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Platelet Rich Plasma (PRP) for Ovarian Function: New Hope for Infertility

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Abstract

The important breakthrough in the recent past has been the use of Platelet –rich plasma (PRP) as a non operative option for a wide spectrum of medical disorders. This autologus PRP is derived from an individual's whole blood after centrifugation, the process that separates red blood cells from plasma. Plasma has a 5- to 10 fold higher concentration of growth factors than whole blood. These growth factors have been found to promote the natural regenerative and healing process by researches across multiple specialties with decent success. There has been a recent introduction of the use of PRP in gynecology especially in cases of infertility. The literature studies on this aspect of management of infertility are although limited. We conducted a thorough literature search for its application as a potential therapeutic agent for infertility in the mini review. The use of PRP for infertility in developing country like India could be promising as it is cost effective and within the reach of majority of population. The issue needs deliberation from practicing Obstetrician - gynecologists so that PRP as a treatment for infertility could be offered.

Keywords: Platelet–rich plasma (PRP), Infertility, gynecologist

Introduction

Infertility is defined as failure to achieve pregnancy within 12 months of unprotected intercourse or therapeutic donor insemination in women younger than 35 years or within 6 months in women older than 35 years. (1) It affects up to 15 % of couples. (2 Conflict of interest- I hereby declare that I have no conflict of interest.

Sources of Funding- None) An infertility evaluation is offered to any couples who by definition have infertility or are at high risk of infertility in near future(3). Women older than 35 years should receive an expedited evaluation and undergo treatment after 6 months of failed attempts to become pregnant or earlier, if clinically indicated. (4) In women older than 40 years, more immediate evaluation and treatment are warranted. (5)

The essential components of initial workup include a review of the medical history, physical examination, and additional tests if indicated. (2) For the female partner, tests will focus on ovarian reserve, ovulatory function and structural abnormalities. (3,5) Imaging of the reproductive organs provides important information on conditions that affect fertility. Imaging of the reproductive organs detects tubal patency, pelvic pathology and assesses ovarian reserve. (6) After ruling out the male factor of the cause of infertility, the main components of the causes of female infertility are addressed i.e the uterus, tubes, ovaries, and any other pathology.

The changing cultural practices have also led to delayed pregnancies. The result is an unfavorable mix of delayed and difficult conception, premature ovarian failure and early menopause. Advanced maternal age and delayed pregnancy planning have all the more added to the numbers of such infertile couples with decrease in ovarian reserve and poor oocyte quality.(7) The presence of thin endometrial lining further complicates the issue, as a healthy and

thick endometrium is crucial for implantation of the embryo. A pivotal role is played by ovarian estrogen hormone, which keeps the endometrium thick and receptive. Premature menopause and low estrogen have many associated negative effects. While PRP used for ovarian rejuvenation does not just resolve fertility issue, it also alleviates some of these adverse effects. (6,7)

Platelet–Rich Plasma (PRP)

The preparation of PRP is an outpatient procedure that involves drawing of blood, preparation of the PRP and subsequently injecting it into the diseased area.

The following steps present a representative method of preparing PRP:

a) Venous blood (15-50 ml) is drawn from the patients arm in anticoagulant containing tubes.

b) The recommended temperature during processing is 21-24 ^{0} to prevent platelet activation during centrifugation of the blood.

c) The blood is centrifuged at 1200 rpm for 12 minutes.

d) The blood separates into three layers: an upper layer that contains platelet and white blood cells, an intermediate thin layer (the buffy coat) that is rich in white blood cells and a bottom layer that contains red blood cells.

e) The upper and intermediate buffy layers are transferred to an empty sterile tube. The plasma is centrifuged again at 3,300 rpm for 7 minutes to help with the formation of soft pellets (erythrocyte and platelets) at the bottom of the tube.

f) The upper two-third of the plasma is discarded because it is platelet poor plasma.

g) Pellets are homogenized in the lower third (5 ml) of the plasma to create the PRP.

h) The PRP may now be used at appropriate site. Approximately 30 ml of venous blood yields 3-5 ml of PRP.

PRP preparation can be classified according to the preparation method, the content of the sample and the proposed application. (Table -1, 2)

Table1. *Platelet containing preparations, as classified by Dohan Ehrenfest et al (8,9)*

Preparation	Acronym	Leucocyte	Fibrin density
Pure platelet rich plasma	P-PRP	Poor	Low
Leucocyte –and platelet rich plasma	L-PRP	Rich	Low
Pure platelet –rich fibrin	P-PRF	Poor	High
Leucocyte –and platelet rich fibrin	L-PRF	Rich	High

Table2. *DEPA classification of PRP preparation (10)*

DEPA	Subgroup	Description
Dose of injected platelets	Very high	.> 5 Billion injected platelets
	High	3-5 Billion injected platelets
	Medium	1-3 Billion inected platelet
	Low	< 1 Billion injected platelets
Efficiency of production	High device efficiency	High device efficiency
	Medium device efficiency	Recovery rate in platelets 70-90 %
	Low device efficiency	Recovery rate in platelets 30-70 %
	Poor device efficiency	Recovery rate in platelets <30%
Purity Of PRP	Very pure PRP	Platelet in PRP >90 %
	Pure PRP	Platelet in PRP 70 -90 %
	Heterogenou PRP	Platelet in PRP 30-70 %
	Whole blood PRP	Platelet in PRP < 30 %
Activation Process	Autologous Thrombin Calcium Chloride	

Platelets contain high concentration of cytokines and growth factors stored within α- granules. These growth factors include platelet derived angiogenic factor, transforming growth factor, insulin –like growth factor, vascular endothelial growth factor, transforming growth factor beta, fibroblast growth factor, epidermal growth factor, connective tissue growth factor and interleukin -8. In addition to growth factors, platelets contain other substances, such as fibronectin, vitronectin, and sphingosine 1 –phosphate, that initiates the process of regeneration. (11) Platelet activation triggers the release of these growth factors. These growth factors and cytokines can stimulate cellular process necessary for endometrial regeneration and activates multi –potent stem cells to generate new, younger tissue and new blood vessels relevant to ovarian regeneration. The stimulus of the release of growth factors may be thrombin, calcium chloride and collagen. However, no evidence has been found regarding the ideal concentration of activator required to trigger the optimal release of growth factors during the activation process of PRP, and different concentrations may therefore lead to different results. (12)

Historical Facts

PRP, developed in the 1970s, was first used in Italy in 1987 in an open heart surgery procedure. (13) Autologous blood products including platelets got popularized in the 1990s in oral and plastic surgery. (14) Chang et al (15) for the first time reported the efficacy of intrauterine PRP infusion for endometrial growth in women having thin endometrium. The use of autologous PRP therapy was prompted by the ability of platelet –derived growth factor (PDGF) in promoting wound healing, angiogenesis, and tissue remodeling. PRP therapy started gaining popularity in the mid -1990s. Since then it has been applied to different medical fields like sports medicine, skin grafting, dentistry, cosmetic surgery and pain management. Many clinical studies have been done worldwide which prove the beneficial effects of PRP therapy in oral-maxillofacial, plastic surgery and orthopedic surgery.

Procedural Details

Pre procedural detailed medical history, clinical examination and laboratory tests including FSH, LH, AMH and Ultrasound is done.Endometrial thickness is measured at the thickest part of the longitudinal axis of the uterus by ultrasound. Endometrial layering and Zone 3 blood flow, ovarian stromal blood flow and ovarian volume ascertained. Patients with pregnancy, cancer, Hb < 8g/dl, platelets < 1,50,000 /cumm, anticoagulant therapy, any co –morbid condition or psychiatric disorder or any contraindications to laparoscopic surgery or general anesthesia should not be subjected to this treatment modality.

In cases requiring ovarian instillations, studies have shown that 20 ml venous blood is drawn and 2- ml of PRP obtained is instilled laparoscopic ally in bilateral ovaries. The laparoscopic procedure is also utilized to ascertain the condition of uterus, tubes and adnexa, to remove peritoneal adhesions or fulgurate endometriotic spots if visible. For the rejuvenation of endometrial tissue, on 7 th -11 th day of the cycle, 20 ml of venous blood is drawn and centrifuged immediately and 2 ml of PRP obtained. 0.1ml injection of calcium gluconate is added per 0.9 ml of PRP before instillation with the help of hysteroscopic needle 12-14 holes are made in endometrium.

PRP in Infertility

The effectiveness of autologous PRP application for endometrial regeneration and repair in patients with thin lining or intrauterine scarring and ovarian rejuvenation in patients with

1. Premature ovarian failure (POF) and low ovarian reserve.
2. In refractory endometrium

Premature ovarian failure (POF) refers to loss of normal function of the ovaries before 40, accompanied by the loss of infertility. Ovarian rejuvenation is still in its infancy stage, but it is already offering new frontiers to hundreds of women who suffer from POF, low AMH, high FSH, poor oocyte quality and in case of ischemia of ovarian tissue as a result of ovarian torsion. Ovarian rejuvenation procedure might create new oocyte in the ovaries of women who are not able to conceive because of low oocyte reserve, premature ovarian failure or premature menopause, yet who desire to have their biological child.

Researchers (16) in Harvard University stated that introduction of isolated growth factor bearing platelet directly into the ovaries might trigger a resurgence in oocyte production. PRP is injected into the ovary under laparoscopic guidance to induce ovarian regeneration. Progesterone receptor activity has been shown to get enhanced with PRP application. Progesterone receptors are the main receptors that help in maintaining healthy endometrium. Harvard scientists(17) show that the PRP application in the ovaries can stimulate the germ cell line developing into ovarian oocytes. Pantos et al,(18) at the annual European Society of Human Reproduction and Embryology conference held in 2016 in Helsinki, Finland, introduced this modality of ovarian regeneration. The PRP injected in women with poor ovarian reserve found successful ovarian rejuvenation within 1-3 months.

The endometrium plays an important role in achieving optimal outcomes of assisted reproductive technologies. The current therapies offer little for patients with intrauterine scarring, adhesions (Ashermann Syndrome) and with failed IVF (In vitro fertilization) treatment due to thin endometrium mandating the urgent need of a breakthrough therapies. Endometrial growth with PRP is a novel and promising method of improving endometrial thickness and improving pregnancy rate. Colombo et al (19) included eight patients who underwent PRP treatment. The inclusion criteria were women with more than 3 cancelled cryo-transfers due to poor endometrial growth (<6 mm), women with a negative hysteroscopic screening for endometrial pathology and women with a negative bacterologic screening. After application of PRP, the endometrial thickness was in severe cases. Out of 8 women 6 were able to conceive. They concluded that the multiple implantation failures were caused by inefficient expression of adhesion molecules, which could potentially be improved by PRP application. Recently the intrauterine infusion of PRP has been described as a way to promote endometrial growth and receptivity.

The minimal endometrial thickness in clinical practices required for embryo transfer is 7mm at the end of follicular phase. Thin endometrium not responding to conventional treatment is a challenge in assisted reproductive techniques (ART) which results in cycle cancellation and repeated IVF failures. Various treatments are available for dealing with thin endometrium, including extended use of exogenous oestrogen (20), use of low dose aspirin (21), vitamin E, (22)vaginal sildenafil citrate (23), electro- acupuncture(24) and application of GCSF (25) (granulocyte colony stimulating factor). However, it is to be noted that women with thin endometrium remain non responsive even after these remedies. PRP has ease of accessibility, affordability and also promising results in comparison. (25,26)

Various studies across the world have highlighted the inclusion of this therapeutic modality as a breakthrough in conditions which are largely unaddressed till now. In a study (27) performed at IVF centre, Tehran, Iran from September 2015 to 2016, ten patients were recruited who had who had a history of cancelled cycles due to poor endometrial growth in the past FET cycles despite conventional treatment. There study showed increased endometrial thickness in 8 hours in 48 hours after the first PRP and the noted thickness was more than 7 mm after the second PRP. Embryo transfer was done for all the patients. Five patients conceived and in four of them, the pregnancy continued normally. In another study by Zadehmodarres et al (28), 20 women with a history of recurrent implantation failure who were candidates for frozen embryo transfer (FET) were recruited. Intrauterine infusion of 0.5 ml of platelet rich plasma was performed 48 hours before blastocyst transfer. The study reported eighteen participants as pregnant with one early miscarriage and one molar pregnancy.

The Inovium Ovarian Rejuvenation Treatment is led by Eric S. Sills (29)at the centre for Advanced Genetics, Caifornia, US. The study started in 2015 with the use of PRP injections for ovarian rejuvenation and increasing fertility on 50 women with menopause or premature ovarian failure. Of the women recruited, 75% developed the option of natural or IVF pregnancy. Overall hormone levels returned to normal in over 75% and nine women conceived.

Callejo et al(30) reported an interesting case in women without ovaries. They injected PRP in the ovarian tissue and observed that after four and a half months

the ovarian function was evident and she had her first spontaneous menstruation. Then after successful drug stimulation, they managed to get good quality eggs and through an IVF trial, the woman got pregnant.

A study published in 2018 by Sills ES et al (31), included extended PRP application to ovarian tissue with a view to document impact on ovarian reserve among women attending for infertility treatment. PRP was freshly isolated from patients (n=4) with diminished ovarian reserve as determined by at least one prior IVF cycle cancelled for poor follicular recruitment response or estimated by serum AMH and / or FSH, no menses for ≥ 1 year. Immediately following substrate isolation and activation with calcium gluconate, approximately 5 ml of autologus PRP was injected into each ovary. For each study subject, AMH, FSH, and serum estradiol data was recorded at two week intervals post PRP and compared to baseline (Pre PRP) values. IVF occurred 78 ± 22 (range = 59-110) days after activated PRP injection, and results appeared independent of patient age, infertility duration, baseline platelet concentration or pretreatment antral follicle count. Each patient had at least one blastocyst suitable for cryopreservation. Evidence of improved ovarian function was noted in all who received intraovarian PRP, possibly as early as two months after treatment.

Conclusion

PRP is an innovative therapeutic modality, as it is affordable, simple, easily performed and effective and gives encouraging results. Women who suffer from low ovarian reserves, POF or premature menopause, low estrogen related issues leading to the thin endometrium and recurrent implantation failures. As in any new and emerging therapies, the efficacy and validity of using PRP worldwide with more acceptance and credibility mandate more stringent statistical back up. Despite its infancy, PRP application has drawn attention among researchers and gynecologists, and this might open new avenues for further and more wide spread use in cases of infertility which are unaddressed by other modalities of treatment.

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