

## CURRENT CLINICAL APPLICATION OF PLATELET- RICH PLASMA (PRP) IN REPRODUCTIVE MEDICINE & A PILOT STUDY OF INSTILLATION OF AUTOLOGOUS PRP FOR SUBOPTIMAL ENDOMETRIUM IN FROZEN EMBRYO TRANSFER (FET) CYCLES .

**Obstetrics & Gynaecology**

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### ABSTRACT

The purpose of this paper is to review the current clinical uses of platelet- rich plasma (PRP) in the field of Reproductive Medicine .All relevant articles published from January 2000 to December 2019 were reviewed and analysed.The article on PRP in field of Reproductive Medicine were mainly case series, pilot studies , or case reports .PRP is currently considered a new therapeutic modality for some disorders that are refractory to conventional drugs .

The pilot study was to evaluate the effectiveness of intrauterine infusion of Platelet Rich Plasma (PRP) in infertile women undergoing frozen embryo transfer cycles with suboptimal endometrium. Material & methods- Instillation of autologous PRP was done in 20 women between 24 & 38 years over 10 months ,with suboptimal endometrial growth , patients with repeated cycle cancellations in addition to Estradiol valerate . Frozen embryo transfer was performed when the endometrium reached an optimal pattern in term of thickness, appearance & vascularity. Results- The mean pre- PRP endometrial thickness(ET) was 5mm which significantly increased to 7mm post-PRP. There was a significant increase in vascularity ,seen by the number of vascular signals seen on Power Doppler , reaching the zones 3 & 4 of endometrium .The positive beta Human Chorionic Gonadotropin (hCG) rate was 70.58% & the clinical pregnancy rate was 58.82%.A total of 2 women are in the second trimester , 8 are in the first trimester with a healthy intrauterine pregnancy , one patient had an ectopic gestation, and one had a biochemical pregnancy .

**Conclusion:** This study suggest that the use of autologous PRP holds promise in treatment of women with suboptimal ET and vascularity in embryo transfer. It would help to reduce the incidence of cycle cancellations and thus even help reduce the financial & psychological burden of repeated cancelled cycles .

### KEYWORDS

platelet rich plasma, reproductive medicine, thin endometrium

#### INTRODUCTION

Platelet-rich plasma (PRP) is becoming more popular as a nonoperative treatment option for a broad spectrum of medical disorders .PRP is widely used in orthopaedic and sports medicine to relieve pain through the natural promotion of healing in musculoskeletal disease such as tendonitis ,arthritis ,ligament sprains & tears .In particular, PRP injections have been used for athletic injuries ,resulting in exceptional healing, a rapid return to regular activities & complete pain relief.<sup>[1]</sup>

Autologous PRP is derived from a individuals whole blood then centrifuged to remove red blood cells . The remaining plasma has a 5-10 fold higher concentration of growth factors than whole blood .these growth factors have been found to promote natural healing responses by researches across multiple specialities ,such as dentistry ,dermatology,urology,& gynaecology especially in Reproductive Medicine.<sup>[2,3]</sup>

The theory underlying this treatment modalities was derived from natural healing process ,as the body first response to tissue injury is to deliver platelets to injured area .Platelets promote healing and attract stem cells to the site of injury..The objective of this paper was to review studies addressing the use of PRP in the field of Reproductive medicine and to evaluate the effects of PRP on suboptimal endometrium in FET cycle.<sup>[4]</sup>

#### PRP in reproductive medicine

##### 1)PRP in premature ovarian failure

Premature ovarian failure (POF) refers to loss of normal function of the ovaries before age 40,accompanied by the loss of fertility, A team of researchers at Harvard University Injected murine ovaries with growth factors ,and mature eggs appears to develop from ovarian stem cells .They stated that the introduction of isolated growth factor bearing platelets directly into the ovaries might trigger a resurgence in oocyte production<sup>[5]</sup>.

PRP therapy is investigated in women with POF, Infertile women more than 35 years of age, and women with low ovarian reserve. Treatment with PRP is referred to as ovarian rejuvenation. In this procedure, PRP is injected into ovary under ultrasound guidance similarly to ovum retrieval in vitro fertilization (IVF). This modality of treatment is still being investigated in trials . Pantos<sup>[6]</sup>, at the annual European Society of Human reproduction & Embryology conference held in 2016 in Helsinki, Finland, introduced this modality (ovarian rejuvenation).They injected PRP in eight peimenopausal/POF women with poor ovarian reserve. They found successful ovarian rejuvenation 1-3 months after PRP treatment . All cases underwent

natural IVF cycles with follicles of (15.20 + - 2.05) mm in diameter , the resulting oocyte were inseminated by intracytoplasmic sperm injection (ICSI) and resulting embryo were cryopreserved.

##### 2)PRP in ovarian torsion

In animal model , 60 adult female rats were subjected ti ischemia and bilateral adnexal torsion for 3 hours .Intraperitoneal PRP was administered 30 mins prior to ischemia in one group , while the other group was not injected with PRP . Detorsion was then done and oxidative stress levels , histopathological changes and reperfusion injuries were lower in the PRP group than in the other group . The researchers concluded that PRP was effective for the prevention of ischemia and reperfusion damage in rat ovary<sup>[7]</sup>.

##### 3)PRP in refractory endometrium

The endometrium plays an important role in achieving optimal outcomes of assisted reproductive technologies . Endometrial growth following inadequate ovarian stimulation may be insufficient ,leading to poor result of IVF/ICSI cycles.Various strategies been suggested to to improve endometrial thickness, especially the resistant cases .PRP is a novel therapy that has been tried in such patients<sup>[8]</sup>.

Colombo et al<sup>[9]</sup> included eight women underwent PRP treatment . The inclusion criteria were women with more than three cancelled cryo-transfers due to poor endometrial growth (≤6mm),women with a negative hysteroscopic screening for endometrial pathology and women with a negative bacteriologic screening .After application of PRP , the endometrial thickness was satisfactory in seven cases. A positive test for Beta –human chionic gonadotropin was found in six women . They concluded that the multiple implantation failure were caused by inefficient expression of adhesion molecules , which could potentially be improved by PRP application .

Similarly , Zadehmoddares et al<sup>[10]</sup>, in their pilot study, included 10 patients who had a history of cancelled cycles due to inadequate endometrial growth (≤7 mm).They found that endometrial thickness increased at 48 hours after the first PRP application and reached more than 7 mm after the second application in all patients . Embryo transferred was then carried out in all patients .5 patients became pregnant (50%), and in four of them the pregnancy progressed normally. They concluded that PRP was effective for endometrium growth in patients with a thin endometrium .

Jang et al<sup>[11]</sup> , in an animal model , investigated the role of PRP in the regeneration of endometrium after ethanol – induced damage . they found that intrauterine administration of autologous PRP stimulated and accelerated regeneration of endometrium , as well as decreasing fibrosis, in a murine model of endometrial damage .

#### 4)PRP in Repeated implantation failure

Repeated implantation failure (RIF) is described as a failure to conceive following several embryo transfers in IVF cycles. Numerous factors are involved in implantation process, including embryo quality, endometrial receptivity, and immunological factors<sup>[12]</sup>.

Several methods have been suggested for RIF management, but little consensus exists on which is most effective. These methods include blastocyst transfer, assisted hatching, hysteroscopy, endometrial scratching, and immune therapy. Recently intrauterine infusion of PRP has been as a way to promote endometrial growth and receptivity<sup>[8,13]</sup>.

Nazri et al<sup>[14]</sup> enrolled 20 participants with a history of RIF to evaluate the effectiveness of PRP in improving the pregnancy rate in RIF patients. The inclusion criteria were being younger than 40 years and having a BMI below 30 kg/m<sup>2</sup>. They reported that 18 of the 20 participants (90%) became pregnant. Sixteen clinical pregnancies were recorded and their pregnancy were ongoing at the time of the study. They concluded that PRP was effective in improving pregnancy outcomes in RIF patients.

#### STUDY

Implantation is a complex process involving multitude of factors and requires a healthy dialogue between the embryo and endometrium. Defining an optimal endometrium prior to embryo transfer has often been a challenge faced by Assisted Reproductive Technology (ART) practitioners, and achieving the parameters apt for ensuring implantation is a task that is wrought with controversies and is poorly defined. For an inadequate endometrial growth, diverse therapeutic approaches have been proposed and tested.

The measurement of endometrial volume has been proposed as a predictor of implantation in the recent years. However, in practice, most clinicians empirically prefer endometrial thickness (ET) >7 mm. The available evidence does not support any specific thickness, and pregnancies with similar successes have been described in endometria from 5-15 mm. Similar findings were revealed in a meta-analysis by Kasius et al published in 2014<sup>[17]</sup>

ET is measured by Transvaginal Sonography (TVS) as the maximum distance between the echogenic interfaces of the myometrium and the endometrium in the plane through central longitudinal axis of the uterine body. Several reports state that more than the thickness or pattern of the endometrium, the vascularity plays an important role in predicting implantation.<sup>[18,19,28,29]</sup>

Studies have stated that absence of blood flow in the endometrial and subendometrial zones is associated with failure of implantation. The pregnancy rates were doubled when the blood flow reached the zones 3 and 4 of Applebaum's grading, as compared to zones 1 and 2.<sup>[28]</sup>

In patients who are unable to achieve an optimal endometrial lining, conventionally many therapies have been tried, such as giving higher doses or extended use of Estradiol valerate (Valest 2mgs; Walter Bushnell, Mumbai, India), adding low dose aspirin, use of Sildenafil (Sildenafil 25 mgs; Emcure Pharmaceuticals, Mumbai, India), Human Chorionic Gonadotropin (hCG), intrauterine Granulocyte-colony stimulating factor (G-CSF) instillation, as well as certain nonconventional therapies such as electroacupuncture, but they lack consistency in delivering results.<sup>[20,27]</sup>

There is a need to evaluate other modalities in this regard, since a suboptimal endometrial growth or vascularity might lead to repeated cycle cancellations or recurrent implantation failure, thus causing not just a psychological but also financial impact on the patient. This drives the patients toward surrogacy as an option, which, considering the medicolegal implications, might not be a viable option now.

The primary objective of the study was to evaluate the role of intrauterine infusion of autologous platelet rich plasma (PRP) on the ET and vascularity of women undergoing frozen embryo transfer cycles with suboptimal endometrial pattern as assessed by transvaginal ultrasound and Power Doppler.

Secondary objectives were to study the implantation rate, clinical pregnancy rate, and outcome of pregnancy, studied up to the second trimester.

We included 20 women between 24 and 38 years of age undergoing frozen embryo transfer cycles over a period of 8 months from January to October 2019, with a suboptimal endometrial pattern, as identified by ET < 7 mm despite standard dose of Estradiol valerate (up to 16 mg/day), or suboptimal endometrial vascularity, defined as <5 vascular signals reaching the central zone (zones 3 and 4 as per Applebaum grading) of the endometrium, as measured by Power Doppler by the same observer. Women with more than two cancelled cycles or recurrent implantation failure due to poor endometrial lining were also included in the study.

Women with any other known cause of implantation failure, such as poor embryo quality, Asherman syndrome, or congenital uterine anomalies, were excluded.

The women were started on Estradiol valerate from day 1 of their menses in a dose of 6 to 8 mg/day, which was gradually increased up to 12 mg/day in divided doses as needed. Serial transvaginal ultrasound examinations were done using transvaginal probe of 5 to 9 MHz, on Samsung R5, starting from day 7/8, and repeated as required.

#### RESULTS

The age group of women included in the study was 24 to 38 years. A total of 20 women were included in the study, of which, frozen embryo transfer was performed in 17. Cycles were cancelled in three women—two due to suboptimal endometrial lining, one due to bleeding 2 days prior to embryo transfer, and one due to viral fever a day before the embryo transfer.

A total of 15 women required a single sitting, whereas 5 women required two sittings of intrauterine PRP infusion. The mean pre-PRP ET was 5 mm and the post-PRP ET was 7 mm (P < 0.00001 at 95% confidence interval) [Figure 1].

8 patients displaying sparse to modest vascularity pre-PRP instillation had an excellent vascularity pattern [Figure 2], whereas, in 10 patients, the vascularity pattern improved to modest from sparse. Only 2 patients persisted to have a sparse vascularity pattern [Figure 3].

Positive beta hCG values were observed in 12 patients (70.58%) after frozen embryo transfer and the clinical pregnancy rate, as defined by the observation of a gestational sac with fetal cardiac activity at 6 weeks gestation by transvaginal ultrasound, was 58.82% [Figure 4].

Among the 12 women with a positive beta hCG value, 2 have crossed into the second trimester of pregnancy, whereas another 8 are in the first trimester with a healthy intrauterine pregnancy. One patient had an ectopic gestation, and biochemical pregnancies (Figure 5)

#### FIG-1 AVERAGE ENDOMETRIAL THICKNESS

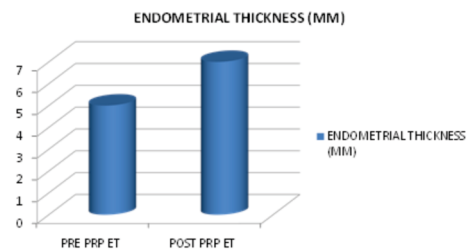
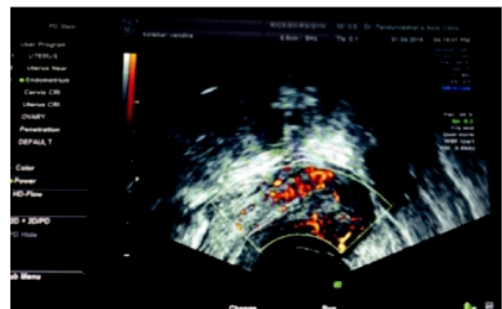
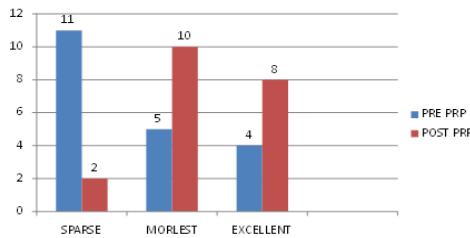


Fig-2 Sonography Of Patients Displaying Sparse To Modest Vascularity Pre-prp Instillation



**Fig-3 endometrial vascular pattern pre & post prp instillation**



**FIG 4-Beta hCG results Implantation rate**

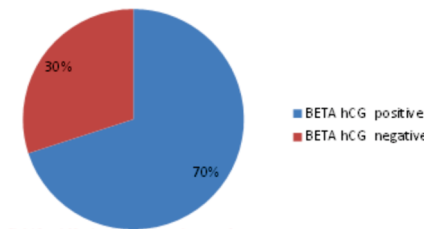
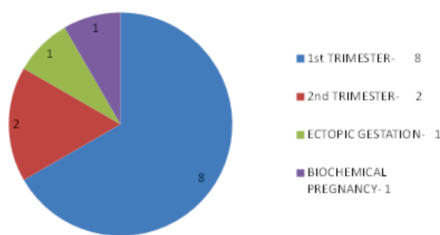


Fig4 → Beta hCG values after frozen embryo transfer

**FIG-5 Pregnancy outcomes with positive beta hCG values**

**FIG 5-OUTCOME OF PREGNANCY**



**DISCUSSION**

PRP is defined as a plasma fraction of autologous blood with the concentration of platelets four to five times above normal. It has been shown to improve regeneration in various tissues with the expression of several cytokines and growth factors. regeneration in various tissues with the expression of several cytokines and growth factors.

A few studies so far have evaluated the role of intrauterine instillation of autologous PRP in suboptimal endometrium. It has been shown to improve ET and vascularity through releasing cytokines and growth factors, including vascular endothelial growth factor (VEGF), transforming growth factor, platelet-derived growth factor, and epidermal growth factor. These factors can regulate cell migration, attachment, proliferation and differentiation, and promote extracellular matrix accumulation.

A single study by Chang et al. published in 2015 evaluated the role of autologous PRP in thin endometrium in five patients undergoing frozen embryo transfer cycles. The ET increased at 48 to 72 h after PRP infusion in all the patients and reached >7 mm on the day of progesterone administration. All the five patients were pregnant. One of the patients had a missed miscarriage secondary to a chromosomally abnormal fetus, whereas the other four had viable intrauterine pregnancies, followed up till the first trimester.<sup>[2]</sup>

A review article by Garcia-Velasco et al. published in 2016 also cites the use of autologous PRP as a potential method of improving ET in women with refractory endometrium.<sup>[1]</sup>

Another pilot study to evaluate the role of autologous PRP in improving ET at the University of California, San Francisco, is still in the Phase 2.

A recent study published in Fertility & Sterility in September 2016 by

Aghajanova et al. evaluates an in vitro model of activated PRP for endometrial regeneration. Activated PRP promoted the migration of all the cells studied, namely human primary endometrial epithelial cells, endometrial stromal fibroblasts, endometrial mesenchymal stem cells (MSC), bone marrow-derived MSC, and Ishikawa endometrial adenocarcinoma cells. These data provide an initial ex vivo proof of principle for the use of autologous PRP to promote endometrial regeneration in Asherman's syndrome and a thin endometrial lining and warrant preclinical studies in animal models and subsequently in the clinical setting.<sup>[16]</sup>

Endometrial vascularity is an important parameter to assess the implantation potential of the endometrium as cited by several studies. A retrospective study of 500 ovum donation-embryo transfer cycles published by Nagori and Panchal in 2012 demonstrated that conception rates were almost doubled when vascularity was seen in zone 3 and 4 of the endometrium than when it reached only zones 1 and 2, with low abortion rates.<sup>[14]</sup> Another recent study in 2014 by Sardana et al. also evaluated 165 women undergoing frozen embryo transfer cycles and concluded that the presence of endometrial vascularity significantly improves the outcome in frozen embryo transfer cycles.<sup>[15]</sup> Autologous PRP is a safe, easily available, and inexpensive treatment modality for women with refractory endo-metrium.

If performed under strict asepsis, the adverse effects are virtually none. This procedure, if used routinely in practice, would reduce not just the physical, but also the financial and psychological burden faced by such patients, who would otherwise face the risk of repeated cycle cancellations or recurrent implantation failures. However, further research in the form of large scale randomized controlled trials is needed, which would help to strengthen our observations and enable practitioners to use this modality clinically to optimize their success rates.

Beta hCG values after frozen embryo transfer.

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