

Effectivity of intrauterine infusion of autologous platelet rich plasma (PRP) in the treatment of thin endometrium in infertile female patients with inadequate endometrial growth during In vitro fertilization (IVF).

Abstract:

Background:

Endometrium is one of the key factors in implantation and pregnancy. Sufficiently great percentage of in vitro fertilization failure is because of the endometrial receptivity therefore, high- quality embryo and adequate receptive endometrial growth are important for successful implantation. Platelet- rich plasma (PRP) is a new model treatment to advance (improvement) the endometrial thickness in women with a thin endometrium. PRP can be used in various medical conditions including patients with refractory endometrium as a complementary therapy to conventional treatment

Objectives:

To determine the effectiveness of intrauterine infusion of autologous PRP in the treatment of thin endometrium during In vitro fertilization (IVF)

Methods: It is a prospective observational study. 50 patients, whose cycles will be cancelled due to inadequate endometrial growth (less than 7 mm) in the past FET cycles despite standard treatments, will be recruited into the study. Endometrial thickness will be measured at the thickest part in the longitudinal axis of the uterus by an expert gynecologist using sonography machine.

Expected Results:

Intrauterine infusion of PRP will prove effective in successful implantation in treatment of patients with thin endometrium. If proven, this protocol can be considered for endometrial preparation in assisted reproduction techniques.

Conclusion:

PRP will be a novel strategy for reproductive medicine with clinical issues, such as endometrium with less thickness (with poor response to conventional therapy), as well as to obtain positive clinical pregnancies and live births.

Keywords:

Platelet-rich plasma, thin endometrium, infertile female patients, In-vitro fertilization.

Introduction:

The percentage of couples suffering from infertility is about 13%, hence infertility became a global public health issue. Since the first in vitro fertilization (IVF) attempts in the mid- 1970s, researchers have found endometrium plays a vital role, in addition to the embryo itself, in achieving a pregnancy.¹ To provide a good coordination between mother and fetus implantation requires a good quality embryo.

Endometrium is one of the key factors in implantation and pregnancy. Sufficiently great percentage of in vitro fertilization failure is because of the endometrial receptivity therefore, high- quality embryo and adequate receptive endometrial growth are important for successful implantation.^{2,3} Increasing the endometrial thickness (ET) helps in improving the pregnancy rates. In numerous studies conducted for embryo transfer, minimum endometrium thickness was found to be 7mm.^{4,5,6}

Platelet- rich plasma has been recently put into operation in reproductive scenarios and is a new model treatment to advance (improvement) the endometrial thickness in women with a thin endometrium. PRP is a centrifuged human blood containing a soaring concentration of platelets. PRP is prepared from the patient's own blood as it has many growth factors and cytokines and many other cytokines that can reform endometrial and embryonic development.^{7,8,9,10}

PRP is easily obtained, low- priced, and rich in growth factors. Since PRP is an autologous preparation, it is non- toxic and non- allergenic. Therefore, it can be used in various medical conditions including patients with refractory endometrium as a complementary therapy to conventional treatment, with generally decent results.¹¹

The effect of platelet growth factors on the relationship between the uterine environment and embryo implantation has been studied, and the results are positive and promising. So the aim of our study is to evaluate the effectiveness of intrauterine infusion of PRP in the treatment of thin endometrium in IVF

Objectives:

1. To identify infertile female patients with inadequate endometrial growth (less than 7 mm) in the past frozen embryo transfer (FET) cycles despite standard treatments.
2. To observe intrauterine infusion of autologous platelet-rich plasma (PRP) in these infertile female patients with thin endometrium.
3. To determine the effectiveness of intrauterine infusion of autologous PRP in the treatment of thin endometrium during In vitro fertilization (IVF).

Methods:

Study design: It is a prospective observational study.

Study setting: It will be carried out in the department of Obstetrics and Gynecology, JNMC and Wardha Test Tube BabyCenter AVBR Hospital, Sawangi, Wardha.

Duration of the study: 2021 to 2022

Study population: 50 patients, whose cycles will be cancelled due to inadequate endometrial growth (less than 7 mm) in the past FET cycles despite standard treatments, will be recruited into the study. Endometrial thickness will be measured at the thickest part in the longitudinal axis of the uterus by an expert gynecologist using sonography machine. From each participant written informed consent will be taken for participation in study.

Sample Allocation: Convenient selection of patients

Autologous PRP preparation:

The preparation of autologous PRP is an outpatient procedure which involves a blood draw from patient herself, preparation of the autologous PRP, and the injection of autologous PRP into the uterine cavity by expert Gynecologist. The method used for preparing autologous PRP is as follows:^{5,6}

{1} sterile tubes having anticoagulant will be taken, for venous blood (15–50 mL) which will be drawn from the patient's arm.

{2} during processing the required temperature will be 21°C–24°C to prevent platelet activation during centrifugation of the blood;

- {3} centrifugation of blood will be done at 1,200 rpm for 12 minutes;
- {4} after centrifugation there will be three layers of blood-
 - i) an upper layer- will contain platelets and white blood cells,
 - ii) an intermediate thin layer (the buffy coat)- this layer will be rich in white blood cells,
 - iii) bottom layer- will contain red blood cells;
- {5} the upper and intermediate buffy layers will be taken out and poured into an empty sterile tube. The plasma will be centrifuged again at 2200 rpm for 10 minutes to help with the formation of soft pellets (erythrocytes and platelets) at the bottom of the tube;
- {6} the upper two-thirds of the plasma will be discarded because it will be platelet-poor plasma;
- {7} in the lower third, pellet will be homogenized (5 mL) of the plasma to create the PRP;
- {8} the PRP will be now ready to inject. Around 30 mL of venous blood can yield 3–5 mL of PRP;
- {9} the affected area will be disinfected before the PRP injection;
- {10} Procedure will be discussed and patient will be assured to make the injection easier and less painful.
- (11) 0.5 ml of PRP will be infused into the uterine cavity with catheter by expert Gynecologist on day 11- 12 of menstrual cycle.^{13,15}
- {11} As the PRP stimulates a series of biological responses, there will be little swelling on injected site and painful for next 3 days.

Then, Hormone replacement therapy (HRT) will be performed for endometrial preparation in all participants: estradiol valera 6 mg/d will be start on the 2nd or 3rd day of the menstrual cycle and it will be increased to 8 mg/d on day 9- 10 due to inadequate endometrial growth (<7 mm).

During the cycle, whenever the endometrial thickness will become more than 7 mm, suppository progesterone 400 mg twice-a-day should be start and embryo transfer (ET) will be carried out per embryonic stage. β hCG value will be measured after day 14 of embryo transfer. Estradiol valerate and progesterone supplementation will be continued for 2 weeks after ET and once the serum β HCG will be positive, hormone supplementations will be continued until 12 weeks of gestation.

The primary outcome will be assessed by endometrial expansion as observed by expert gynecologist and the secondary outcomes will be chemical and clinical pregnancies, determined by positive serum β HCG, 2 weeks after ET and the presence of fetal heart beat in the transvaginal ultrasound 5 weeks after ET.

Effectivity of intrauterine infusion of autologous PRP in the treatment of thin endometrium during In vitro fertilization (IVF) will be determined based on primary and secondary outcome of this study.

Statistical Methods: Student's paired and unpaired t test, one way ANOVA, Pearson's correlation coefficient.

Software: SPSS 27.0 version.

Expected Outcome:

Intrauterine infusion of PRP will prove effective in successful implantation in treatment of patients with thin endometrium. If proven, this protocol can be considered for endometrial preparation in assisted reproduction techniques.

Discussion:

According to Samy A, Abbas AM, Elmoursi A, Elsayed M, Hussein RS¹² the local administration of PRP is notably effective in increasing pregnancy rates of infertile women with refractory endometrium with ET < 6 mm, as PRP contains growth factors and other cytokines that have positive effects on local tissue repair and endometrial receptivity; it helps to increase endometrial receptivity and vascularity.

Farimani M, Poorolajal J, Rabiee S, Bahmanzadeh M¹³ found that the local administration of PRP shows effectiveness in successful implantation. Further studies will be needed in infertility treatment to justify the benefits and clinical application of PRP.

According to Colombo GV, Fanton V, Sosa D, Lotti J, Aragona SE, Lotti T¹⁴ due to the inefficient expression of adhesion molecules, multiple implantation failures were caused, after PRP application which can hypothetically be more represented.

According to Zadehmodarres S, Salehpour S, Saharkhiz N, Nazari L¹⁵ PRP was effective for endometrial growth in patients with thin endometrium and had a history of cycle cancellation due to thin endometrium. PRP is a safe procedure, with minimal risks of transmission of infectious disease and immunological reactions since it is made from autologous blood samples.

Eftekhar M, Neghab N, Naghshineh E, Khani P¹⁶ stated that endometrial thickness increased significantly to $8.67 \pm$ in PRP group than in controls.

So, several studies conducted in past have shown that PRP may be effective in improving the endometrial growth and possibly pregnancy outcomes in women with a thin endometrium.

Conclusion:

PRP will be a novel strategy for reproductive medicine with clinical issues, such as endometrium with less thickness (with poor response to conventional therapy), as well as to obtain positive clinical pregnancies and live births.

PRP is an innovative therapeutic modality which is simple, financially affordable, easy to perform, and effective. It is also a non-invasive modality with encouraging results and no side effects.

Future Scope and Implications:

This study will evaluate the effectiveness of intrauterine infusion of PRP in successful implantation in treatment of patients with thin endometrium. If proven, this protocol can be considered for endometrial preparation in assisted reproduction techniques

References:

1. Bos-Mikich A, de Oliveira R, Frantz N. Platelet-rich plasma therapy and reproductive medicine. *Journal of assisted reproduction and genetics*. 2018 May;35(5):753-6.
2. Zeyneloglu HB, Onalan G. Remedies for recurrent implantation failure. In *Seminars in reproductive medicine* 2014 Jul (Vol. 32, No. 04, pp. 297-305). Thieme Medical Publishers.

3. Chang Y, Li J, Chen Y, Wei L, Yang X, Shi Y, Liang X. Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during in vitro fertilization. *International journal of clinical and experimental medicine*. 2015;8(1):1286.
4. Zadehmodarres S, Salehpour S, Saharkhiz N, Nazari L. Treatment of thin endometrium with autologous platelet-rich plasma: a pilot study. *JBRA assisted reproduction*. 2017 Jan;21(1):54.
5. El-Toukhy T, Coomarasamy A, Khairy M, Sunkara K, Seed P, Khalaf Y, Braude P. The relationship between endometrial thickness and outcome of medicated frozen embryo replacement cycles. *Fertility and sterility*. 2008 Apr 1;89(4):832-9.
6. Richter KS, Bugge KR, Bromer JG, Levy MJ. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos. *Fertility and sterility*. 2007 Jan 1;87(1):53-9.
7. Richter KS, Bugge KR, Bromer JG, Levy MJ. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos. *Fertility and sterility*. 2007 Jan 1;87(1):53-9.
8. Zwiép 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 duration of air leak: a randomized controlled trial. *Ann Surg Int*. 2016;2:2-011.
9. Chang Y, Li J, Chen Y, Wei L, Yang X, Shi Y, Liang X. Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during in vitro fertilization. *International journal of clinical and experimental medicine*. 2015;8(1):1286.
10. Wasterlain AS, Braun HJ, Dragoo JL. Contents and formulations of platelet rich plasma. In *Platelet Rich Plasma in Musculoskeletal Practice 2016* (pp. 1-29). Springer, London.
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 Jan;22(1):42.

12. Samy A, Abbas AM, Elmoursi A, Elsayed M, Hussein RS. Effect of autologous platelet-rich plasma transfusion in the treatment of infertile women with thin endometrium and its implications in IVF cycles: a literature review. *Middle East Fertility Society Journal*. 2020 Dec 1;25(1):5.
13. Farimani M, Poorolajal J, Rabiee S, Bahmanzadeh 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 Biomed*. 2017;15(12):803-806.
14. Colombo GV, Fanton V, Sosa D, Lotti J, Aragona SE, Lotti T. Use of platelet rich plasma in human infertility. *Journal of biological regulators and homeostatic agents*. 2017 Apr 1;31(2 Suppl. 2):179-82.
15. Zadehmodarres S, Salehpour S, Saharkhiz N, Nazari L. Treatment of thin endometrium with autologous platelet-rich plasma: a pilot study. *JBRA assisted reproduction*. 2017 Jan;21(1):54.
16. Eftekhar M, Neghab N, Naghshineh E, Khani P. Can autologous platelet rich plasma expand endometrial thickness and improve pregnancy rate during frozen-thawed embryo transfer cycle? A randomized clinical trial. *Taiwanese Journal of Obstetrics and Gynecology*. 2018 Dec 1;57(6):810-3.