RESEARCH ARTICLE

Role on Intraovarian Platelet-rich Plasma in the Poor Ovarian Responder

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Abstract

Objective: To evaluate the effects and safety of monthly intraovarian injection of autologous platelet-rich plasma (PRP) for three consecutive cycles prior to *in vitro* fertilization and embryo transfer (IVF–ET) on ovarian reserve markers in women with poor ovarian response (POR) as per Bologna criteria.

Materials and methods: This is a prospective cohort study of 66 women diagnosed as poor ovarian responders as per the Bologna criteria were recruited in the study from 1 January 2018 to 31 December 2020. Platelet-rich plasma was prepared from peripheral blood and PRP activation was achieved with 0.1 cc of 10% calcium gluconate. Platelet-rich plasma injection into ovarian stroma was performed transvaginally, under ultrasound guidance, into both ovaries using a 35 cm single lumen 17G needle between D7 and D12 of the menstrual cycle in three consecutive cycles. Antral follicle count (AFC) and anti-müllerian hormone (AMH) were measured from the second to fourth day of menstruation before the first PRP treatment and immediately following last PRP administration. Ovarian stimulation and *in vitro* fertilization (IVF) embryo transfer were performed immediately following the last PRP administration. The primary outcomes were the change in AFC post-PRP treatment. Secondary outcomes were increased in serum AMH, the total number of oocytes retrieved and clinical pregnancy rates.

Results: Sixty-six women were included. After three cycles of ovarian PRP administration, there was a significant increase in AFC mean (SD) 4.85 (4.65) compared to pre-treatment AFC. There was a statistically significant difference between pre- and post-treatment AFC (5.21 vs 10.06, $p < 0.0001$). Improved post-treatment AFC was not limited to younger patients; when stratified by age, significant AFC improvements were seen in patients of age less than 35 years as well as more than 35 years ($p < 0.0001$). Furthermore, the significant increase was seen in mean serum AMH level following PRP treatment (1.18 vs 0.93, $p < 0.0001$). The improvement in serum AMH levels were seen across all age patients (<35 years vs ≥35 years). The mean (SD) number of oocytes retrieved were 7.49 (4.45) and pregnancy rate was 25%. The PRP treatment was well tolerated.

Conclusion: Intraovarian PRP injections are effective to improve AFC, AMH prior to antiretroviral therapy (ART) in poor ovarian responders, and results in reasonable number of oocytes retrieved following ovarian stimulation.

Keywords: Assisted reproductive technology, Ovarian reserve, Platelet-rich plasma, Poor ovarian responder.

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Introduction

The ovarian reserve defines the quantity and quality of the ovarian primordial follicular pool. In women with poor ovarian reserve, there is decrease in number and quality of oocytes. While poor ovarian reserve usually occurs in female with advanced age, it is not limited to them and can also be seen in young women. Ovarian stimulation for IVF in such women often results in poor ovarian response (POR) and the success of the treatment is often very limited. The management of these infertile patients remains difficult and they are usually recommended IVF with oocytes donated from young women with normal ovarian reserve.[1,2] Oocyte donation is an efficacious way of management of these patients but is not acceptable to all. There is a requirement for a new modality of treatment for these patients.

Platelet-rich plasma is a concentrate which is obtained from centrifuged whole blood.[3] Platelet-rich plasma contains up to 7-fold higher platelet concentrations than that in the serum. The platelets are involved in triggering cellular proliferation and tissue differentiation.[4] There are several soluble mediators in PRP that coordinate cellular repair after tissue injury.[5] Platelet-rich plasma also plays a role in regeneration of tissue, proliferation of cell, remodeling of extracellular matrix, apoptosis, cell differentiation, and angiogenesis.[6] Platelet-rich plasma comprises of high levels of several growth factors such as insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), vascular endothelial growth factor (VEGF), β-transforming growth factor, epidermal growth factor (EGF), essential growth factor for fibroblast, and hepatocyte growth factor (HGF). It has been suggested that these growth factors present in PRP are responsible for the regenerative properties of PRP.[3,4]

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The PRP therapy is being used in various branches of medicine. Platelet-rich plasma helps to improve hemostasis in patients with thrombocytopenia. Therefore, it is commonly used for the management of thrombocytopenia.[5,6] In orthopedics and sports medicine, PRP is used in many musculoskeletal disorders, helping to minimize discomfort as it facilitates natural healing. In athletic injuries, the use of PRP injections results in relief of pain allows the patient to return to normal activities early.[7] The use of PRP is also investigated in cosmetic surgery.[8,9] and in the management of diseases of the eye,[10] and even myocardial infarction.[11]

Platelet-rich plasma therapy is now being utilized in the management of infertility also. Justo Callejo et al. showed that PRP treatment improved quality and vascularization of implant in ovarian autologous transplantation.[12] Molina A et al. investigated the effect of intrauterine injection of PRP in women with a thin endometrial lining. They demonstrated an increase in endometrial thickness and implantation rates in these patients. However, the statistical significance was unclear.[13] Other investigators also reported that administration of PRP in uterus in patient with thin endometrium improved the endometrial thickness.[14] Some small studies have investigated the benefits of intra-uterine instillation of PRP in women with recurrent implantation failure with encouraging results.[15]

Application of PRP is also being investigated in ovarian tissue regeneration. The treatment comprising of intraovarian injection of PRP is known as ovarian rejuvenation. Platelet-rich plasma is injected into the ovary under the USG (ultrasound) guidance.[4] This intervention is investigated in many clinical trials all over the world.[5] Bakacak et al. demonstrated how PRP prevents ischemia and reperfusion damage in rats following bilateral adnexal torsion and surgical detorsion, mainly through an increase in VEGF.[16] Pantos et al. first introduced ovarian rejuvenation treatment using PRP. They injected PRP into the ovary using ultrasonography (USG) guidance. They demonstrated that intraovarian injection of PRP in eight perimenopausal/premature ovarian failure (POF) female with poor ovarian reserve resulted in ovarian rejuvenation after 1–3 months of PRP injection.[17] A recent non-randomized interventional study on the use of autologous PRP compared with intraovarian injections of PRP to no treatment in women with diminished ovarian reserve. The study demonstrated that PRP injections resulted in significant improvement in ovarian reserve markers such as follicle-stimulating hormone (FSH), AMH, and AFC.[18] The use of intraovarian PRP for ovarian rejuvenation is a new modality of treatment and the evidences on its efficacy and safety of use are still emerging. Our study aims to evaluate the safety and effects of intraovarian injections of autologous PRP for three consecutive cycles on ovarian reserve markers in Indian women diagnosed as poor ovarian responders as per the Bologna criteria.

Materials and Methods

This prospective cohort study was conducted from 1 January 2018 to 31 December 2020 in our fertility clinic. Women planning to undergo ART who fulfilled the following patient selection criteria were included in the study: Inclusion criteria: 1) Signed and dated informed consent, 2) Normal karyotype, and 3) POR as per Bologna criteria. Exclusion Criteria: 1) Ovarian insufficiency secondary to sex chromosome etiology, 2) Pelvic adhesions which would preclude the safe injection of PRP into the ovary, and 3) Any medical condition for which plasma infusion is contraindicated. Antral follicle count (AFC) and AMH were measured from the second to fourth day of menstruation before first PRP treatment and immediately following last PRP administration. Ovarian stimulation and IVF–embryo transfer was performed immediately following the last PRP administration.

Method of PRP Preparation

Five milliliters of 3.2% sodium citrate was taken in a 20-cc syringe. A sterile butterfly scalp vein set was attached to this syringe and 15 mL of blood was drawn through the antecubital vein of one forearm. The sample was labeled and placed in room-temperature centrifuge set to 1,200 remote patient monitoring (RPM) for 12 minutes. The visible supernatant with the buffy coat was aspirated out and placed in a separate tube. This tube was centrifuged at 3,300 RPM for 7 minutes. The top of the column was then aspirated since it is likely to be dilute and does not contain adequate platelets and the bottom 4 mL reserved for PRP. Platelet-rich plasma activation was achieved with 0.1 cc of 10% calcium gluconate which was done just prior to the intraovarian injection.

Method of Intraovarian PRP Injection

All patients who fulfilled the inclusion criteria received PRP injections treatment once between days 7 and 12 of the menstrual cycle for three consecutive cycles. The procedure was done under intravenous sedation similar to that done during egg retrieval. Platelet-rich plasma was injected into the ovarian stroma using a 35 cm single lumen 17G needle under transvaginal ultrasound guidance. Once tip placement was confirmed, the PRP was slowly injected into the stroma while avoiding injection into ovarian follicles. Both ovaries were systematically injected. At the end of the procedure, ultrasound assessment of the pelvis was performed to confirm absence of vascular injury. The procedure lasted about 10–15 minutes. After the procedure, the patients were monitored for 2 hours and then discharged. All procedures were uneventful.

The IVF and Embryo Transfer

The IVF and embryo transfer was carried out in the cycle following the third and last intraovarian PRP treatment by a gonadotropin-releasing hormone (GnRH)-antagonist protocol starting on day 2 of the cycle. Gonadotropin dose was calculated based on patient age, BMI, AMH, and previous response to ovarian stimulation, if applicable. The patients received recombinant FSH (rFSH) 200–300 IU, in addition to 75–150 IU of human menopausal gonadotrophin (hMG) for 9–12 days. Then, GnRH antagonist was given in a flexible protocol based on follicular size at a dose of 0.25 mg once daily until the day of oocyte trigger. Once transvaginal ultrasound confirmed one or more follicles measuring ≥17 mm, recombinant human chorionic gonadotrophin 0.2 mg was administered subcutaneously to induce oocyte maturation. Ovum pick up was performed 35–36 hours post-rhCG, and 1–3 embryos were transferred between days 2 and 5 depending on patient response and embryo quality.

Outcome Variables

The primary outcome measured was the change in AFC between beginning of first PRP and start of IVF stimulation. The secondary outcomes measured were as follows: 1) Change in serum AMH between the same duration, 2) The total number of oocytes retrieved at oocyte retrieval, and 3) The clinical pregnancy rate.

Table 1: Comparison of AFC pre- and post-intraovarian PRP

Variable	*N*	*Mean*	*SD*	*p*
Pre-PRP AFC	66	5.21	1.82	–
Post-PRP AFC	66	10.06	5.23	–
AFC Difference	66	−4.85	4.65	<0.0001

Table 2: Comparison of AMH pre- and post-intraovarian PRP

Variable	*N*	*Mean*	*SD*	*p*
Pre-treatment_AMH	66	0.93	0.49	–
Post-treatment_AMH	66	1.18	0.69	–
AMH_Difference	66	−0.24	0.61	0.0159

Table 3: Comparison of AMH and AFC pre- and post-intraovarian PRP in women aged more than or 35 years ($N = 28$)

	Pre-PRP	*Post-PRP*	*p*
AMH	0.83	1.11	0.0212
AFC	5.07	9.54	<0.0001

Table 4: Comparison of AMH and AFC pre- and post-intraovarian PRP in patients aged less than 35 years ($N = 38$)

	Pre-PRP	*Post-PRP*	*p*
AMH	1.01	1.23	0.3527
AFC	5.32	10.45	<0.0001

Statistical Analyses

The specialty and associate specialist (SAS) (SAS Institute Inc., USA) was used for statistical analysis. Both AMH and AFC were summarized as continuous variables with count, mean, and standard deviation. The *p*-value was calculated using Wilcoxon signed–rank test.

Results

All 66 women who underwent PRP had measurements of their AFC and AMH at the start of treatment and in the cycle immediately after the last PRP. The results of our study are as follows: After three cycles of ovarian PRP administration there was significant increase in AFC mean (SD) 4.85 (4.65) compared to pre-treatment AFC as seen in Table 1. Table 1 also demonstrates that there was a statistically significant difference between the pre- and post-treatment AFC (5.21 vs 10.06, $p < 0.0001$). Table 2 shows the difference in the AMH levels before and after PRP therapy and shows a significant difference. Women in our study were stratified into two groups: Those who were 35 years and over, and those below it. Improved post-treatment AFC was not limited to younger patients; significant AFC improvements were seen in patients less than 35 years of age (5.32 vs 10.45) as shown Table 3 as well in patients more than or 35 years of age (5.07 vs 9.54) after treatment ($p < 0.0001$) as seen in Table 4.

The mean (SD) number of oocytes retrieved post PRP treatment in 45 patients out of 66 patients were 7.49 (4.45). Out of the 66 patients, 38 patients (57.58%) developed embryos. The mean (SD) number of embryos obtained were 3.33 (4.45). Pregnancy rate achieved was 25%. The PRP treatment was well tolerated. No patient reported any adverse effect.

Discussion

Our study evaluated the effect of 3-month intraovarian injection treatment course of PRP on ovarian reserve markers in women classified as poor ovarian responders before undergoing ART. The results of our study demonstrated that a 3-months treatment course with PRP improved ovarian reserve markers. These women achieved reasonable pregnancy rates of 25% after IVF–ET. However, our research study did not evaluated effect of PRP on miscarriage and live birth rate. Our study demonstrated that intraovarian injection of PRP results in rejuvenation of ovarian activity in all women (both <35 and ≥35 years of age). These are very encouraging results. The results of our study are similar to a recent prospective trial conducted by Melo et al. in 83 women with known diminished ovarian reserve.[18] They evaluated the effect of ovarian PRP injection on ovarian reserve and pregnancy outcomes. A total of 46 women received PRP treatment and 37 were in control group (no intervention). Women who underwent treatment with PRP had a significant improvement in pre and post treatment AMH (0.62 vs 1.01) and AFC (3 vs 7), respectively, at 3 months following PRP administration. The rates of biochemical pregnancy were 26.1% and clinical pregnancy were 23.9% in the PRP group which were higher than the group with no treatment.[19] Recently, Sills et al. investigated US-guided transvaginal intraovarian injection of PRP in case series of four patients with poor ovarian reserve having history of at least one previous canceled IVF cycle due to poor response. They reported increased AMH and /or significantly decreased FSH levels in all patients.[19]

Platelet-rich plasma promotes the development of pre-antral follicles, which responds to gonadotropins administered for controlled ovarian stimulation leading to development of ovulatory follicles. These outcomes of intraovarian PRP injection could be due to multiple growth factors present in PRP which may promote neo-angiogenesis in ovary and increase intraovarian blood supply. The improved intraovarian blood supply facilitates the development of pre-antral follicles.[20,21] However, the mechanism by which PRP improves the ovarian reserve is not clear thus far. Growth factors derived from PRP include multiple regulatory proteins. The multiple regulatory proteins present in PRP mediate chemical messages by attaching to cell membrane receptors. By this interaction, the signaling pathways are enabled which govern cell growth, proliferation, and differentiation.[22]

The presence of multiple growth factors in PRP such as platelet-derived growth factor, tissue growth factor-β, IGF-1/2, VEGF, and EGF play a role in development of the pre-antral follicles following treatment with intraovarian PRP injection, which leads to an increase in the AMH levels and also in the antral follicular count as suggested by Melo et al.[18] Sfakianoudis et al. demonstrated that autologous intraovarian PRP infusion may enable reactivation of folliculogenesis leading to restoration of ovarian function with recovery of the menstrual cycle in women with premature ovarian insuffiency.[21]

The procedure of PRP injection was well accepted by all the patients in our study. There were no adverse effects, suggesting that intraovarian PRP instillation is a safe technique. However, the randomized controlled trials involving large number of patients are required to establish efficacy and safety of intraovarian PRP injection in poor ovarian responders. The future RCT are also required to establish a protocol for intraovarian PRP administration.

This novel treatment using intraovarian PRP injection would be a welcome treatment option in poor responders seeking IVF treatment. The chief limitation of our study is that it was a non-randomized, non-comparative study involving a small number of patients.

Conclusion

This study demonstrated that the intraovarian injection of PRP in the poor responder improves ovarian reserve markers of AFC and AMH. It resulted in reasonable number of oocytes retrieval following ovarian stimulation and pregnancy following IVF–ET. It may be offered as an investigational treatment option for women who are found to be poor ovarian responders if they wish to use their own eggs instead of opting for donor oocytes. Further randomized controlled trials are required to demonstrate its benefits and if confirmed, to optimize PRP protocols in the future.

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