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Transuterine Perfusion of Platelet-Rich Plasma in Infertile Women with Diminished Ovarian Reserve: A Case Series with In Vivo Conception and Continuing Pregnancies

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ABSTRACT

Purpose: *To report successful in vivo fertilization in four out of five women with diminished ovarian reserve (DOR) after transuterine perfusion of the ovaries and pelvis with autologous platelet-rich plasma (PRP).*

Methods: *Patients that exhibited DOR (defined as AMH <1.1 ng/mL and antral follicular count of < 7 follicles) and had failed in vitro fertilization and/or artificial insemination in the past year were offered transuterine perfusion of PRP with oral letrozole and timed intercourse as an alternative therapy. Five patients accepted the protocol. Patients took 10 mg of letrozole orally from day 3 to day 7 of the menstrual cycle to increase chances of ovulation. They underwent one transuterine perfusion of the pelvis during the follicular phase of each cycle with a 10 mL volume of fresh autologous platelet-rich plasma under ultrasound guidance to allow periovarian spillage from the fallopian tubes.*

Result: *In 4 out of 5 cases patients conceived without the need for in vitro intervention. Three patients had a successful pregnancy. One patient had a miscarriage at 19 weeks. One patient did not conceive within the four months of observation.*

Conclusion: *Delivering platelet-rich plasma to the ovaries by transuterine perfusion may be a safe and cost-effective approach to enhance fertility in women with impaired ovarian reserve undergoing fertility therapies.*

Keywords

Diminished ovarian reserve, Platelet-rich plasma, Letrozole, Intrauterine insemination (IUI).

Introduction

Autologous platelet rich plasma (PRP) was first injected into ovaries by Pantos and his group in Greece (2016). They describe the technique as being similar to an egg retrieval, where ultrasound guidance is utilized while the patient is under anesthesia, to inject 2mL of PRP into each ovary. They reported improvement of ovarian function and pregnancy in perimenopausal and menopausal patients [1,2]. A laparoscopic approach to reaching the ovaries has also been described [3].

Since these publications appeared in the medical literature, PRP has gained attention as a potential adjuvant in assisted reproductive technology. PRP has been shown to enhance reproductive function by improving outcomes in patients undergoing *in vitro* fertilization (IVF) after intraovarian injections of platelet -rich plasma [4,6]. PRP has also been used to improve implantation after embryo transfers, with some authors describing up to a 30% increased implantation rate after previously documented thin endometrium [5]. The multiple uses of PRP to improve fertility hold a promising future, however there are a few challenges to consider when ovarian

rejuvenation is intended using these previously described methods. Both the ultrasound guided approach as well as the laparoscopic approach must be done under anesthesia, in a controlled setting. This poses increased costs and limited access to patients that could not afford these additional costs. Also, there are anesthesia risks as well as procedural risks due to the inherent nature of surgery. As with all invasive procedures, there are increased risks of bleeding, infection and/or damage to adjacent tissues that must be addressed. As we closely explore the physiology of the ovaries as well as their response and relationship to the peritoneal environment, we understand that the pelvis is a dynamic ecosystem.

The peritoneal fluid content influences the ovaries and surrounding organs, and the ovaries at the same time excrete proteins and hormones into the peritoneal fluid that affect other pelvic organs and the fertility process [7]. They have collected the peritoneal fluid of infertile women showing that women suffering with infertility of unknown origin have less progesterone concentration and less proteins overall, thus less activation of spermatozoa [8]. The ovarian surface is a dynamic membrane with autocrine and paracrine functions. Its proliferative factors, interleukins, cytokines, and hormones are diffused through the pelvic cavity inducing changes in adjacent tissues [9]. These interactions are clearly seen and have been biochemically mapped in ovarian epithelial cancer behavior [10]. In a similar fashion, we see the intricate relationship of the peritoneal fluid and the ovaries in the pathophysiology of endometriosis. The peritoneal fluid is a dynamic environment that connects the immune system with the endocrine system by providing a fluid network through which these organs communicate. A recent study compared the content of the peritoneal fluid in women with infertility and newly found endometriosis with a control group, and the biomarkers were very different [11].

It is clear that the ovaries respond to their environment. Also, PRP seems to have a positive effect on ovarian function and fertility. However, the methods that have been used until now are costly and invasive. We hypothesized that exposing the ovaries to PRP by intraperitoneal deposition would have a result comparable to injecting the ovaries directly. The potential value of this approach is suggested by the improvement in ovarian function in rats given PRP intraperitoneally [12,13].

By utilizing the uterus and fallopian tubes as a channel to reach the peritoneal cavity, we eliminate not only costs, and surgical driven risks, but also discomfort while increasing patient satisfaction.

In this report we describe five cases in which we successfully used a less invasive and more affordable way to deliver autologous PRP to the ovaries and the pelvis. PRP was injected transvaginally under ultrasonography guidance.

Methods

This is a pilot, open label, prospective study. The protocol was approved by the Institutional Review Board (IRB) of the University of Georgia (PROJECT00005794. July 5,2022). The inclusion criteria were healthy patients having idiopathic diminished ovarian reserve with plasma antimüllerian hormone (AMH) <1.1 ng/ml and with antral follicle count of less than 7 follicles. (Jayaprakasan et al., 2010), with patent fallopian tubes and a normal endometrial cavity confirmed by saline ultrasound hysterography and a x-ray hysterosalpingogram, failed infertility treatments for at least one year including various standard medical interventions, IVF and/or monitored cycles with intrauterine insemination (IUI). Toxic habits such as smoking, alcohol consumption or use of recreational drugs were exclusion criteria.

Patients were given a full explanation of the procedure, the information available on PRP and fertility until that moment and were allowed to ask questions until they fully understood what the process would be. They were all counseled by a trained professional and signed the informed consents following the Helsinki guidelines. Complete blood count, blood biochemistry, coagulation tests, HIV, Rapid Plasma Reagin (RPR), Hepatitis B surface antigen and PCR Covid test were also performed to the included patients prior to performing the procedure.

Platelet Rich Plasma Preparation

The required volume of PRP was obtained aseptically from 60 mL of the patient's peripheral blood. The method of preparation was based on the protocol described by Dhurat and his group [14]. The samples were drawn into sterile blue-top tubes with sodium citrate as anticoagulant. Tubes were spun in a centrifuge at room temperature for 5 min at 200 rpm. The plasma supernatant was then transferred to two sterilized glass conical tubes, and a second centrifugation was performed at 400 rpm for 10 min. The platelet-poor supernatant plasma was saved and the sedimented platelet pellets were then reconstituted in 5 mL of it. The final volume was 10 mL. That volume was chosen to ensure spillage into the pelvic cavity around the ovaries based on what has been observed during saline ultrasound hysterosalpingography.

Procedure

PRP transuterine perfusion was performed monthly during the follicular phase for a period of four months. We used this time frame based on common recommendations for dermatological disorders. We used the PRP immediately after preparation, on cycle days 6-10. The procedure was performed on an ambulatory basis without anesthesia. An inflated hysterosalpingogram catheter (Cooper surgical 5 fr 1.5 cc balloon) was used to prevent backflow from the cervical canal. The transvaginal probe was introduced into the patient's vagina. The PRP was injected slowly over 3-5 minutes into the uterine cavity under ultrasound observation. All women tolerated the procedure well with minimal discomfort and were able to return to normal activities immediately.

Additionally, the patients were given letrozole 10 mg on cycle days 3-7 to ensure ovulation, were on a low carbohydrate diet, and took prenatal multivitamins, vitamin D3, omega-3 fatty acids and coenzyme Q10. They were having periovulatory timed intercourse during this period. Ovulation was verified with a cycle day 21 progesterone evaluation. After the original baseline visit,

Table 1: Demographic, Pretreatment and Results Data.

	Case 1	Case 2	Case 3	Case 4	Case 5
Age	33	42	43	41	38
Ethnic group/race	caucasian	caucasian	caucasian	african-american	hispanic
BMI	22	25	21	23	20
Place of Residence	Large city	Large city	Large city	Large city	Large city
Time Trying to Conceive (years)	1	3	3	2	2
Level of Education	Master's degree	Master's degree	4 years of college	4 years of college	4 years of college
Comorbidities				Leiomyomas (<3cm)	
Previous Fertility treatments	IUI	IUI/ IVF	IUI	IUI/IVF	IVF
AMH (ng/mL)	0.16	0.68	0.30	0.90	0.33
Antral Follicle Count	3	5	4	6	5
CD3 FSH (mIU/mL)	27.9	21.2	15.0	9.8	7.2
Pregnancy	yes	yes	yes	yes	no

the patients had no follicular monitoring or hormonal evaluation other than the cycle day 21 progesterone check during the three-month period.

Results

Five Patients were included in this pilot study. Their demographic and pretreatment infertility data are shown in Table 1.

Case 1

A 33-year-old caucasian woman who presented with secondary infertility for over 1 year. She had a 3-year-old daughter on evaluation her plasma AMH level was 0.16 ng/mL and cycle day three FSH was 27.9 mIUm/L. Antral follicle count was three. No male component was found, and her radiologic studies revealed normal uterus and patent tubes. She proceeded to undergo two monitored IUI cycles with letrozole 5 mg and 75 units of FSH (gonal F). These did not result in pregnancy despite documented adequate ovulation with a post insemination progesterone of 12 ng/mL.

She agreed to try this alternative protocol before proceeding to more aggressive methods. She followed the established protocol and took letrozole 10 mg on day 3 to day 7 of her menstrual cycle. On cycle day 7, 10 mL of PRP was introduced into the patient's peritoneal cavity under ultrasound visualization. Appropriate ovulation was documented. This process was repeated each month for three consecutive months. She conceived in her third month of treatment and delivered a healthy male fetus at 39 weeks of gestation via a normal vaginal birth. No progesterone support was required during her uneventful pregnancy.

Case 2

A 42-year-old caucasian woman who presented with one-year history of primary infertility prior to seeking fertility care. She underwent 3 IUI monitored cycles with Letrozole and progesterone supplementation that did not result in pregnancy. After these, she proceeded to have IVF. The first IVF resulted in 4 viable eggs and 1 day 6 embryo that became an unsuccessful frozen embryo transfer. She decided to do IVF with genetic testing on the embryos to select genetically normal embryos through karyotyping screening. The following 4 IVF cycles produced 3 normal embryos out of 15 blastocysts. The frozen embryo transfers from the 3 normal embryos resulted in 1 biochemical pregnancy and a pregnancy loss at 7 weeks. The fifth IVF yielded 6 eggs, with 1 blastocyst that was genetically abnormal. At this point, her serum AMH concentration was 0.68 ng/mL and day-three FSH was 21.1m IU/mL. Her antral follicle count was 5. She underwent three sessions of monthly PRP transuterine perfusion while taking letrozole 10 mg on day 3-7 and having timed intercourse. She conceived during the third cycle and delivered a normal full-term male infant at 40 weeks of gestation via cesarean delivery. Pregnancy was uneventful.

Case 3

A 43-year-old nulligravid caucasian woman presented with a three-year history of primary infertility. She started her fertility care after the first 6 months of unsuccessful timed intercourse at home. She had three unsuccessful cycles of clomiphene and timed intercourse followed by two Monitored cycles of ovulation induction with FSH (gonal F) 75-100 units daily with HCG ovulation trigger and IUI 30-35 hours after trigger. She wanted to try our protocol prior to attempting IVF due to financial reasons. On evaluation, her plasma AMH concentration was 0.30 ng/mL and her day-three FSH level was 15.0 mIU/mL. Her antral follicle count at baseline was 4. She was treated with letrozole 10 mg on cycle days 3-7 and timed intercourse as well as a monthly follicular phase (CD6-10) PRP transuterine perfusion. She conceived in the third month with intrauterine insemination. Pregnancy was uneventful. She delivered a normal full term male infant through a vaginal birth at 37 weeks of gestation.

Case 4

41-year-old african-american woman that came in after a second failed IUI. She had a 11 week spontaneous abortion from a monitored cycle with IUI and oral Letrozole 5 mg at age 39. Upon presentation her AMH was 0.90 ng/mL and her cycle day 3 FSH was 9.8 mIU/mL. Her saline ultrasound also revealed 4 leiomyomas ranging from 1.3 cm to 3 cm. None were submucosal. Antral follicle count was 6. She opted to proceed with IVF with preimplantation genetic testing which resulted in 5 eggs and 1 non-tested cycle day 3 embryo that was transferred fresh and did not result in pregnancy. She agreed to try our protocol at this point. She was treated with Letrozole 10 mg orally taken during cycle day 3

Table 2: Summary of Results.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Treatment	**Letrozole 10mg on day 3-5 of cycle**	**Letrozole 10mg on day 3-5 of cycle**	**Letrozole 10mg on day 3-5 of cycle**	**Letrozole 10mg on day 3-5 of cycle**	**Letrozole 10mg on day 3-5 of cycle**
PRP	**During cycle day 6-10**	**During cycle day 6-10**	**During cycle day 6-10**	**During cycle day 6-10**	**During cycle day 6-10**
Repeated	**Monthly for 3 months**	**Monthly for 3 months**	**Each month for 3 months**	**Monthly for 3 months**	**Monthly for 4 months**
Pregnancy	**Third month**	**Third month**	**Third month**	**Third month**	**none**
Delivered	**39 weeks**	**40 weeks**	**37 weeks**	**19 weeks**	**n/a**
Additional medical intervention	**none**	**none**	**none**	**none**	**none**
Clinical Evolution	uneventful	uneventful.	uneventful.	Loss	yes
Complications	**none**	**none**	**none**	**none**	**none**

through cycle day 7 together with the monthly PRP transuterine perfusion and timed intercourse at home. She conceived after the second PRP. Unfortunately, she suffered a spontaneous loss at 19 weeks. Genetic testing on the fetus revealed a normal female karyotype.

Case 5

38-year-old nulliparous hispanic woman who presented to the clinic after two years of unprotected sex. Upon initial evaluation, her AMH was 0.33 ng/mL and her cycle day 3 FSH was 7.2 mIU/mL. Antral follicle count was 5. Radiologic studies revealed a normal uterus and open tubes. She decided on IVF as her choice of treatment. She underwent 3 IVF cycles that did not result in pregnancy. During the first IVF cycle, 5 eggs were retrieved that resulted in 2 day three embryos and 1 day 5 embryo. She underwent 2 frozen embryo transfers; both day 3 embryos were transferred together. Neither transfer resulted in a pregnancy. The second IVF resulted in 2 eggs that did not produce embryos. The third IVF produced 3 eggs that resulted in 1 day 3 embryo that was transferred fresh without positive results. She agreed to try our protocol. She underwent four rounds of monthly follicular phase transvaginal PRP infusions with oral letrozole 10mg during day 3-7 of her cycle without resulting in a pregnancy.

Discussion

The need to find innovative methods to enhance fertility outcomes with or without assisted reproduction techniques is on the rise. As our fast-paced lives push the envelope of our biological limitations, more women are seeking pregnancies later in life. That in itself poses the challenges associated with the physiologic aging of the female reproductive organs regardless of any other comorbidities that could affect fertility. The most common option given for older women and/or women with decreased ovarian reserve is donor eggs. However, most women would prefer to use that as a last resource and will seek any form of assistance to give them a chance for a biological offspring. PRP has been proven as an useful tool to help women with diminished ovarian reserve reach the goal of motherhood without resorting to donated eggs [15]. However, until now the literature only presented case series either a laparoscopic or a transvaginal ultrasound guided injection to approach. We have presented an innovative approach to deliver autologous PRP to the periovarian region without the associated costs or risks. Just like the animal models, we have seen a clinical improvement in fertility outcomes with this approach [12,13].

We understand that is a very small sample of patients, and that a more structured evaluation is needed. However, these preliminary clinical results are very promising and can be added to the data that points to PRP as a great adjuvant in fertility management. There are a few questions that arise from our findings, and that should be addressed in later studies. First, we cannot be certain that our delivery system caused positive changes in follicular development. Because the follow-up of the patients was minimal, there was no tangible way that measured the effect of the peritoneal PRP on the ovaries. We suggest that in further projects, strict monitoring of the patients with recurrent evaluation of biomarkers is done. Second, It can even be argued that because this method involves exposure of the uterus and the fallopian tubes to PRP, it could impact the integrity and functionality of those organs as well, resulting in the improvement of the entire reproductive system. On the other hand, the outcomes of these five patients where four out of five became pregnant with genetically normal fetuses is a great qualitative indicator that probably ovarian function was indeed improved. Whatever the mechanism, this small series strongly suggests a potential benefit of transuterine PRP infusion in the management of infertility in women with diminished ovarian reserve. In that role, it could obviate the need for more expensive and complicated ART techniques. A randomized trial is in order.

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