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A novel technique- subendometrial autologous platelet rich plasma injection in patients with unresponsive thin endometrium undergoing frozen-thawed embryo transfer: a prospective cohort study

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Abstract

Background The purpose of this study was to investigate the effects of subendometrial PRP injection on endometrial thickness and pregnancy outcomes in patients with a history of unresponsive thin endometrium undergoing frozen-thawed embryo transfer (FET).

Methods This prospective cohort study was conducted at Acibadem Mehmet Ali Aydinlar University-Istanbul, Turkey. Women with a history of suboptimal endometrial proliferation (< 7 mm) were offered to participate in the study. Group 1 consisted of 100 individuals who consented to subendometrial PRP injection, while Group 2 consisted of 100 individuals who did not accept PRP injection. Within ten days of the menstrual cycle ending, autologous PRP was produced by centrifuging peripheral blood and administered transvaginally into the subendometrial region under ultrasound monitoring. After the PRP procedure, 14 days of oral estradiol supplementation were started as part of the hormonal treatment on the 2-4th day of the second menstrual cycle. Women determined to have adequate endometrial thickness following the initiation were scheduled for embryo transfers. Embryo transfer was scheduled for women who were found to have adequate endometrial thickness after the initiation of progesterone. Pregnancy (positive serum hCG) and livebirth rates were followed.

Results A total of 200 women (age 36.4 ± 5.8) were included in the study. Endometrial thickness increased after PRP therapy as compared to the control group (7.7 ± 1.9 mm vs. 6.1 ± 1.2 mm; $p < 0.01$). Three women (3.0%) in the PRP group conceived spontaneously, whereas 97 women (97.0%) attempted FET; no spontaneous pregnancies occurred in the control group. Compared to 75/100 (75% of the total) in the control group, 33/97 women (34.0%) in the PRP group were unable to undertake ET because of persistently unresponsive thin endometrium or fluid in the endometrial cavity ($p < 0.001$). The PRP group had a considerably higher percentage of positive serum hCG tests than

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the control group (25.8% vs. 9.0%; $p=0.002$). Additionally, the PRP group had a higher clinical pregnancy rate (22.7% vs. 7.0%; $p=0.002$). The live birth rate was significantly higher in the PRP group than the controls (17.5% vs. 2.0%; $p<0.001$).

Conclusions In women with a history of suboptimal endometrial development, subendometrial PRP injection was associated with improved endometrial thickness and livebirth rate.

Trial registration Baskent University institutional review board and ethics committee (KA-20/23) http://www.ClinicalTrials.gov, (NCT04424160), 2020.06.15.

Keywords Thin endometrium, Endometrial PRP, Frozen embryo transfer

Background

Successful embryo implantation requires a receptive endometrium, a viable embryo, and effective cross-talk between them. In women undergoing IVF treatment, several studies have shown that endometrial thickness is the most important key factor related to the outcome of frozen-thawed embryo transfer (FET) cycles [1, 2]. Live birth rates plateau after an endometrial thickness of 7 to 10 mm and an endometrial thickness of less than 6 mm is associated with a reduction in live birth rates after embryo transfer [3]. In FET cycles, a decline in clinical pregnancy and live birth rates is generally observed as endometrial thickness falls below the 7 mm threshold [4].

Managing thin, treatment-resistant endometrium remains a challenging aspect of reproductive medicine. Despite the lack of a consensus regarding the clinically most effective treatment modalities or adjuvants, numerous strategies to promote endometrial proliferation have been introduced in the literature [5, 6]. According to Canadian Fertility and Andrology Society, there is insufficient evidence for the use of any adjuvants (aspirin, luteal oestradiol, sildenafil citrate, gonadotropin-releasing hormone agonist, platelet-rich plasma, stem cells) and additional adjuvants (including pentoxifylline, vitamins C, vitamins E and L-arginine) to increase pregnancy or live birth rates in patients with thin endometrium [7].

Platelet-rich plasma (PRP) consists of a high concentration of platelets found in plasma produced by centrifugation of peripherally collected blood [8, 9]. Platelets contain over 800 types of proteins, cytokines, growth factors, and hormones, which are crucial role in promoting cellular proliferation, growth, and differentiation [10, 11]. Autologous platelet sources have been employed in various fields, including plastic and orthopedic surgery, for tissue repair and regeneration, and for the maturation of isolated human primordial and primary follicles [12, 13].

Recent advancements have been made in addressing endometrial hypoproliferation through intrauterine PRP infusions. Chang et al. have first presented the effect of PRP on five patients with thin endometrium (≤7 mm) and infused 0.5-1 ml of PRP on day 10 of the hormone replacement therapy (HRT) [14]. This PRP intervention led to enhanced endometrial thickness and successful pregnancies in all patients. Kim et al. included twenty-four women with histories of two or more unsuccessful IVF cycles and a thin endometrium (<7 mm) who received two or three intrauterine PRP infusions starting on the 10th day of their menstrual cycle [15]. They reported an average increase in endometrial thickness of 0.6 mm compared to their previous cycle and a clinical pregnancy rate of 12.7%. In a prospective single-arm self-controlled trial conducted in Japan, PRP administration was performed in 36 patients with endometrial thickness <7 mm in the prior cycle (mean 6.04 with blinded measurement) [16]. After PRP administration, the mean endometrial thickness significantly increased compared to the prior cycle by 1.27 mm and 0.72 mm for unblinded and blinded measurements, respectively, allowing 88.9% (32 patients) to proceed with FET and achieving a clinical pregnancy rate of 15.6%. In a trial including 85 women with endometrial hypoproliferation, infusion of PRP into the endometrial cavity have resulted in a significant increase in endometrial thickness of 1.2 ± 0.21 mm compared to the prior cycle and a 37% clinical pregnancy rate per embryo transfer after intrauterine infusion of PRP [17].

In this study, we hypothesized that in women with thin refractory endometrium, subendometrial injection of autologous PRP may improve endometrial thickness and IVF outcomes after FET. Our objective was to examine the impact of subendometrial PRP injections on endometrial thickness and pregnancy outcomes among patients with histories of unresponsive thin endometrium undergoing FET.

Methods

Study design and patient selection

This non-randomized controlled trial was conducted at Acibadem University IVF Center in Istanbul, Turkey, from Jun 15, 2020, to Apr 30, 2022. Inclusion criteria encompassed women of reproductive age undergoing FET with at least one PGT-A tested or untested embryo from the previous IVF trial and a history of suboptimal endometrial proliferation less than 7 mm after hormone replacement therapy in at least one previous cycle. Exclusion criteria included individuals with a history of

malignancy, poor embryo quality, hematological, immunological, or hormonal disorders, chromosomal or genetic abnormalities, congenital or acquired (fibroid, polyp) uterine abnormalities, presence of intrauterine adhesions on hysterosalpingography, contraindications for plasma infusion due to anticoagulant use, and previous or current IgA deficiency.

Participants with a history of suboptimal endometrial proliferation (<7 mm) were invited to join the study. The patients were given detailed information about PRP that it was yet an unproven experimental intervention. Those who met the inclusion and exclusion criteria and who consented to undergo subendometrial PRP injection were classified into Group 1 ($n = 100$), and those who opted out formed the control group (Group 2; $n = 100$). Only PRP intervention was performed for the improvement of endometrial thickness without any other adjuvant methods such as sildenafil, pentoxifylline, vitamin E, G-CSF, or aspirin to figure out the exact effect of PRP.

PRP Preparation

Autologous PRP was prepared by drawing blood from each patient, followed by centrifugation under the manufacturer's protocol using the T-lab autologous platelet-rich plasma kit (T-Biotechnology Laboratory, Bursa, Turkey). Each patient's blood was collected under sterile conditions, divided into two tubes, and centrifuged at 830 g for 8 min. A 16 G needle attached to a 5 ml syringe was then used to withdraw PRP, rich in growth factors, from the buffy coat layer without disrupting the clot. A total volume of 4–8 ml PRP was collected, transferred to a separate container, and gently shaken for 30–60 s.

Subendometrial injection

Following the cessation of menstruation, autologous PRP was injected transvaginally into the junctional zone of the subendometrial region under conscious sedation in the operating room within two hours of PRP preparation (Fig. 1). Under ultrasound guidance, PRP injections were performed starting from the fundal region and passing through the myometrium using a 35 cm 17 G single lumen needle (Cook, USA). PRP was injected in the subendometrial region, on both sides of the cavity, spaced about 1 cm apart, and moved towards the lower uterine segment on both sides of the uterine cavity. The opposite myometrium was accessed by traversing the endometrial cavity from anterior to posterior. Each patient received a total of 4–6 ml PRP.

Endometrial Preparation for frozen embryo transfer

Patients with irregular cycles undergoing FET began treatment with oral contraceptives between days two and five of their cycle. On the second menstrual cycle following PRP injection, a mid-luteal phase injection of 3.75 mg leuprolide acetate depot (Lucrin; Abbott) was administered, and estradiol replacement therapy was started in the subsequent cycle. Oral estradiol (Estrofem; Novo Nordisk) was initially prescribed at 4 mg per day for five days and gradually increased to 8 mg daily. Ultrasound examination was performed on the midsagittal transvaginal image at the maximum distance between the endometrial-myometrial interface of the anterior to the posterior wall of the uterus by the same operator (YC) [18, 19]. If the endometrial thickness did not reach 7 mm or the appearance was non-trilaminar after 14 days of estradiol therapy, an additional 2 mg intravaginal estradiol and a

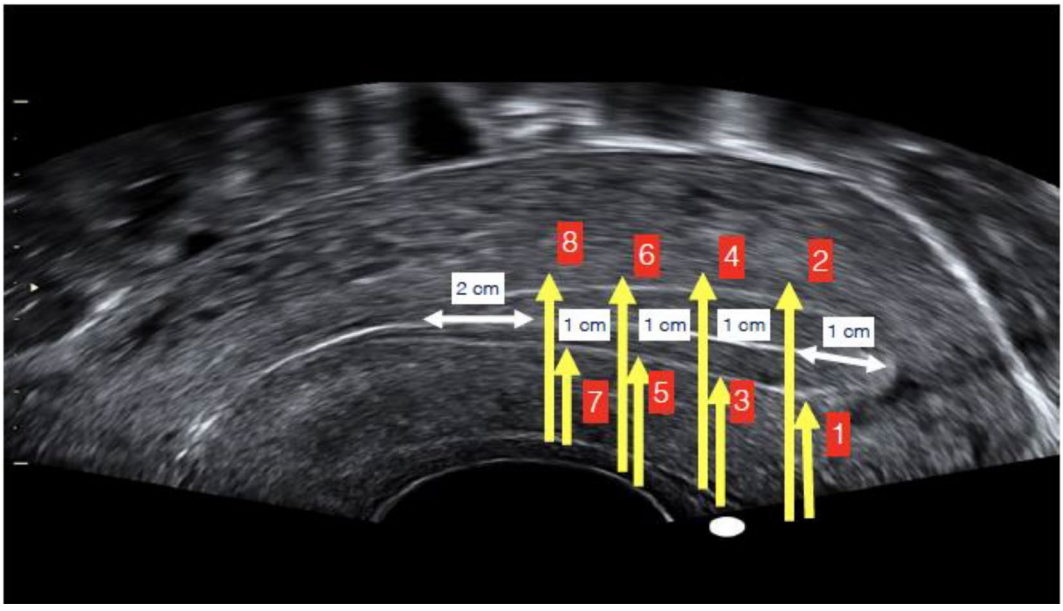

Fig. 1 Ultrasound image demonstrating the transvaginal subendometrial PRP injection technique

7.8 mg estradiol transdermal patch (Climara; Bayer) were added, with a follow-up assessment after four days. The cycle was canceled if the thickness remained below 7 mm with a non-trilaminar structure. When a thickness over 7 mm with trilaminar appearance was achieved, 8% vaginal progesterone (Crinone gel 8% BID; Merck) and 50 mg intramuscular progesterone (Progestan Kocakfarma) twice daily were added in a high dose for the prevention of probable miscarriage due to vaginal only use [20]. Frozen thawed blastocyst transfer was scheduled 116–120 h following progesterone initiation.

Blastocyst quality was assessed according to the criteria presented by Gardner and Schoolcraft [21]. The categorization was based on expansion of the blastocyst, grades of inner cell mass (ICM) and trophoectoderm (TE) cells. The ICM was assigned as grade "A" if composed of many cells that are all compacted; grade "B" if composed of fewer cells with less compaction; and grade "C" if composed of very few cells that may not show compaction at all. The TE was assigned as grade "A" if composed of many cells; grade "B" if composed of fewer cells; and grade "C" if composed of very few cells. With the combination of these parameters, blastocysts were categorized in four groups: excellent, (≥ 3AA); good, (3,4,5,6, AB and BA); average, (3,4,5,6 BB, AC and CA); and poor (≤ 3BB) [22]. The embryos that had undergone PGT-A were good quality blastocysts except from poor quality embryos. Excellent, good, or average quality embryos or PGT-tested euploid embryos were thawed and were planned for single frozen embryo transfer.

Pregnancy outcome was assessed 12 days after ET by assessing serum ß-HCG level. Clinical pregnancy was identified by the presence of a gestational sac or fetal pole on transvaginal ultrasound following a positive pregnancy test. Livebirth was defined as the delivery of a live infant after 28 weeks of gestation.

Statistical analysis

Data were processed using SPSS (SPSS-IBM 2.3, Inc., Chicago, IL, USA) and MedCalcsoftware version 18.11.6 (MedCalc Software, Broekstraat 52, 9030 Mariakerke, Belgium). Normality was assessed with the Shapiro-Wilk test. Paired Student's t-tests were applied for pre- and post-PRP comparisons, while independent Student's t-tests were used for between-group comparisons. Continuous variables are presented as mean ± standard deviation (SD), while categorical data are shown as frequencies and percentages. A p-value of less than 0.05 was considered statistically significant. The diagnosis of thin endometrium is still controversial in the literature, but it is defined as being less than 4 mm on the day of implantation [4]. At this stage, in patients with the diagnosis of thin endometrium implantation rates are low and in order to reach values of at least 7 mm and above where implantation can be achieved a total of 176 patients, at least 88 in each group, are needed with an alpha error of 0.05% and a power of 90%. For this reason, taking into account the patients who would be lost to follow-up during the study period, a study group of 200 patients in total was designed, with at least 100 patients in each group.

Results

The study included 200 women with an average age of 36.4 years (± 5.8), all with a history of suboptimal endometrial proliferation (≤ 7 mm) following hormone replacement therapy for FET (Fig. 2). The study population was divided into two groups, with 100 patients in the PRP group and 100 patients in the control group. There were no significant differences between groups in terms of mean age, body mass index (BMI), previous IVF attempts, or prior hysteroscopy procedures (Table 1). Additionally, there were no adverse effects (i.e. hemorrhage, infection…) reported with the PRP procedure.

Endometrial thickness significantly improved in the PRP group compared to the control group, with the PRP group achieving an average thickness of 7.7 mm (± 1.9) compared to 6.1 mm (± 1.2) in the control group ($p < 0.01$) (Table 2). Additionally, three women (3.0%) in the PRP group achieved spontaneous pregnancy, while no spontaneous pregnancies occurred in the control group. (Table 3). The rate of positive serum hCG test, indicating pregnancy, was higher in the PRP group than in controls (25.8% vs. 9.0%; $p = 0.002$), with a similarly higher clinical pregnancy rate (22.7% vs. 7.0%; $p = 0.002$). Furthermore, the live birth rate was substantially higher among those receiving PRP injections (17.5% vs. 2.0%; $p < 0.001$).

In the PRP group, 33 out of 97 women (34.0% of the total) could not proceed with embryo transfer due to persistently unresponsive thin endometrium or fluid in the endometrial cavity, compared to 75/100 (75.0% of the total) in controls.

In the PRP group, 19 of 97 patients underwent PGT-A. In the control group, this number was 8 out of 100 (Table 4). Subgroup analysis of PGT-A tested patients did not reveal a difference between the groups. The quality of transferred embryos were similar in between the PRP and control groups (excellent quality: 62.5% vs. 56.0%; good quality: 17.2% vs. 24.0%; and average quality: 20.3% vs. 20.0%, $p = 0.76$ respectively). Pregnancy and live rates were 47.4% (9/19) and 36.8% (7/19) of euploid embryo transfers for the PRP group, compared to 50.0% (4/8) and 12.5% (1/8) in controls ($p = 1.0$ and $p = 0.364$, respectively).

Discussion

The data in our study indicates that subendometrial autologous PRP injection appears to improve endometrial thickness and live birth rates in couples undergoing

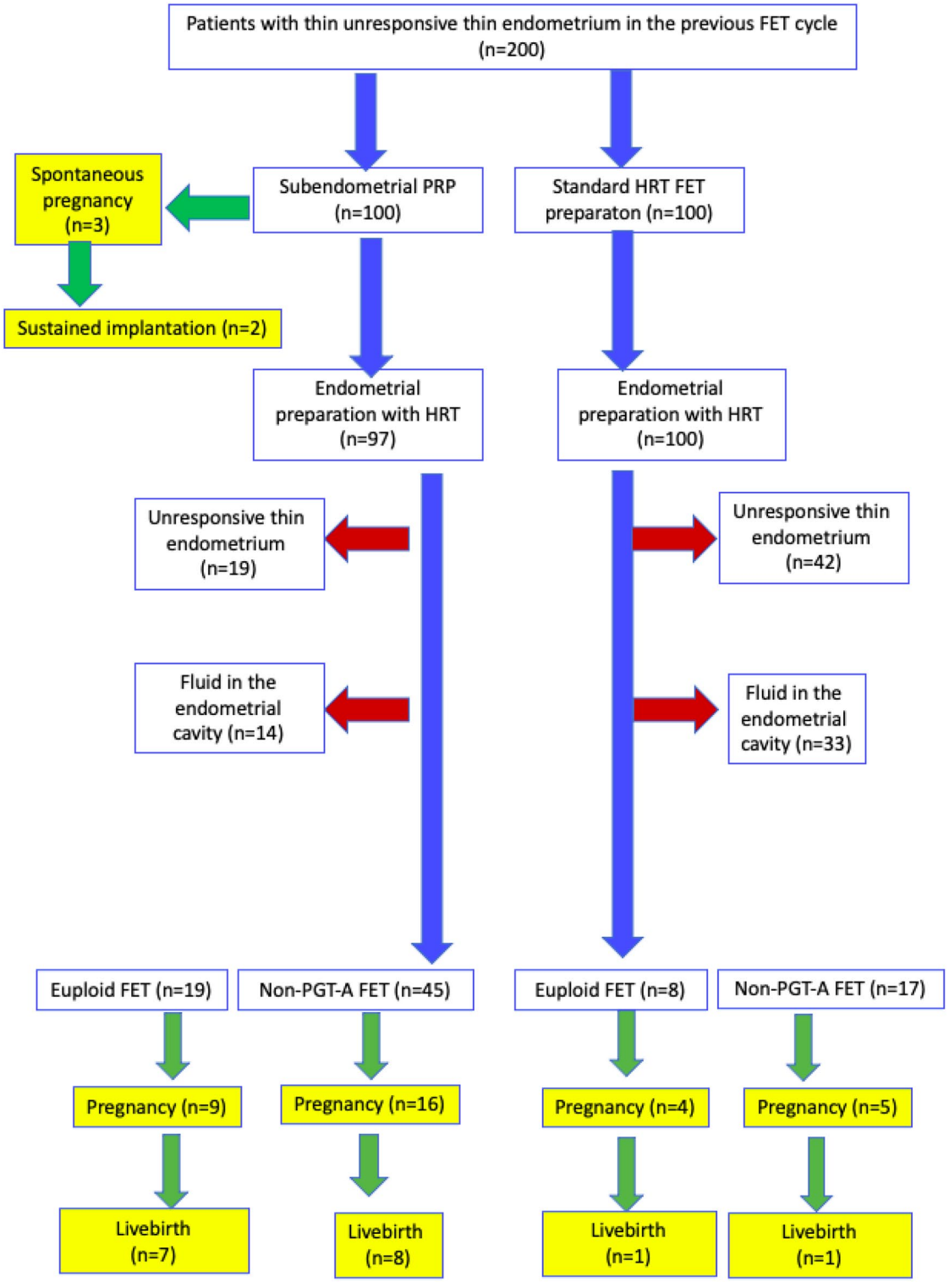

Fig. 2 Flowchart of the endometrial PRP protocol

Table 1 Comparison of variables between PRP and control groups

	PRP Group (*n* = 100)	Control Group (*n* = 100)	*p*
Age	36.9 ± 5.7	35.9 ± 5.7	0.25
BMI (kg/m^2)	26.7 ± 5.8	26.0 ± 4.9	0.35
Number of previous IVF trials	4.2 ± 3.0	3.9 ± 3.2	0.58
Number of previous hysteroscopy procedures	2.8 ± 1.6	2.3 ± 1.5	0.37

Table 2 Endometrial thickness before and after PRP injection

Outcome	PRP Group (*n* = 97)	Control Group (*n* = 100)	*p*
Endometrial thickness (mm) prior to PRP	5.3 ± 0.9	5.6 ± 1.2	0.04
Endometrial thickness (mm) after the PRP treatment	7.7 ± 1.9	6.1 ± 1.2	< 0.01

Table 3 Pregnancy outcomes in PRP and control groups

	PRP Group (*n* = 97) (%)	Control Group (*n* = 100) (%)	*p*
Spontaneous pregnancy (n)	3	0	0.08
Live birth/Spontaneous pregnancy (n)	2/3 (66.7%)	0	
Positive hCG test	25/97 (25.8%)	9/100 (9%)	0.002
Clinical pregnancy	22/97 (22.7%)	7/100 (7.0%)	0.002
Live birth	17/97 (17.5%)	2/100 (2.0%)	< 0.001
Cycle cancellation	33/97 (34.0%)	75/100 (75.0%)	< 0.001

Table 4 Pregnancy outcomes in PRP and control groups who underwent PGT-A tested euploid embryo transfer

	PRP Group (*n* = 19) (%)	Control Group (*n* = 8) (%)	*p*
Positive hCG test	9/19 (44.4%)	4/8 (50%)	0.901
Clinical pregnancy	8/19 (42.1%)	3/8 (37.5%)	0.824
Live birth	7/19 (36.8%)	1/8 (12.5%)	0.364

FET with a history of endometrial hypoproliferation. We hypothesized that since the endometrial cells start to grow from the basal layer, the administration of PRP through the cervix directly into the uterine cavity might not interact with the basal cells. Therefore, we injected the PRP into the subendometrial layer, which passed through the myometrium.

Mechanism of PRP in regards of endometrial proliferation has not been defined precisely in the literature. Some hypothetical pathways that might take a role in endometrial receptivity include chemokines and cytokines, growth factors, clotting factors and their inhibitors, adhesive proteins, integral membrane proteins, and immune mediators [23]. Wang et al. investigated the effect of PRP on endometrial mesenchymal stem cells (EnMSCs) from human menstrual blood on cell proliferation, migration, and adhesion by using CCK8, scratch, and adhesion tests [24]. They have reported that the stimulation of endometrial growth, migration, and adhesion of EnMSCs in a dose-dependent manner was more effective than that of the control group. Aghajanova et al. have investigated the effect of PRP on human endometrial stromal fibroblasts (eSF), endometrial mesenchymal stem cells (eMSC), bone marrow-derived stem cells (BM-MSC), and Ishikawa endometrial adenocarcinoma cells (IC) [25]. Their results have revealed increased stromal and mesenchymal cell proliferation and upregulation of transcripts for inflammation markers/ chemokines. Bos-Mikich et al. have pointed out the importance of final plasma products like cytokines and growth factors during the preparation of PRP [26]. They have proposed the positive effects of signaling molecules binding to specific receptors on epithelial cell surfaces, improving endometrial receptivity.

There have been a few case series with a small number of patients assessing the role of PRP in infertile women with endometrial hypoproliferation (Table 5). Intrauterine infusion of PRP has increased endometrial thickness in all ten patients with five pregnancies (50%) among patients with a history of inadequate endometrial growth [27]. Colombo et al. have reached adequate endometrial thickness in 7 out of 8 patients with thin endometrium after PRP infusion, resulting in 6/7 pregnancies [28]. In another case series study, Frantz et al. administered PRP every two days- in total 3 times- to 24 patients with thin endometrium persisting after 14 to 17 days of endometrial preparation of FET [29]. They have reported 54% ongoing pregnancy with 12.5% of miscarriage. Molina et al. have infused the PRP into the uterine cavity two times- on day 10 of HRT and 72 h later- in patients with a history of refractory endometrium [30]. In all cases, they have reported an endometrial thickness of > 9 mm with a 73.7% positive pregnancy rate. In a randomized controlled trial including 83 women with poor endometrial response, intrauterine PRP infusion resulted in a significantly increased endometrial thickness (from 6.09 ± 0.47 to 8.67 ± 0.64) in the PRP group compared to the control group (from 6.15 ± 0.37 to 8.04 ± 0.27) ($p = 0.001$) with a significantly higher clinical pregnancy rate per cycle in the PRP group (32.5% vs. 14.0%; $p = 0.044$) [31]. In another randomized controlled trial, Abduljabbar et al. reported improved endometrial thickness and clinical pregnancy rates in women who have received PRP after OPU compared to controls [32]. Chang et al. have investigated 64 women with thin endometrium and have reported significantly thicker endometrium compared to the control group (7.65 ± 0.22 mm vs. 6.52 ± 0.31 mm; $p < 0.05$) with a significantly higher clinical pregnancy rate in the PRP group compared to the control group (44.12% vs. 20%; $p < 0.05$) with the infusion of PRP into the endometrial cavity [33].

Table 5 Studies investigating endometrial PRP injection in patients with endometrial hypoproliferation

Study	Country	Study Design	Number of Cases	Population (incl/excl)	Intervention	Outcome
Intrauterine Instillation						
Case Series						
Chang et al., 2015 [14]	China	Case series	5	Age 31–39 History of ET < 7 mm on hCG day	PRP intrauterine infusion (0.5-1 cc) on the 10th day of HRT cycle. If endometrial thickness failed to increase 72 h later, PRP infusion was done 1–2 times in each cycle.	ET increased in all patients OPR 4/5 Miscarriage 1/5
Zadehmodarres et al., 2017 [27]	Iran	Case series	10	Age 30–37 History of ET < 7 mm	PRP intrauterine infusion (0.5 cc) on cycle days 11-12th and 13-14th	ET increased in all patients Five patients were pregnant and in four of them the pregnancy progressed normally. CPR (5/10) 50% OPR (4/10) 40%
Colombo et al., 2017 [28]	Italy	Case series	8	History of at least 3 cryo-transfers due to ET < 6 mm	PRP intrauterine infusion	ET increased for 7 patients with an endometrial thickness greater than 6.5 mm (mean 6.9 mm) Positive beta-HCG was positive in 6 women, the pregnancy was progressing normally in 2 women, and one had an early miscarriage.
Frantz et al., 2020 [29]	Brazil	Case series	21	Age 23–41 History of ET < 5 mm persisting after days 14–17 days of endometrial preparation	PRP infusion (0.5 cc every second day, for a total of three infusions) on cycle days 14–17 until ES > 5 mm	OPR or birth (54%) Miscarriage rate (12.5%)
Cohort Studies						
Kim et al., 2019 [15]	South Korea	Prospective cohort compared to prior cycle	24	Age 20–45 History of ET < 7 mm >=2 prior failed IVF cycles Frozen embryo available for ET	0.7–1.0mL of PRP infusion PRP on cycle day 10, repeated every 3 days until ES > 7 mm	ET increased (0.6 mm higher compared to prior cycle) IR increased CPR increased OPR/LBR 20%
Kusumi et al., 2020 [16]	Japan	Prospective cohort compared to prior cycle	36	Age 20–50 History of ET ≤ 7 mm	1 ml PRP infusion PRP on cycle days 10th and 12th	ET increased CPR 15.6% Safety
Russell et al., 2022 [17]	Canada	Retrospective cohort copared to prior cycle	85	Age 24–52 History of RIF and/or persistent thin endometrium Have cryopreserved PGT-A-tested euploid embryos	PRP infusion (0.5–0.75 cc, one or more times) on cycle days 10–15 until ES > 7 mm	ET increased CPR increased (37% vs. 20%) LBR increased (19% vs. 2%)
Molina et al., 2018 [30]	Venezuela	Prospective Cohort	19	Age 33–45 previous history of refractory endometrium at least one failed IVF attempt	PRP infusion (1 cc) intrauterine cavity on day 10 and day 12 of HRT	ET increased in all cases (> 9 mm) after the second administration. PPR 73.7% LBR 26.3% OPR 26.3% Biochemical 10.5% Anemrbyonic 5.3% Fetal death 5.3%

Table 5 (continued)

Study	Country	Study Design	Number of Cases	Population (incl/excl)	Intervention	Outcome
Chang et al., 2019 [33]	China	Prospective cohort study with control group	64	Age < 40 Basal serum FSH < 10IU/L; History of cancelled embryo transfer due to ET < 7 mm in hormone replacement therapy No obvious intrauterine adhesion, no submucosal uterine myoma or endometrial polyps; No history of hematological disorders (e.g., leukopenia, thrombasthenia et al.) At least 2 frozen good-quality blastocyst-stage embryos	Infusion of 0.5-1mL PRP PRP on cycle day 10, repeated at 3 day intervals until ES > 7 mm	ET increased Cancellation rate decreased IR increased CPR increased
Randomized Controlled Trials						
Eftekhar et al., 2018 [31]	Iran	RCT	83	History of ET < 7 mm in an FET cycle	Infusion of 0.5-1 cc of PRP PRP on cycle day 13 when ES < 7 mm in the study group and repeat after 48 h if ES still < 7 mm	ET increased IR increased (21% vs. 9.37%) CPR/cycle increased (32.5% vs. 14.0%) OPR/cycle increased (27.0% vs. 14.0%)
Abdul-jabbar et al., 2022 [32]	SAU	RCT	70	Age 18–44 Subjects undergoing IVF/ICSI-frozen embryo transfer (FET) History of repeated failures, endometrial thicknesses between 0.4 and 0.7 cm.	Infusion of 0.5 mL of PRP into the uterine cavity using an intrauterine insemination (IUI) catheter after the OPU.	ET increased more compared to control group (From 0.59 ± 0.089 to 0.86 ± 0.090 vs. from 0.58 ± 0.09 to 0.75 ± 0.07) Pregnancy more compared to control group (34.3% vs. 14.3%)
Hysteroscopic Injection						
Agarwal et al., 2020 [37]	India	Retrospective cross-sectional	32	Age 27–39 History of ET < 7 mm	A total of 4 mL of PRP was injected with an ovum pickup needle into the subendometrial region in all four walls of the cavity (1.0 mL in each wall) under hysteroscopic guidance 7–10 days after the injection of leuprolide	ET increased ≥7 mm in 24 (75%) PPR 12(50%) CPR 10 (41.6%) Biochemical 2 (8.33%) OPR 3(12.5%) LBR 5 (20.8%) Missed abortion 2(8.3%)
Yu et al., 2024 [38]	Taiwan	Prospective, case control study	116 ($n = 55$ intrauterine; $n = 38$ hysteroscopic; $n = 23$ control)	At least one unsuccessful EFET cycle, which included either cancellation of the EFET cycle due to thin EM or failure to achieve pregnancy after receiving euploid embryo transfer.	IU infusion: A 2 mL amount of autologous PRP was infused into the uterine cavity twice, with a 48 h interval between each infusion Hys infusion: With the guidance of hysteroscopy, 2 mL of autologous PRP into the endometrium at a depth of 2–3 mm in four directions including upper, lower, right, and left side of uterine cavity, 0.5 mL in each direction using a 19 GA single-lumen ovum aspiration needle with its beveled edge serving as a guide.	EM thickness exceeding 7 mm (IU vs. HYS) 78.2% vs. 55.3% Higher IR (HYS:52% vs. Control:18%) Higher CPR (HYS:52% vs. Control:22%) Higher LBR (HYS:38% vs. Control:4%)
Subendometrial Injection						

Table 5 (continued)

Study	Country	Study Design	Number of Cases	Population (incl/excl)	Intervention	Outcome
Noushin et al., 2021 [39]	India- UK	Prospective observational cohort study.	318 ($n=109$ intra-uterine; $n=55$ sub-endo-metrial; $n=154$ control)	<40 A history of recurrent implantation failure un-dergoing frozen embryo transfer (FET)	Subendometrial PRP: A volume of approximately 2 mL of PRP each injection into the subendometrial space in the ante- rior and posterior walls of the uterus. IU PRP: Approximately 1 mL of PRP infusion into the uterine cavity via ET catheter under transabdominal USG guidance.	Higher OPR/LBR in the SE-PRP and IU-PRP groups compared to control group (40% and 41.3% vs. 22.1%) Higher CPR in the SE-PRP and IU-PRP groups compared to control group (51% and 52.3% vs. 33.8%) Similar miscarriage rates (24.45% vs. 22.23% vs. 22.07%)

After these publications, a systematic review by Maleki-Hajiagha et al. commented that PRP is an alternative treatment strategy in patients with thin endometrium and pointed out the need for further prospective, large, and high-quality randomized controlled trials (RCTs) to identify the subpopulation that would most benefit from PRP [34]. Similarly, a narrative review by Sharara et al. reported that limited literature shows promise in increasing endometrial thickness; however, it warned that the lack of standardization of PRP preparation and randomized controlled trials need to be addressed in future studies [35]. The authors further emphasized that PRP use should be considered experimental until definitive large RCTs are available. Liu et al. analyzed a total of eight randomized controlled trials and reported superior outcomes in the PRP group in regard to endometrial thickness, clinical pregnancy, live birth, and cycle cancellation rates [36]. Their results haven't shown the same outcomes with spontaneous abortion and chemical pregnancy rates.

Different routes of PRP administration have been introduced in the literature. Intrauterine instillation has been the most common way of administration described in the literature. Other than the intrauterine route, hysteroscopic injection or subendometrial administration has also been reported. Agarwal et al. have performed hysteroscopic guided endomyometrial junction PRP injections in 32 patients with a history of embryo transfer cancellation due to a thin endometrium [37]. Their results have revealed an adequate endometrial thickness of >7 mm in 75% of the patients, resulting in a 50% positive pregnancy test. Yu et al. have investigated the effects of intrauterine infusion and hysteroscopic injection of autologous PRP in couples with persistent thin endometrium undergoing euploid embryo transfer [38]. They have reported an increase in endometrial thickness with both techniques but a more significant increase with the hysteroscopic injection in implantation rate, clinical pregnancy rate, and live birth rates. Noushin et al. have compared the effectiveness of subendometrial or intrauterine PRP administration with controls in recurrent implantation failure patients [39]. They have reported a higher ongoing pregnancy rate/ live birth rate in the subendometrial (22/55; 40%) and intrauterine PRP groups (41.3%) compared to the controls (22.1%). They have also compared the subendometrial group with the intrauterine group in terms of clinical pregnancy rate, reporting no statistical difference (51% vs. 52.3%). They have concluded an improvement in FET outcome with the administration of PRP without any difference in the administration method (subendometrial or intrauterine). Our study revealed improved endometrial thickness and higher live birth rates with subendometrial injection compared to the control group.

While this was a prospective cohort study, the non-randomized design of our study might be one of the limitations. Some but not all of the transferred frozen embryos were genetically tested, which might be commented as another limitation of the study. Repeated sub-endometrial PRP injections may induce endometrial injury that may alter subsequent endometrial measurement and potentially improve receptivity per se. A control group including women performed endometrial scraping or injection of saline could be necessary to demonstrate the efficacy of PRP but this was not possible from the ethical point of view which can be accounted as another limitation of the study. Another limitation might be the potential for observer-dependent discrepancies among patients. De Geyter et al. have analysed the reproducibility of the sonographic measurement of endometrial thickness on the same patient and on the same day by two different investigators [40]. They have reported a rate of outliers outside the double SD margins above or below the mean as 4.3%. The strength of our research is the instillation of PRP through a novel route passing by the myometrium and reaching the subendometrial region in patients with thin endometrium. This unique method had only been reported in a previous manuscript but in another group of patients- the recurrent implantation failure group [39]. Besides, the number of patients

recruited in each group in our study was higher than the published manuscripts. Also, only patients with a history of thin endometrium were administered PRP, resulting in a homologous group of patients.

Conclusions

Our results revealed that in women with a history of endometrial hypoproliferation, subendometrial PRP injection was associated with improved endometrial thickness and a cumulative (spontaneous and following FET) implantation rate of 18%. Based on the findings, subendometrial injection of autologous PRP might be considered in women with unresponsive thin endometrium. However, this intervention needs to be studied in prospective randomized clinical trials prior to wider clinical application.

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Author contributions
YC, BT designed the study; YC, YAT, HBZ, BT analyzed the data and wrote the manuscript; AY, OK, IOA, SYK, ZEUK, CY recruited patients. All authors read and approved the final manuscript.

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Data availability
Data can be maintained upon request from the corresponding author.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Baskent University's institutional review board and ethics committee (KA-20/23) and supported by Baskent University Research Fund. Also, the study registered at the http://www.Clinicaltrials.gov, (NCT04424160). The consent that was obtained from all of the participants was informed. Our study adhered to the Declaration of Helsinki to this effect.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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