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Original Research Article

Efficacy of autologous platelet rich plasma for ovarian rejuvenation in infertile women having poor ovarian reserve

Jesmine Banu[1]*, Mostafa Tarique[1], Nishat Jahan[2], Nastaran Lasker[1], Nighat Sultana[1], Chowdhury Faisal Alamgir[3], Maliha Darmini[4], Sirajum Munira[1]

[1]Department of Reproductive Endocrinology and Infertility, BSMMU, Dhaka, Bangladesh
[2]Department of Gynecology, Nabinagar Upazila Health Complex, Brahmanbaria, Bangladesh
[3]Department of Urology, New Cross Hospital, The Royal Wolverhampton NHS Trust, United Kingdom
[4]Department of Gynecology, Mymensingh Medical College and Hospital, Mymensingh, Bangladesh

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***Correspondence:**
Dr. Jesmine Banu,
E-mail: drjesminebanu@gmail.com

ABSTRACT

Background: Poor ovarian reserve (POR) is a condition in which the ovary loses its normal reproductive potential and compromising fertility. Normal function of the ovaries and adequate good quality follicles are responsible for the reproductive process of a woman. Various treatment methods exist for POR, but the present study was conducted to observe the effectiveness of platelet rich plasma infusion through measurement of AMH and AFC values and pregnancy outcomes.
Methods: This prospective observational study was done in the department of reproductive endocrinology and infertility from July 2019 to June 2022. A total of 60 patients with poor ovarian reserve were recruited maintaining inclusion and exclusion criteria.
Results: Mean age of the participants was 36.4 years, with 78.32% of the participants being housewives and 21.68% being service workers. 65.38% had education below SSC levels. 72.46% had primary infertility while 27.54% had secondary infertility. At first cycle, compared to baseline counts, mean±SD AMH had increased by 0.04±0.15 ng/dl, and mean±SD AFC had increased by 1.34±1.89 in number. During second cycle post-PRP, the mean difference of AMH and AFC was 0.18±0.21 ng/dl and 2.17±1.71 in a positive manner. By final follow-up, pregnancy rate was 20% among patients.
Conclusions: The study observed significant improvement in AMH and AFC values following PRP infusion. The improvement of both values was gradual, and increase of AMH values were observed up to second post-PRP menstrual cycle, while AFC increased till third cycle post-PRP. Among 60 patient twelve (12) had pregnancy (20%).

Keywords: Infusion, Platelet, Plasma, Rejuvenation, Ovarian, Infertile

INTRODUCTION

Ovarian aging is a physiological process that causes a reduction in the quantity and quality of oocytes.[1] It has substantial consequences for fertility and is becoming a more prevalent reason for women to seek reproductive treatment.[2] Not only is the amount of oocytes decreasing, but so is their quality, resulting in poor reproductive results in older women, including decreased fertilization and blastocyst formation rates, as well as higher aneuploidy rates.[3] The age-related physiological decline in follicle number has major implications for fertility and is becoming an increasingly prevalent reason for women seeking reproductive treatment. Ovarian aging has risen in an infertile population subset. These women are referred to as POR or poor responders due to a combination of low ovarian reserve measurements and prior low egg yield after ovarian stimulation. In women of reproductive age, POR is described as a reduction in the amount of ovarian

follicular pool. It is a significant cause of infertility in many couples and a significant limiting factor for the success of any infertility treatment strategy. POR can be caused by disease, injury, or trauma, although it is most commonly caused by age. The European Society for Human Reproduction and Infertility (ESHRE) advised that two of the three criteria mentioned below result in a POR diagnosis to standardize the definition of POR: (i) advanced maternal age (40 years) or any other POR risk factor; (ii) a previous cycle with 3 oocytes harvested using conventional stimulation; and (iii) an abnormal ovarian reserve test (i.e. AFC, 5-7 follicles or AMH, 0.5-1.1 ng/ml).[4] Following the introduction of the POSEIDON criteria, a more complicated classification system that accounts for population heterogeneity, the terminology used to designate these individuals changed from POR to poor prognosis.[5] Women who use POR account for 15% of all assisted reproductive technology (ART) cycles performed in the United States. Reproductive endocrinologists have a harder time treating these patients because their cycles are more likely to be cancelled, resulting in fewer embryos available for transfer and lower pregnancy rates.[6,7] As a result, this group has tried a number of experimental strategies to boost follicle activation, enhance egg retrieval and improve IVF success. Kawamura et al recommended ovarian fragmentation for *in vitro* activation (IVA) in women with primary ovarian insufficiency in combination with Akt-stimulating drugs, and several subsequent trials have demonstrated favourable results.[8-10] Ovarian fragmentation increases actin polymerization, which disrupts intracellular Hippo signalling and promotes cell proliferation and primordial follicle activation.[11] Another study found that autologous stem cell ovarian transplantation (ASCOT) improved ovarian function and raised the number of antral follicles and oocytes in poor responders.[12] While these novel treatments are exciting, they are also quite invasive and have yet to be proven in randomized clinical trials. Another less invasive way of improving ovarian response in weak responders is intra-ovarian injection of platelet rich plasma (PRP). PRP is derived from whole blood centrifugation and contains a high concentration of growth factors and cytokines. Several of these factors promote healing and tissue regeneration by increasing chemotaxis, cell migration, and differentiation. PRP also stimulates follicle development in vitro, and small case studies show that it might be a helpful treatment for women with POR.[13-15] The current study was carried out to investigate the effects of ovarian rejuvenation using the PRP approach in individuals with low ovarian reserve.

Objective

General objective

The general objectives were to observe the effect of PRP therapy by measure of AMH level; to observe the effect of PRP therapy by measuring of AFC count; to observe the effect of PRP therapy by measuring of serum FSH level; to observe the effect of PRP therapy on pregnancy outcome.

METHODS

This prospective observational study was conducted at the department of reproductive endocrinology and infertility, BSMMU, from July 2019 to June 2022 flowing the inclusion and exclusion criteria in the study. During this period, a total of 72 cases were initially selected among them, 12 participants had been lost at follow-up through the study, either immediately after PRP or later. These cases were then excluded and the final study sample size was determined as 60. Patient selection was done following random selection method from any sub-fertile women of sound physical and mental health visiting the study hospital. Data was collected using a pre-tested data collection sheet containing structured questionnaire with all the variables of interest by interviewing detailed history, physical examination, laboratory investigation. Informed written consent was obtained from each participant and ethical approval of the study was also obtained from the ethical review committee of the study hospital. All baseline laboratory investigations were done during primary infertility work-up including TVS for D4/5 AFC, AMH, D2 FSHl. After pre-anesthetic check up with counselling, informed written consent for laparoscopic tubo-peritoneal evaluation under general anesthesia was taken. During laparoscopic evaluation, 5 ml of pre prepared autologous PRP was injected into each ovary by aspiration needle. After laparoscopy and PRP injection, post-operative patient management was done accordingly and advice was given for monthly follow up for three months on OPD of REI department. Collected data was analyzed using SPSS software. In order to monitor the effect of PRP injection into the ovary, AMH (anti-mullerian hormone), AFC (antral follicular count) and ovarian volume were measured at four weeks' intervals in women who did not menstruate and during the menstrual flow in menstruating women for a period of three months. Pregnancy outcome was observed either spontaneous or ovulation induction or intrauterine insemination.

Inclusion criteria

Women between the age of 25-40 years; AMH levels <0.7 ng/ml; AFC <5 in number were included in the study.

Exclusion criteria

·Premature ovarian failure (POF) due to genetic origin. such as Turner's syndrome or chromosomal abnormality; patient on anticoagulant therapy; those affected with other chronic diseases were excluded.

PRP process

Step 1: Collect the patient's own blood; step 2: centrifuge the blood; step 3: process and collect the platelets; step 4: inject the PRP into the desired site.

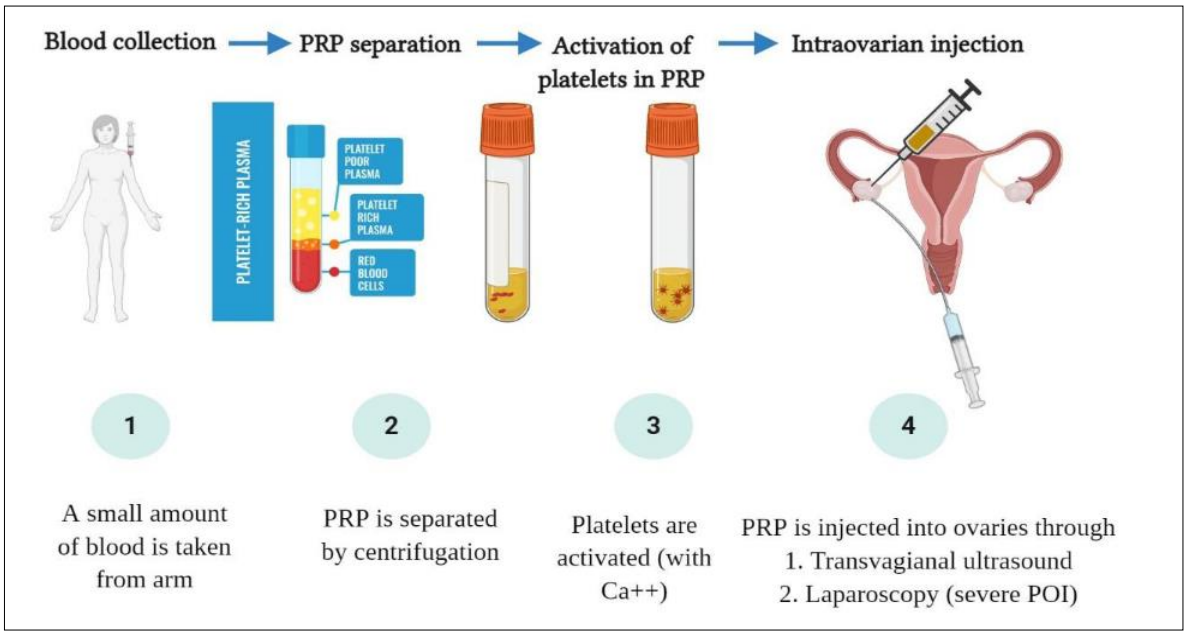

Figure 1: PRP process.

The follow up procedure

In order to monitor the effect of PRP injection into the ovary, AMH (anti-Mullerian hormone), AFC and serum FSH will be measured at four weeks intervals in women who do not menstruate and during the menstrual flow in menstruating women for a period of three months. If the AMH and AFC raise, and serum FSH become low there is objective evidence of ovarian rejuvenation is demonstrated. Parameters clinical data was collected for 3 year regarding changes of menstrual pattern, pregnancy outcome. Statistical analysis was done with Microsoft excel.

RESULTS

Among the participants, mean age was 36.4 years, with 78.32% of the participants being housewives and 21.68% being service workers. 65.38% had education below SSC levels, while the remaining had education up to or above SSC levels. 72.46% had primary infertility while 27.54% had secondary infertility. Menstrual pattern was normal for 83.8%, 16.2% had oligo-menorrhea and 5.9% had a family history of early menopause.

AMH values and AFC count was measured after PRP at different menstrual cycles. At first cycle, compared to baseline counts, mean±SD AMH had increased by 0.04±0.15 ng/dl, and mean±SD AFC had increased by 1.34±1.89 in number. Mean FSH had decreased by 2.08±0.31 mIU/ml.

During second cycle post-PRP, the mean difference of AMH and AFC was 0.18±0.21 ng/dl and 2.17±1.71 in a positive manner. The mean difference of FSH was 2.14±0.14 in a negative manner.

Table 4 shows a percentage presentation of AMH and AFC values at different menstrual cycles post-PRP compared to baseline. The values had continued to increase at different cycles. At 1st cycle, the mean increase was 58.62% and 55.17% in AMH and AFC respectively. At 2nd cycle, it was 86.21% and 79.31% respectively. At the 3rd post-PRP cycle, the AMH values did not rise any further, but the AFC values increased by 95.65%. The FSH values had a mean decrease of 67.27% at first follow-up, 81.19% at second and 92.61% at third follow-up, compared to baseline findings.

It was observed that at the final follow-up, 20% of patients had gotten pregnant, 3.33% of patients had gotten an abortion and 76.67% of the participants remained non-pregnant.

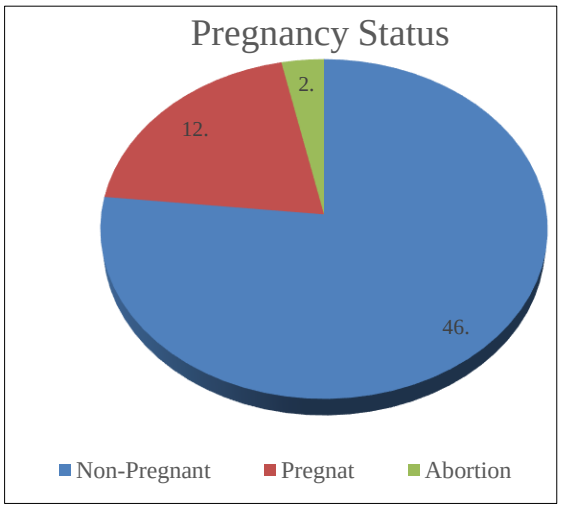

Figure 2: Distribution of the study participants by pregnancy status.

Table 1: Distribution of participants by demographic and clinical parameters (n=60).

Parameters	Percent
Age	
Mean±SD	36.4±2.8
Occupation (%)	
House wife	78.32
Service	21.68
Education (%)	
<SSC	65.38
≥SSC	34.62
Type of infertility (%)	
Primary	72.46
Secondary	27.54
Duration of infertility	
Mean±SD	7.2± 2.9
Menstrual pattern (%)	
Normal	83.8
Oligomenerrhoea	16.2
Family history of early menopause	5.9

Table 2: Changes of AMH and AFC values between baseline and first-cycle post-PRP (n=60).

Parameters	Baseline; mean± SD	First cycle post-PRP mean±SD	Difference of mean±SD
AMH (ng/dl)	0.32±0.16	0.37±0.14	0.04±0.15 (+)
AFC (number)	3.41±0.73	4.76±2.06	1.34±1.89 (+)
FSH (mIU/ml)	10.57±0.25	8.48±0.56	2.08±0.31 (-)

Table 3: Changes of AMH and AFC values between baseline and second-cycle post-PRP (n=60).

Parameters	Baseline; mean±SD	Second cycle post-PRP; mean±SD	Difference	95% CI of mean difference	
				Lower	Upper
AMH (ng/dl)	0.32±0.16	0.51±0.2	0.18±0.21 (+)	0.10	0.26
AFC (number)	3.41±0.73	5.59±1.76	2.17±1.71 (+)	1.52	2.82
FSH (mIU/ml)	10.57±0.25	8.43±0.11	2.14±0.14 (-)	1.48	0.29

Table 4: Percentage of changed AMH and AFC values at baseline compared to different cycles post-PRP (n=60).

Parameters	Baseline; mean±SD	At first cycle (%)	At second cycle (%)	At third cycle (%)	Mean percentage (%)
AMH (raised)	0.32±0.16	(58.62)	(86.21)	(86.21)	78.71
AFC (raised)	3.41±0.73	(55.17)	(79.31)	(95.65)	76.71
FSH (decreased)	10.57±0.25	(67.27)	(81.19)	(92.61)	80.36

DISCUSSION

The present study was conducted with the goal of studying the efficacy of intra-ovarian infusion of PRP on the improvement of ovarian reserve parameters in infertile women with POR or premature ovarian insufficiency. The study was conducted with a total of 60 patients who were properly followed up after infusion of PRP for a duration of three months or three menstrual cycles. PRP has been explored and used as a rejuvenating agent in several disciplines of medicine, including orthopedics, plastic surgery, dermatology, and dentistry.[16] Pregnancy rates in women with POR diagnosed using the POSEIDON criteria (groups 3 and 4) range from 12.7 percent to 35.5 percent.[17] In another study of 26,697 cycles, the first-cycle live birth rates in POSEIDON groups 3 and 4 were 14.73 percent

and 6.58 percent, respectively.[18] PRP's influence on pregnancy rates in POR patients has also been studied. In a prospective controlled non-randomized research, Melo et al investigated the impact of intra-ovarian PRP injection in 83 women with POR.[19] They discovered that the PRP group had higher biochemical (26.1% vs. 5.4%, p=0.02) and clinical (23.9% vs. 5.4%, p=0.03) pregnancy rates. In the present study among 60 participants, mean age was 36.4 years. This was within the reproductive age, and measuring the AMH and AFC counts revealed decreased values compared to normal findings. At the baseline, the patients had a mean AMH value of 0.32 and a mean AFC value of 3.41. Considering the mean age of the participants, the AMH values were well below the normal range.[20] The mean AFC count at baseline was much lower even compared to the infertile groups of other studies, at 3.41. Some studies observed AFC count of 10 and above even among infertile women, and direct relation between decreased AFC and infertility was observed in multiple studies.[20-22] After intra-ovarian infusion of PRP, positive improvement was observed during the first menstrual cycle, mean AMH had increased by 0.37 ng/dl, while AFC had increased by 4.76 in number. During the second cycle, AMH had increased by 0.18 ng/dl and AFC by 2.17 count. It was observed that compared to baseline values, mean AMH had increased by 58.62% at the first cycle, by 86.21% at second cycle, but showed no further improvement at third cycle. The increase of AMH was similar to the findings of other studies using PRP for ovarian rejuvenation.[23,24] In other comparative studies, incidence of pregnancy had increased during the study period, but the present study was conducted while excluding any pregnancy cases to observe the measurable effect of PRP in terms of AMH and AFC count. The mean increase in AFC at first cycle was 55.17%, lower than AMH increase, but by the third cycle, mean AFC had increased by 95.65% compared to baseline. PRP had been observed to increase the AFC count in women with severely decreased AFC.[13,19,23,24] Compared to AFC and AMH, mean FSH values decreased post-PRP. At first cycle, the mean FSH decreased by 2.08 mIU/ml and at second post-PRP cycle, it decreased by 2.14 mIU/ml compared to baseline. Among the total 60 participants, by the time of the 3rd post-PRP cycle, 12 patients had been pregnant and 2 more had been pregnant but opted for pregnancy. During this short period of time, ovarian PRP infusion had a pregnancy success rate of 23.33%, which was similar to other previous studies with similar short follow-up history.[18,21-23] PRP had been proven to be an effective method for ovarian rejuvenation in terms of AMH and AFC in the present study. Some studies have observed even further improvement after combining the autologous bone marrow-derived stem cells and PRP for ovarian rejuvenation.[25]

Limitations

The study was conducted in a single hospital with a small sample size. So, the results may not represent the whole community.

CONCLUSION

The study observed significant improvement in AMH and AFC values following PRP infusion. The improvement of both values was gradual and increase of AMH values were observed up to second post-PRP menstrual cycle, while AFC increased till third cycle post-PRP. 2/8th of the patients had been pregnant by the final data collection of the study.

Recommendations

Further studies with dedicated control group are necessary to better understand the effect of infusion of PRP among infertile participants.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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