of a visualized prediction model for early miscarriage risk in patients undergoing IVF/ICSI procedures: a real-world multi-center study. Frontiers in Endocrinology. 2024; 14: 1280145. https://doi.org/10.3389/fendo.2023.1280145.