



Efficacy of Platelet-Rich Plasma Combined with Wen Shen Formula in the Treatment of Thin Endometrium-Related Infertility

Xiaofang Liu^{1, #}, Xinyuan Dai², Xiaolan Li³, Xiaoqin Sun⁴

¹ Reproductive Endocrinology Department, Yinchuan Guolong Orthopedic Hospital, Yinchuan 750011, China

² School of Traditional Chinese Medicine, Xi'an Medical College, Xi'an 710000, China

³ Department of Ultrasound Diagnostics, Yinchuan Second People's Hospital, Yinchuan 750011, China

⁴ Department of Ultrasound Diagnostics, Yinchuan Guolong Orthopedic Hospital, Yinchuan 750011, China

Objective: To explore the effectiveness of Platelet-Rich Plasma (PRP) combined with Wen Shen Formula in treating thin endometrium.

Methods: From January 2019 to January 2023, 90 patients with thin endometrium-related infertility who showed poor response to standard Hormone Replacement Therapy (HRT) in our Reproductive Endocrinology Department were studied. Patients receiving PRP infusion + Wen Shen Formula enema (Group A, n = 45) were compared with those receiving only PRP infusion (Control Group B, n = 45) in terms of endometrial thickness, embryo implantation rate, clinical and ongoing pregnancy rates.

Results: Compared to the control group, Group A showed a significant increase in endometrial thickness after 13 days, with statistically significant differences between the groups ($P < 0.05$). There were also significant differences in biochemical pregnancy, miscarriage, and ongoing pregnancy rates ($P < 0.05$).

Conclusion: Platelet-rich plasma (PRP) combined with Wen Shen Formula enema effectively promotes the growth of thin endometrium in patients and improves pregnancy outcomes. It can be considered a safe and effective method for clinical treatment of patients with thin endometrium-related infertility.

Keywords: Platelet-rich plasma; Thin endometrium; Pregnancy outcome.

Introduction

The negative impact of thin endometrial lining on pregnancy outcomes in infertile women is gradually receiving attention in the field of reproductive medicine. More studies support a diagnostic threshold for thin endometrium thickness before ovulation or on the trigger day as 7 mm^[1]. There are many treatment methods to improve endometrial thickness before ovulation induction or transplantation. Currently, clinical endometrial proliferation regimens include increasing estrogen dosage, prolonging estrogen use^[2], combining with low-dose aspirin^[3], Sildenafil Citrate^[4], neuromuscular electrical stimulation^[5], and intrauterine infusion of granulocyte colony-stimulating factor (G-CSF)^[6]. However, some patients with persistent thin endometrium do not respond to these treatments and fail to reach the thickness required for implantation. The problem of thin endometrium has always troubled many clinicians. Platelet-rich plasma (PRP), which contains a high concentration of growth factors, is increasingly being studied to improve pregnancy outcomes in infertile patients. While more research is being conducted on PRP intrauterine infusion to

treat persistent thin endometrium, studies focusing solely on PRP infusion are limited, and the quality of evidence could be better. Therefore, this study combines modern disease understanding with traditional Chinese medicine to identify targeted treatment clues; it utilizes clinical knowledge and experience to find targeted medication clues and clinically validates specific symptom relief effects. This approach reconstructs targeted treatment in modern medicine and diagnosis in traditional Chinese medicine. Adding Wen Shen Formula enema on top of PRP intrauterine infusion, this study adopts a combined Chinese and Western medicine treatment approach for patients with thin endometrium-related infertility, researching endometrial thickness, embryo implantation rate, clinical and ongoing pregnancy rates, providing reference evidence for clinicians.

Materials and methods

Study subjects and inclusion criteria

90 patients treated in our hospital's Reproductive Endocrinology Department with thin endometrium-related infertility from January 2019 to January 2023 were selected. The thin endometrium had to meet the following criteria: in a natural ovulation cycle or in a cycle where ovulation was stimulated with gonadotropins, the endometrial thickness was < 7 mm when at least one follicle developed to a diameter ≥ 14 mm; or in anovulatory patients, the endometrial thickness remained < 7 mm after > 12 days of medication in an artificial cycle. Hysteroscopic examination in the past 6

Correspondence: Xiaofang Liu. E-mail: liuxiaofang516@163.com. Address: Reproductive Endocrinology Department, Yinchuan Guolong Orthopedic Hospital, Yinchuan 750011, China.

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months revealed no obvious abnormalities. The patients were divided into two groups of 90: Group A (45 patients): hormone replacement + PRP intrauterine infusion combined with Wen Shen Formula enema (Group A); Group B (45 patients): hormone replacement + PRP intrauterine infusion (Group B). Inclusion criteria included: ① Age 25-37 years; ② Endometrial thickness < 7 mm on the day of ovulation in natural or ovulation induction cycles; ③ Hysteroscopic examination showing normal uterine

morphology; ④ Normal ovarian function [Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Anti-Müllerian Hormone (AMH) all within normal range, normal thyroid function, blood sugar and blood lipids]; ⑤ Infertility factors mainly include pelvic tubal factors, ovulatory disorders, male factors, and no comorbid internal or external medical conditions. The differences in these conditions were not statistically significant ($P > 0.05$). See [Table 1](#).

Table 1. Comparison of Endometrial Thickness Before and After Intervention in Both Groups ($\bar{x} \pm s$).

Group/Day	10 Days Before Intervention	13 Day After Intervention
A group (n = 45)	5.21 ± 1.12	8.32 ± 1.50
B group (n = 45)	5.18 ± 1.05	4.11 ± 1.35
<i>t</i>	0.14	13.98
<i>P</i>	0.89	< 0.001

Note: The data of both patient groups were tested and confirmed to follow a normal distribution with homogeneity of variance. Independent sample t-tests and paired sample t-tests were used for group comparisons.

Exclusion criteria

The exclusion criteria are as follows: ① History of estrogen-related tumors; ② Hysteroscopic examination revealing abnormal uterine cavity morphology, intrauterine adhesions, endometrial polyps, submucosal uterine fibroids, etc.; ③ Endometrial lesions: abnormal endometrial hyperplasia, endometrial tuberculosis, endometritis, etc.; ④ Adenomyosis; ⑤ Bilateral tubal hydrosalpinx, etc.

Grouping and treatment methods

Among the 90 patients who responded poorly to standard hormone replacement therapy (HRT) (endometrial thickness <7 mm) during ovulation induction cycles, they were divided into Group A: PRP + Wen Shen Formula enema group (45 cases): PRP (45 cases). During the HRT cycle, medication (oral estradiol/estradiol dienogest tablets in red and yellow compound packaging, 17β-estradiol 2 mg per dose, once daily) + oral Wen Shen Formula enema (cinnamon twig 15 g, ground beetle 10 g, black medicine 15 g, turtle shell 30 g, bupleurum 15 g, prunella 30 g, spiny restharrow 9 g, licorice 9 g, moutan bark 15 g, pseudostellaria root 30 g, clematis root 9 g, peony root 15 g, red vine 20 g, astragalus 15 g, one dose per day, decocted twice totaling 200 ml, at 38 °C, administered as a retention enema for >30 min) for 14 days. On the 12th day of menstruation, if the endometrial thickness was <7 mm, 1 ml PRP was injected into the uterine cavity. If the endometrial thickness did not increase after 72 h, PRP infusion was repeated in the same cycle. When the endometrial thickness reached ≥ 7 mm, ovulation was induced, and ultrasound was used to check for ovulation. Stop the red pills; switch to oral yellow pills of estradiol dienogest tablets 2 mg/10 mg per dose per day, check blood HCG >6 miu/ml 14 days after ovulation, retest to double pregnancy 48 hours later, observed until 12 weeks of pregnancy; if blood HCG <6 miu/ml, continue the next treatment cycle. This was repeated for 3 consecutive cycles.

Group B, as Control (45 cases) only oral HRT medication during the cycle and did not undergo the Wen Shen Formula enema. On the 12th day of menstruation, if the endometrial thickness was <7 mm, PRP was infused into the uterine cavity using the same method.

Efficacy Criteria

Observation Indicators: The endometrial thickness, biochemical pregnancy, iscarriage, and ongoing pregnancy rates of the two groups of patients were observed after treatment. The assessments were conducted by checking blood HCG and B-ultrasound examinations, and the endometrial thickness, biochemical pregnancy, miscarriage, and ongoing pregnancy rates were statistically analyzed at the end of the study for both groups.

Statistical Methods

Statistical analysis was performed using SPSS 20.0 software, employing t-tests and paired sample t-tests. Due to insufficient sample size, Fisher's exact test and a non-parametric rank sum test were adopted for two independent samples. Scatter plots and correlation analyses were created using GraphPad Prism7 software. Significance difference analysis and SPSS multivariate ordinal regression analysis were performed with GraphPad.

Results

Comparison of Endometrial Thickness on Day 10 and After Day 13 of the Menstrual Cycle Between the Two Groups

Before day 10 of the menstrual cycle, there was no statistically significant difference between the groups ($P > 0.05$), indicating comparability. After day 13 of the menstrual cycle, there was a statistically significant difference between the groups ($P < 0.05$). See [Table 1](#) for details.

Comparison of Endometrial Thickness

On day 16 of the menstrual cycle, a second infusion of endometrial thickness was required (13 patients in Group A needed a second infusion; 21 patients in Group B). Multivariate ordinal regression analysis and scatter plot construction were used; there was a statistically significant difference between the groups, with Group A having a significantly higher endometrial thickness than Group B, see [Fig.1](#).

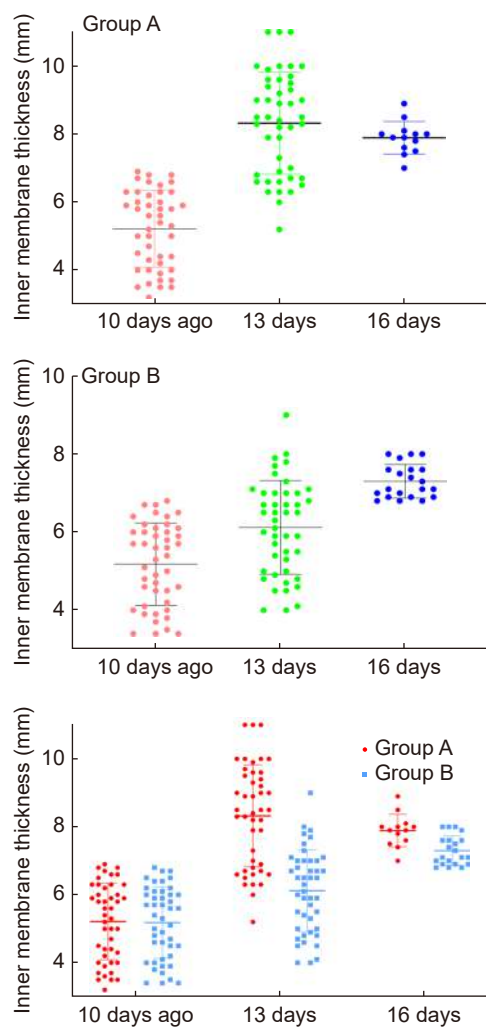


Fig. 1. Comparison of endometrial thickness after perfusion. Group A and Group B: Red spots: On day 10 of the menstrual cycle, endometrial thickness; Green spots: On day 13 of the menstrual cycle, the first infusion of endometrial thickness was required; Blue spots: On day 16 of the menstrual cycle, a second infusion of endometrial thickness was required Group C: Comparison of membrane thickness within the 10th day of menstruation in group A and Group B; Comparison of endometrial thickness after the first perfusion on the 13th day of menstruation; Comparison of endometrial the thickness after the second perfusion on the 16th day of menstruation.

Comparison of Pregnancy Outcomes After Treatment

After the intervention, the comparison of biochemical pregnancy, miscarriage, and ongoing pregnancy between the two groups showed statistically significant differences ($P < 0.05$), as shown in Table 2.

Table 2. Comparison of Pregnancy Conditions (%).

Group	Biochemical Pregnancy	Miscarriage	Ongoing Pregnancy	Ongoing Pregnancy Rate
Group A	3	9	30	66.66%
Group B	6	7	18	40%
χ^2	9.000	16.000		
Z			-6.856	-8.602
P	0.012	< 0.001	< 0.001*	< 0.001*

Note: For the comparison of pregnancy conditions between the two groups, Fisher's exact test was used due to the insufficient sample size for biochemical pregnancy and miscarriage. A non-parametric rank sum test was used for two independent samples. *: Pay close attention.

Discussion

Patients with thin endometrium infertility suffer partly due to the aging of endometrial epithelial cells, decreased proliferation, and some epithelial functional abnormalities that hinder the establishment of a receptive state, affecting embryo implantation^[7]. This study utilized intracavitary perfusion of platelet-rich plasma combined with traditional Chinese medicine (TCM) 'Warm Kidney' enema to repair damaged epithelial functions while protecting undamaged endometrium, re-establishing a receptive state, and enhancing pregnancy rates in patients with thin endometrium. From a clinical perspective, the study explored the 'Warm Kidney' formula in TCM, which includes ingredients like Cinnamomum cassia 15 g, Eupolyphaga 10 g, Lindera aggregata 15 g, turtle shell 30 g, Bupleurum chinense 15 g, Prunella vulgaris 30 g, Gleditsia sinensis 9 g, Glycyrrhiza uralensis 9 g, Salvia miltiorrhiza 15 g, Poria cocos 30 g, Caulis spatholobi 9 g, Paeonia lactiflora 15 g, Millettia reticulata 20 g, and Astragalus membranaceus 15 g. Turtle shell, for instance, contains keratin, bone collagen, vitamins, amino acids, polysaccharides, etc., and its pharmacological effects include enhancing immune function, natural killer cell activity, macrophage phagocytosis, preventing cell mutation, promoting hematopoiesis, increasing hemoglobin content, and providing antioxidant effects.

Prunella vulgaris contains organic acids such as rosmarinic acid, triterpenes like ursolic acid, and oleanolic acid, with pharmacological effects including reducing cell early death rates and anticoagulant actions, anti-inflammatory, and immunosuppressive effects. Poria cocos has inhibitory effects on Staphylococcus aureus, Streptococcus hemolyticus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexneri, Corynebacterium diphtheriae, and Bacillus anthracis; it inhibits immune cell responses and has antithrombotic effects. Caulis spatholobi enhances hematopoietic function (maintaining red blood cell stability, promoting bone marrow cell proliferation), inhibits platelet aggregation, and has anti-inflammatory, antioxidant, immune-regulating, antiviral, and antibacterial effects.

These TCM ingredients warm and nourish kidney yang, enhancing immune function. The ancient text "Ge Zhi Yu Lun·Conception Theory" states: "The union of the father's essence and the mother's blood occurs due to mutual attraction. The essence is endowed. The blood captures the essence to form the child, originating from Qian Yuan (Heavenly principle); the blood forms the womb, originating from Kun Yuan (Earthly principle)." This implies that the womb, like soil, is the ground for reproduction, and the fertility of this 'soil' is key to the successful implantation of an embryo^[8]. According to the TCM diagnostic and treatment sys-

tem for thin endometrial infertility, the principles are: depression; deficiency; damage. The treatment involves supporting the healthy, replenishing the deficient, securing the foundation to prevent relapse, acting proactively, treating the damage throughout, warming and nourishing kidney yang, and harmonizing body fluids, which can promote the expression of endometrial vascular endothelial factors and endometrial endothelin, thereby repairing abnormal endometrium and protecting the normal endometrium.

The function of endometrial epithelial cells in thin endometrial infertility is a crucial indicator of the receptive state^[9]. Protein translation modifications in epithelial cells regulate the receptive state^[10]; immune cells can directly promote the expression of epithelial cell adhesion molecules, or affect epithelial function by regulating stromal cell decidualization^[11]. The intersection of platelet-rich plasma perfusion and 'Warm Kidney' enema creates a targeted approach for refractory thin endometrial infertility patients. Choosing state-targeted combined medication can investigate the cause, cut off the origin of the disease, and prioritize unaffected areas, thereby enhancing therapeutic efficacy. This reconstructs a modern integrative Chinese and Western medicine diagnosis and treatment system.

In this study, 90 women with thin endometrium infertility received treatment. On the third day of the menstrual cycle, they orally took 2 mg of estradiol valerate per day. After the menstrual cycle, Group A underwent Warm Kidney enema treatment for 14 days. Ultrasonic examination during the natural cycle showed dominant follicles ≥ 14 mm or, on the 12th day of the HRT cycle, the endometrial thickness was still <7 mm. If 1 mL of platelet-rich plasma (PRP) intrauterine perfusion did not significantly change the endometrium within 72 hours, another 1 mL of PRP was infused. After monitoring ovulation, the estradiol valerate was stopped and replaced with oral dydrogesterone 2 mg/10 mg per day. On the 14th day post-ovulation, serum human chorionic gonadotropin (blood HCG) was measured. After 48 hours, a repeat test was done to confirm doubling and pregnancy, observed until 12 weeks of gestation. If blood HCG was <6 mIU/mL, the next treatment cycle continued; this was repeated for 3 consecutive cycles. Group B only received HRT cycle medication without Warm Kidney enema, monitoring endometrial thickness <7 mm on the 12th day of the menstrual cycle and then following the same PRP intrauterine perfusion method. The comparison of endometrial thickness before and after intervention, biochemical pregnancy, miscarriage, and ongoing pregnancy was made between the two groups. The combination of Warm Kidney enema treatment significantly improved outcomes, with a notable increase in endometrial thickness and pregnancy rates, and lower biochemical pregnancy and miscarriage rates. All subjects were patients with thin endometrial infertility, providing clinical evaluation information for the endometrial receptivity state. This study has certain limitations, such as a small sample size and a short observation period, which may lead to sampling bias.

Conclusion

Among the cases tracked in this case report, the state-targeted differentiation treatment and warm kidney enema supplements the TCM for the treatment of infertile women with thin endometri-

um, could short the treatment time for concentrated PRP intrauterine perfusion, effectively reduces the increase in epithelial cell aging, enhances regenerative capacity, re-establishes a receptive state, and improves the pregnancy rate in patients with thin endometrial infertility.

Abbreviations

AMH, Anti-Müllerian Hormone; FSH, Follicle Stimulating Hormone; G-CSF, granulocyte colony-stimulating factor; HRT, Hormone Replacement Therapy; LH, Luteinizing Hormone; PRP, Platelet-Rich Plasma; TCM, Traditional Chinese Medicine.

Ethical approval and consent to participate

The study protocol was approved by the Medical Ethics Committee of Yinchuan Guolong Hospital, with ethics number 2020-002, and informed consent was obtained from each patient.

Authors' contributions

XFL analyzed data, prepared figures and wrote the manuscript. XFL and XYD edited the manuscript. XFL and XYD, XLL, XQS were responsible for the concept and design of the study. XFL designed the experiments, wrote and edited the manuscript.

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