

Sub-endometrial PRP Injection Under Hysteroscopic Guidance-A boon with thin endometrium with Implantation Failure.

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Abstract

Background: A healthy uterine milieu is a must criterion for the successful embryo implantation in patients undergoing in vitro fertilization (IVF)/ intracytoplasmic insemination (ICSI). Endometrial thickness is considered as an important parameter for a good embryo transfer outcome. Despite recent advances and various modalities to improve endometrial thickness in the form of hormone replacement therapy (HRT) and intrauterine granulocyte colony stimulating factor (G-CSF) instillation, only minimal improvement is seen in endometrial thickness & subsequent implantation.

Objective: This study described an innovative technique in the form of single sub-endometrial PRP (platelet rich plasma) injection through hysteroscopy to improve the

endometrial thickness thereby an embryo transfer outcome in women with recurrent implantation failure (RIF) with thin endometrium in IVF cycles.

Material and Methods: Present observational study was conducted at Omega hospital, Nagpur, Maharashtra on fifteen women subjects who had failure of implantation might be due to thin endometrium. They were given a single dose of 1.5ml autologous platelet rich plasma (PRP) injection into the sub-endometrium under hysteroscopic guidance on day 3 of menses of present IVF cycle.

Results: In this study mean age of study subjects was 34.4 years (range 32-42 years). There was improvement in endometrial thickness and vascularity with a single sub-endometrial PRP injection. Out of fifteen women,

fourteen were β hCG positive i.e., chemical pregnancy rate was 93.33%. Eleven women have positive clinical pregnancy with appearance of cardiac activity at 5-6 weeks and now ongoing with uneventful pregnancy, clinical pregnancy rate was 73.33%.

Conclusion: Use of single sub-endometrial autologous PRP injection is safe and effective technique which helps in improving endometrial thickness, thereby increasing embryo transfer outcome and clinical pregnancy rate.

Keywords: PRP, Hysteroscopy, Infertility, thin endometrium, implantation failure

Introduction

A healthy uterine endometrium causes transformation of endometrial cells into decidual cells. It is required for implantation of high-quality embryo and rapid growth of placenta. It is called as a good receptive endometrium. (1)

In clinical practice of ART (assisted reproductive technique) either IVF or ICSI, a thin endometrium is a cause for cycle cancellation. It can cause unsuccessful embryo transfer. At present there are various treatment options emphasizing on improving the endometrial thickness & endometrial receptivity. These are use of exogenous Estrogen², use of low-dose aspirin³, vitamin E⁴, vaginal sildenafil citrate⁵ and instillation of granulocyte colony stimulation factor (G-CSF). (2)

In spite of combination treatment, endometrium is not responsive leading to recurrent implantation failure. (3,4) PRP is a group of soluble and diffusible polypeptide substances. It regulates the growth, differentiation and phenotype of numerous cell types. PRP is an autologous source of many factors and substances. For e.g., platelet-derived growth factor and transforming growth factor- β . These growth factors released after platelet degranulation provide the mechanisms required to produce the

necessary biological synthesis for the tissue regeneration process. (5)

PRP contains several growth factors and cytokines. It includes fibroblast growth factor (FGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VGEF), transforming growth factor (TGF), insulin-like growth factor I, II (IGF-I, II), connective tissue growth factor (CTGF) and interleukin 8 (IL-8). PRP has been investigated as a therapeutic approach for regeneration of endometrial cell. Thus, it improves thickness and receptivity. (6)

Endometrium contains receptors for all the factors that help in endometrium proliferation, decidualization and placental angiogenesis. These are required for embryo implantation & help us to explore the specific effect of PRP on human endometrium regeneration. (7)

Considering these regenerative properties, this study was designed to investigate the outcome of embryo transfer after PRP injection in sub-endometrium under hysteroscopic guidance with an aim to improve the endometrial thickness and embryo transfer outcome in IVF/ICSI patients with thin endometrium.

Material and Methods

This was a prospective observational study conducted at Omega Hospital Pvt Ltd, Nagpur, M.S., India from 1st January 2018 to 30th September 2018. Informed written consent of all the patients was taken before recruitment. Details of previous 6 ART cycles of 238 patients were noted and fifteen patients were selected.

Inclusion criteria

- Age 20–45 years at the time of enrollment
- Endometrial thickness (EMT) of <7 mm on the human chorionic gonadotropin (hCG) administration day
- Two or more failed IVF cycles

- Women with recurrent implantation failure or negative B-hcg who had thin endometrium.
- Women willing to participate in the study

Exclusion criteria

- Hematologic disorders, hemoglobin level of <9.0 g/dL or platelet count of <100,000/ μ L
- Auto-immune disease,
- Chromosomal abnormality in the patient or spouse
- Peripheral NK cell proportion of $\geq 12\%$
- Body mass index (BMI) of ≥ 30 kg/m²
- Uncontrolled endocrine or other medical conditions, such as prolactinemia or thyroid diseases.
- Women not willing to participate in the study

Written informed consent was taken of all the women who had undergone this PRP injection under hysteroscopic guidance. Patients were appointed for PRP injections under hysteroscopic guidance on day 3 of menses of present IVF cycle.

On the day of hysteroscopy, 15 ml of venous blood was drawn and sent to laboratory immediately before the procedure. Venous blood sample was collected in a syringe pre-filled with 5 ml of anticoagulant solution (ACD-A), and centrifuged at 1200 rpm for 12 min to separate the red blood cells. The plasma was centrifuged again at 3300 rpm for 7 min to obtain the PRP. The resulting pellet of platelets was mixed with 1 ml of supernatant, and then 1.5 ml of PRP was obtained. This PRP was injected into the sub-endometrium under hysteroscopic guidance into the anterior wall, posterior wall, and lateral walls with the help of Bet Tochi hysteroscope using a 17 Gauge Reproline needle. The patient was discharged on the same day. Also, women from frozen embryo transfer (FET) cycles were started with estrogen tablets minimum dose of 2mg thrice a day

to maximum of 4mg thrice a day from day 3 of stimulation.

The primary outcome was endometrial expansion and the secondary outcomes were chemical and clinical pregnancies, determined by positive serum β HCG, 2 weeks after embryo transfer and the presence of fetal heart beat in the transvaginal ultrasound 5 weeks after embryo transfer.

Table 1: Study proforma

Age
Primary or Secondary Infertility
Any disorder like controlled DM, HTN or hypothyroidism
No. of previous IVF cycles
No. of embryos transferred in previous cycles
No. of embryos transferred in present cycle after PRP
No. of chemical pregnancies
No. of clinical pregnancies
Successful pregnancies

Statistical analysis

The data obtained was cleaned, coded and further statistical analysis was done using STATA version 10.1 (2011) by STATA Corp, Texas (USA). Descriptive data was summarized using means, frequency and percentages. Appropriate statistical tests were applied like Pearson correlation coefficient, Spearman’s rank-order correlation coefficient (Rho), Kendall’s Tau, etc. Results In this study the age of the 243 study participants range

Results

Table 2: Age & Type of infertility

Age & Type of infertility	Mean age	Range
Age	36 years	30-42 years
	No. of	Percentage

	subjects n=15	(95% CI)
Primary Infertility	06	40% (16.3-67.7%)
Secondary infertility	09	60% (32.3 -83.7%)

In present study, out of total 15 subjects with recurrent implantation failure with thin endometrium, mean age of the subjects were 36 years (range 30-42 yrs.).

Out of fifteen subjects, 6 (40%) (95% CI 16.3- 67.7%) women had primary infertility and 9 (60%) (95% CI 32.3 -83.7%) women had secondary infertility. (Table 2)

Table 3: No. of embryos transferred

No. of embryos transferred	In previous cycle n=15	In present cycle after PRP n=15
Primary infertility	2-3	3
Secondary Infertility	2-3	3

In present study, out of total 15 subjects, number of embryos transferred in previous cycle was 2-3 while number of embryos transferred in present cycle after inj PRP were 3 in all patients. (Table 3)

Table 4: Appearance of Endometrium

Appearance of Endometrium	Endometrium on day 3	Endometrium on day 10
Endometrium appearance	Scanty & pale, avascular	7-9 mm, zone 2 & 3 vascularity

In present study, out of total 15 patients, on hysteroscopy, no structural uterine abnormalities were detected. There were no endometrial polyp or fibroids. The endometrial appearance was scanty, pale and avascular on hysteroscopy. On follow up of these patients on day 10 (HCG Day), there was an improvement in endometrial thickness and vascularity (zoning) on TVS and Doppler flow after injecting PRP. The endometrial thickness improved to more than 7mm up to 8-9mm with

zone 2 and zone 3 vascularity till day 10 (HCG Day), thus making it favorable for embryo transfer. (Table 4)

Table 5: Chemical & Clinical pregnancy rate

Pregnancy rate	No. of subjects of Primary infertility n=6	No. of subjects of Secondary infertility n=9
Chemical pregnancy in Primary infertility	6 (100%)	8 (88.8%)
Clinical pregnancy	4 (66.6%)	7 (77.7%)

In present study, out of total 15 subjects, fourteen were Bhcg positive i.e., chemical pregnancy rate was 93.33%. Out of nine secondary infertility cases, 8 were chemical pregnancy positive (88.88%) and 7 had successful clinical pregnancy (77.77%). Out of six primary infertility cases, all 6 were chemical pregnancy positive (100%) and 4 out of 6 had successful clinical pregnancy rate (66.66%).

Total eleven women had positive clinical pregnancy with appearance of cardiac activity at 5-6 weeks. All eleven women had uneventful pregnancy, with maximum age of gestation reached up to 38 weeks. Total clinical pregnancy rate in this study is 73.33%. (Table 5)

Discussion

In present study, out of total 15 subjects with recurrent implantation failure with thin endometrium, mean age of the subjects was 36 years (range 30-42 yrs.).

Out of fifteen subjects, 6 (40%) (95% CI 16.3- 67.7%) women had primary infertility and 9 (60%) (95% CI 32.3 -83.7%) women had secondary infertility. (Table 2)

Kim H et al found that average age of the patients was 38.4 years. The mean duration of infertility in the 20 women was 5.7 years. Mean number of dilatation and

evacuation performed was 1.3. (8) In present study, out of total 15 subjects, number of embryos transferred in previous cycle were 2-3 while number of embryos transferred in present cycle after inj PRP were 3 in all patients. (Table 3) Leila N et al found that participants had a history of failed previous ET attempts between 3-7. (9) In present study, out of total 15 patients, on hysteroscopy, no structural uterine abnormalities were detected. There were no endometrial polyps or fibroids. The endometrial appearance was scanty, pale and avascular on hysteroscopy. On follow up of these patients on day 10 (HCG Day), there was an improvement in endometrial thickness and vascularity (zoning) on TVS and Doppler flow after injecting PRP. The endometrial thickness improved to more than 7mm up to 8-9mm with zone 2 and zone 3 vascularity till day 10 (HCG Day), thus making it favorable for embryo transfer. (Table 4)

Zadeh Modarres S et al found that endometrial thickness increased at 48 h after the first PRP. It reached more than 7 mm after the second PRP in all patients. (10)

In present study, out of nine secondary infertility cases, 8 were chemical pregnancy positive (88.88%) and 7 had successful clinical pregnancy (77.77%). Out of six primary infertility cases, all 6 were chemical pregnancy positive (100%) and 4 out of 6 had successful clinical pregnancy rate (66.66%). Total eleven women had positive clinical pregnancy with appearance of cardiac activity at 5-6 weeks. All eleven women had uneventful pregnancy, with maximum age of gestation reached up to 38 weeks. Total clinical pregnancy rate in this study is 73.33%. (Table 5)

Zadeh Modarres S et al in their study on 10 patients with history of refractory endometrium found 5 patients with clinical pregnancy after two infusions of PRP into uterine cavity with pregnancy rate of 50%. (10)

Molina et al found that platelet-rich plasma as an adjuvant in the endometrial preparation of patients with refractory endometrium, in 19 patients with implantation failure, improves endometrium thickness after two infusions. 14 women had a clinical pregnancy with pregnancy rate of 73.6%. (11) Farimanie et al in 2017 reported a case of 45-year-old women with recurrent implantation failure since 2013-2016. She was given autologous PRP infusion. She was pregnant with successful delivery of a healthy male child weighing 2450 gm. (12) In present study, as the cases selected were known cases of thin endometrium with implantation failure in their previous ART (assisted reproductive technique) cycles, we did not wait for day 10 endometrial status in the current IVF cycle. Instead of instilling PRP on day 10 of cycle in thin endometrium through IUI catheter, that may damage the endometrium before embryo transfer, in our study. Day 3 hysteroscopic sub endometrial injection gave an adequate time and stimulus to the endometrium for proliferative regeneration, without disturbing endometrial milieu. With this technique, we found adequate endometrial growth in all the women with thin endometrium as assessed on TVS on day 10(HCG Day).

Other studies, which have been discussed before, have used PRP instillation through IUI catheter on day 10, have got comparable results with our study but, they required two PRP infusions in the uterine cavity.

Present study couples both invasive procedures of hysteroscopy and PRP instillation in a single setting and produces similar results. There were no complications in present study.

Conclusion

The use of the PRP injections and its regenerative effects on the endometrium microvasculature seems to benefit

the refractory endometrium, thus increasing endometrial receptivity and a consequent increase in implantation rates. Being an autologous sample, it is safe to the patient, easy to obtain and cost effective.

Limitations

- small sample size
- lack of control group

References

1. Yajie C, Jingjie Li, Yuqing C, Lina W. Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during in vitro fertilization *Int J Clin Exp Med* 2015;8:1286-1290
2. Gleicher N, Vidali A, Barad DH. Successful treatment of unresponsive thin endometrium. *FertilSteril* 2011; 95: 2123.e13-7.
3. Maryam E, Nasim T, Afsar AT. The thin endometrium in assisted reproductive technology: An ongoing challenge. *Middle East Fertility Society Journal* 2018; 23:1-7
4. Garcia-Velasco JA, Acevedo B, Alvarez C, Alvarez M, Bellver J, Fontes J, Landeras J, Manau D, Martinez F, Munoz E, Robles A, Rodriguez-Tabernero L. Strategies to manage refractory endometrium: state of the art in 2016. *Reprod Biomed Online*. 2016; 32:474-89.
5. Zwiep T, Humphrey R, Fortin D, Inculet RI, Malthaner RA. Autologous platelet rich plasma and concentrated platelet poor plasma are safe in patients requiring lobectomies but do not reduce the the duration of air leak: a randomized controlled trial. *Ann Surg Int*. 2016; 2:2–11.
6. Farimani M, Bahman Zadeh M, Azami N, Mohammad poor N. High pregnancy rates with administration of autologous platelet-rich plasma in ART patients with recurrent implantation failure. *Proceedings of 15th international congress of obstetrics and Gynecology*; 4-7 October; Tehran; 2016: 68. duration of air leak: a randomized controlled trial. *Ann Surg Int* 2016; 2: ASI-2-011.
7. Farimani M, Bahman Zadeh M, Pooro lajal J. A New Approach Using Autologous Platelet-Rich Plasma to Treat Infertility and To Improve Population Replacement Rate. *J Res Health Sci*. 2016; 16(3): 172-173.
8. Kim H., Shin EJ, Koo HS, Kwon H, Choi DH, Kim JH, Effect of Autologous Platelet-Rich Plasma Treatment on Refractory Thin Endometrium During the Frozen Embryo Transfer Cycle: A Pilot Study, *CLINICAL TRIAL,article,Front.Endocrinol.,14,February,2019* | <https://doi.org/10.3389/fendo.2019.00061>
9. Nazari L, Saleh pour S, Hosseini S, Zadeh Modarres S, Ajori L. Effects of autologous platelet-rich plasma on implantation and pregnancy in repeated implantation failure: A pilot study. *Int J Reprod Biomed*. 2016 Oct;14(10):625-628. PMID: 27921085; PMCID: PMC5124324.
10. Zadeh Modarres S, Saleh pour S, Saharkhiz N, Leila N, Treatment of thin endometrium with autologous platelet-rich plasma. *JBRA Assisted Reproduction* 2017;21(1):54-56
11. Molina A, Sánchez J, Sánchez W, Vielma V, Platelet-rich plasma as an adjuvant in the endometrial preparation of patients with refractory endometrium. *JBRA Assisted Reproduction* 2018;22(1):42-48
12. Faramani M, Poorolajal J, Rabiee S, Bahman Zadeh M, Successful pregnancy and live birth after intrauterine administration of autologous platelet-rich plasma in a woman with recurrent implantation failure: A case report. *Int J Reprod Bio Med* 2017 Vol. 15. No. 12. pp: 803-806.