Effect of intra ovarian injection of platelet rich plasma to patients with diminished ovarian reserve (DOR)- A Prospective Study

Author

Dr Vipin Kumar[1],Dr Kasturi R Nath[2],Dr Swati Dongre[3],Dr Asem Tokenova[4],Dr Nargis Ashurova[5]

[1]Consultant Gynecologist, Miracle IVF Clinic Almaty, Kazakhstan
[2]Assistant Professor, Department of Community Medicine, Dr SMCSI Medical College, Karakonam, Thiruvananthapuram, India
[3]Chief Reproductologist, Santati IVF Centre, Thane, India
[4]Reproductologist, Miracle IVF, Almaty, Kazakhstan
[5]Reproductologist, Miracle IVF, Tashkent, Uzbekistan

Corresponding Author: Dr Kasturi R Nath, Assistant Professor, Department of Community Medicine, Dr SMCSI Medical College, Karakonam, Thiruvananthapuram, India

ABSTRACT

Background: Diminished ovarian reserve (DOR) is among the common findings in infertile women with no significant underlying condition especially women with advanced age. The aim of this study was to investigate the intra-ovarian potential of platelet-rich plasma (PRP) administration on oocytes-dependent variables in the DOR women.A few years ago, we used intrauterine PRP injection in patients with recurrent implantation failure (RIF) which led to noticeable findings.Nowadays, PRP is used more widely in reproductive medicine due to its regenerative potentials; however, not enough data is available on this issue.One of the new strategies for facing primary ovarian insufficiency is PRP therapy.

Material and Methods: This is a Prospective cohort study conducted in three IVF Clinics Thane-India, Almaty-Kazakhstan and Tashkent-Uzbekistan from October 2021 to September2022. All couples with failed IVF with diminished ovarian reserve as per ARSM definition between the age group of 35 to 45 (both inclusive).To standardize the definition of poor ovarian response, a European Society of Human Reproduction and Embryology Working Group convened in Bologna and proposed that two of the following criteria be present to define whether a given low response to stimulation is truly representative of poor ovarian response:1) Advanced maternal age (R40 years). 2) A previous poor ovarian response. 3) An abnormal ovarian reserve test. (AMH less than 1)

Result: Demographic characteristics, baseline ovarian reserve markers and ultrasound findings of women included in the analysisTable 1.Women treated with PRP had significant improvement in biochemical and ultrasound markers of ovarian reserve. Notably, AMH levels were on average 72% higher following PRP ($P < 0.001$). Of the 124 women who underwent IVF/ICSI following participation in the study, those with previous PRP treatment yielded an average more than 1.5× the number of oocytes

collected. Nevertheless, the rate of medium- and top-quality embryos in participants who received PRP treatment was significantly higher.

Conclusion:This study revealed that the injection of PRP into human ovaries is safe and improves ovarian reserve markers as measured by antral follicle count and serum levels of AMH and FSH. Nevertheless, further studies are needed to evaluate the impact of PRP on pregnancy outcomes in women undergoing ART.

Keywords: Platelet-rich plasma, Ovarian reserve, Assisted reproductive techniques.

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INTRODUCTION

Aging causes different changes in the physiology of the human body and is associated with increased risk of infertility in females. [1] Over the course of time, ovaries experience a decrease in their follicular quantity (ovarian reserve) also knowns as poor ovarian reserve. [2] Nowadays, assisted reproductive techniques (ART) are being widely used in the clinic to overcome this problem. [3] However, some patients do not respond to the stimulators administrated (such as gonadotropin) before ART which has been described as poor ovarian response (POR). [4]

Despite age, so far, different laboratory tests and ultrasound investigations have been used as routine techniques to predict the probability of ART success in these cases. [5] Also, anti-Mullerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin, and estradiol levels are among the helpful laboratory tests for this aim. [6] However, invasive methods such as ovarian biopsy have also been mentioned by studies. [7] So far, a notable number of investigations have tried to defineDOR according to the clinical and para-clinical status of cases as a standard definition. [8]

So far, platelet-rich plasma (PRP) has been used in many trials for accelerating the healing of acute and chronic wounds, plastic surgeries, tendinopathies and other regenerative goals. [9] A few years ago,intrauterine PRP injections were used in patients with recurrent implantation failure (RIF) which led to noticeable findings. [10] Then, it was aimed to use this autologous product for women with poor response to gonadotropin stimulation as well. [11] Nowadays, PRP is used more widely in reproductive medicine due to its regenerative potentials; however, not enough data is available on this issue. [12] One of the new strategies for facing primary ovarian insufficiency is PRP therapy. A report on 23 women (of 311) with primary ovarian insufficiencywho conceived spontaneously has shown that PRP could be a proper treatment option for these patients. [13]

Recently, PRP therapy has been used as a possible treatment for 17 women diagnosed with poor ovarian response (POR) which seemed potent enough to be a possible future treatment according to the obtained results. [14]Through this study we aimed to assess the outcome of PRP therapy on the hormonal profile (serum AMH, LH and FSH) and to determine the the number and quality of oocytes retrieved after PRP therapy.

MATERIALS AND METHODS:

This is a Prospective Cohort study conducted in three IVF Clinics fromOctober 2021 to September2022.

PLACE OF STUDY: 3 IVF clinics in

a. Thane, India
b. Almaty, Kazakhstan
c. Tashkent, Uzbekistan

SAMPLE SIZE:297 patients who registered in either of the clinic in Thane, Almaty, or Tashkent. 163 were excluded time to time based on the exclusion criteria. Finally, 124

patients were enrolled for the study to draw the required conclusions

INCLUSION CRITERIA:

Couples with failed IVF and with diminished ovarian reserve as per ARSM definition who falls in between the age group of 35 and 45 (both inclusive) were enrolled for the study by convenient sampling method and underwent PRP therapy after a proper consent was obtained.

To standardize the definition of poor ovarian response, a European Society of Human Reproduction and Embryology Working Group convened in Bologna and proposed that two of the following criteria be present to define whether a given low response to stimulation is truly representative of poor ovarian response:

1) Advanced maternal age (R40 years).
2) A previous poor ovarian response.
3) An abnormal ovarian reserve test.(AMH less than 1)

EXCLUSION CRITERIA:

a) Lost to follow up
b) Incomplete hormonal assessment
c) Other medical disorders which can be cause of infertility rather than only diminished ovarian reserve. It's excluded at first meeting/consultation with the patient in the clinic.
d) Positive viral markers (HIV/Hepatitis B and C)

CONDUCT OF STUDY

After the patients were enrolled a recent lab analyses of following hormones were made. The analyses were done on day 2 of menstruation .

a. Serum LH
b. Serum FSH
c. Serum Estradiol
d. Serum AMH

A Record was obtained from previous IVF cycle to as the number of Oocytes retrieved and also the number of MII (means good quality oocytes) among them.

Then patient within first seven days of menstruation underwent Platelet rich plasmaadministration to both the ovaries under sedation using a 24-gauge size aspiration needle. The procedure was done after obtaining the consent from the patient about the novelty of procedure and risk and complications associated.

After the procedure, patient underwent the stimulation protocol (standard antagonist) on the second menstruation post procedure. Time was given for the action to start.

On the second day again following hormones were repeated.

a. Serum LH
b. Serum FSH
c. Serum Estradiol
d. Serum AMH

On the day of egg retrieval, number of oocytes retrieved was noted and confirmed byexperienced embryologist and also the number of MII was gathered.

PRP preparation

PRP preparation was performed by separating plateletrich plasma after centrifugation. Approximately 20 ml of blood sample was collected under sterile conditions, and PRP was prepared using T-lab autologous plateletrich plasma kit according to the manufacturer's instructions. Briefly, after blood collection, the tubes were centrifuged at 830 rpm for 8 minutes. Then, a 16 G needle connected to a 5 ml syringe was inserted into the tube and advanced to the buffy coat layer. The PRP was collected by rotating the needle tip. After collecting approximately 2-4 cc PRP from the first tube, the second tube was processed similarly (a total 4-8 cc PRP was collected). The collected solution was transferred to the re-suspension tube and shaken gently for 30s-1 min.

Intraovarian injection

The same day, within 2 hours of sample preparation, PRP injection was performed transvaginally under ultrasound guidance and under sedation anesthesia into at least one ovary using a 35 cm 24G single lumen needle. The injection was done underneath the ovarian cortex to the subcortical and stromal areas. Although women with POI have small fibrotic ovaries making intraovarian injection of 2-4 ml of PRP challenging, this was achieved through distention, possibly by creation of new planes and making the injection in multiple sites within the ovaries. After the procedure, the patients were taken to the recovery room and observed for 30-40 minutes and discharged home on the same day.

Timing of PRP injection, patient assessment and follow-up

PRP injection was timed randomly in women who were amenorrheic, while in women who reported oligomenorrhea, PRP was injected on the seventh day of menstrual bleeding. Baseline antral follicle count (AFC), serum anti-mullerian hormone (AMH) and follicle stimulating hormone (FSH) levels were determined prior to PRP injection, on the same day. Intra-assay coefficient of variation for AMH was < 0.055 ng/mL. Intraassay coefficient of variation for FSH was <5.1%.

After PRP injection, both amenorrheic and oligomeorrheic women were managed expectantly for 6 weeks. Those who did not have a menstrual bleed for 6 weeks underwent pregnancy testing and (if not pregnant) menses was hormonally induced (2x (2 mg estradiol valerate) for 15 days and 2x (0.5 mg norgestrel) for 5 days). The same strategy was repeated if menstruation was delayed in the subsequent cycle. On the 2-4th days of the second menstrual cycle after the PRP procedure, AFC and serum AMH and FSH levels were re-assessed. Those who were found to have antral follicle(s) at that point were started on controlled ovarian hyperstimulation (COH), while those who did not were followed monthly, up to 6 months, and underwent COH when/if they developed antral follicle(s). Following each assessment, patients who did not develop antral follicles were treated with cyclic estrogen and progesterone (2 mg estradiol valerate for 10 days and 2 mg estradiol valerate + 0.5 mg norgestrel for 11 days). This was done for ease of scheduling and to control cycle progress

Positive response to PRP was considered when at least one antral follicle was seen on ultrasound in women without any antral follicles at baseline or an increase in AFC compared to baseline measurements or an increase in AMH or a decrease in serum FSH levels in women with the same AFC when compared to pre-treatment measurement.

Women who were found to have at least one antral follicle (even if the number was not higher compared to pre-PRP evaluation) were started on ovarian stimulation for IVF-ICSI. In cases with an increase in AMH or decrease in FSH, but no antral follicles compared to basal measurements, stimulation was not started.

Controlled ovarian hyperstimulation and IVF

Controlled ovarian hyperstimulation (COH) was started on the second or third day of the induced menstrual cycle. Gonadotropin stimulation was started at 300 IU recombinant FSH or 300 IU human menopausal gonadotrophin (hMG). When the dominant follicle reached a mean diameter of 14 mm, cetrorelix 0.25 mg /d s.c was administered. Patients were monitored with serial serum E2 and progesterone measurements and transvaginal ultrasonographic examinations. When at least one leading follicle reached a mean diameter of 18 mm, 250 mcg recombinant chorigonadotrophin alfa (rHCG) was used to induce follicle maturation.

Statistical analysis

Data distribution was tested with the Shapiro–Wilk test. Differences between pre- and post-treatment values were compared using the

Wilcoxon test. Logistic regression was used to estimate the odds ratio of having at least one mature oocyte or at least one cleavage stage embryo in percentile categories after adjusting for age. Stratified analysis was also performed to investigate the odds ratio among women in different age groups. As post-PRP outcomes have not yet been determined in cohort studies of adequate samples size, we were not able to perform a reliable power analysis prior to the initiation of the study. All data were analyzed using SPSS (SPSS-IBM 2.3, Inc., Chicago, IL, USA). p values less than 0.05 were considered statistically significant.

RESULTS

Table 1 Demographic characteristics of study population

	PRP (n = 124)
Median Age (years)	43 (41-45)
Body mass index (kg/m^2)	26.5 (23.9-27.8)
Smoker, n (%)	11(8.8)
Nulliparity, n (%)	89 (71.7)
Previous miscarriage, n (%)	
0	109 (87.9)
1	12 (9.6)
≥ 2	3 (2.4)
Baseline AMH (ng/mL)	0.73 (0.51-0.81)
Baseline day 3 FSH (mIU/mL)	14.9 (13.8-18.6)
Baseline AFC	6 (5-7)

In Table 1, Demographic characteristics, baseline ovarian reserve markers and ultrasound findings of women included in the analysis were observed.

Table 2: Comparison of ovarian reserve parameters pre- and post-treatment

	PRP (n = 124)		
	Pre-treatment	**Post-treatment**	**P value**
AMH (ng/mL)	0.73 (0.51 to 0.81)	1.09 (0.8 to 1.9)	<0.001
FSH (mIU/L)	14.9 (13.8 to 18.6)	10.09 (8.7 to 11.8)	<0.001
Total AFC (n)	6 (5 to 7)	9 (8 to 10)	<0.001

Women treated with PRP had significant improvement in biochemical and ultrasound markers of ovarian reserve (Table 2). Notably, AMH levels were on average 72% higher following PRP (P < 0.001).

Table 3 Cycle characteristics of participants undergoing IVF/ICSI

	IVF/ICSI (n = 124)
Number of oocytes collected	6.0 (3.0-11.0)
Number of fertilized oocytes	5.0 (2.0-9.0)
Fertilization rate	0.7 (0.44-2.0)
Embryo quality*, n (%)	
Top and medium	79 (100)
Low	0

Of the 124 women who underwent IVF/ICSI following participation in the study (Table 3), those with previous PRP treatment yielded on average more than 1.5× the number of oocytes collected. Nevertheless, the quality (M11) of oocytes retrieved after PRP is also significantly increased in participants who received PRP treatment was significantly higher.

DISCUSSION

This study of 124 extremely poor prognosis patients between the ages of 35–45 yearsrevealed little objective hormonal and/or IVF outcomerelated effects of PRP after comparing IVF cycle outcomes before and after PRP and, within the study population, comparing outcomes between women with regular menstrual cyclicity and women with oligo-amenorrhea. Two 40-year-old patients with prior unproductive IVF cycles at other IVF centers, however, conceived and delivered viable offspring. We also observed a statistically significant increase in AFCs post-PRP in comparison to pre-PRP

Considering how adversely patients in this prospective cohort study were selected, these observed results must be interpreted cautiously. This study must also be viewed as only one of three currently ongoing registered PRP studies at our center, with the two others having much more rigid patient selection. This interim analysis, therefore, presents a more diverse patient population since this prospective cohort study was open to every patient who did not qualify for the other two clinical trials and only had third-party oocyte-donation left as a potential alternative to PRP.

In this study, and in all of our PRP studies, we use a thin 24-gauge needlefor the PRP infusion to minimize mechanical effects in the ovary which have been shown by others to activate dormant follicles However, it is still possible, even with the thin needle, that with 7 to 12 punctures per ovary, the mechanical disruption could cause some follicle activation.

That this study, therefore, in several aspects, did not match previously reported PRP outcomes (Cakiroglu et al., 2020) should not surprise.[14] It, therefore, at least as of this moment, should not be interpreted as a rejection of all PRP treatments in women with LFOR. This study, however, and again, unsurprisingly, offers preliminary evidence that, like most fertility treatments, any success of intraovarian PRP may be patient dependent on patient age, with younger patients doing better than older women.

In a prospective non-randomized trial, Melo et al. (2020)[15] compared, 39 to 44year-old patients who chose PRP before IVF to 37 who rejected the treatment. Like in this study, they found no significant difference in AMH, Day-2 FSH and AFCs. However, they reported, in post-PRP cycles, a significant improvement in oocyte yield (5 versus 3 oocytes), improvements in embryo quality and higher clinical pregnancy rates (23.9% versus 5.4%), but no difference in live birth rates (8.7% versus 2.7%). Although the lack of randomization and absence of provider-blinding obviously weakens their findings, considering how much younger their patients were in comparison to the women in the

present report, their results can indeed be viewed as surprisingly similar.

Sills et al reported a prospective, though not randomized, trial of 182 patients who received PRP after a previous failed IVF cycle. These investigators did find a statistically significant improvement in AMH in women both under and over the age of 42-years-old, with a peak response 4 weeks after PRP. However, with the increases in AMH in women under the age of 42-years-old only going from 0.21 to 0.32 ng/ml, their positive findings may have questionable clinical significance and may only reflect a simple regression to the mean. A more recent cohort study of 311 young women with evidence of LFOR found increased AFCs and AMH levels following PRP treatment. In that study, 23 women conceived spontaneously, and an additional 201 women underwent an IVF cycle after undergoing PRP treatment, resulting in 13 pregnancies and 9 (4.5%) live births (Cakiroglu et al., 2020).[14] Therefore, that study's results in certain aspects again approximated the here-reported results.

Additionally, the studies published so far are difficult to compare (Sharara et al., 2021)[15] because of technical differences in how the PRP was prepared, difference in PRP volumes injected into ovaries and, indeed, different locations of injection. So, for example, some earlier studies used activated PRP (Sills et al., 2020),[16] while we chose not to use activated PRP based on studies in other medical fields in which activation did not improve results (Raeissadat et al., 2015).[18] Because of the experimental nature of this study, we also tried to minimize the volume of injected PRP into the ovary and, therefore, used smaller amounts than reported in most earlier studies. Finally, other studies injected intramedullary into the ovary, expecting diffusion into the subcortical layers (Sills et al., 2019)[16] but since primordial follicles are in the ovarian capsule, we felt that, and possibly also because of their mechanical impact, multiple subcapsular injections may be more appropriate.

Conclusions

This study revealed that the injection of PRP into human ovaries is safe and improves ovarian reserve markers as measured by antral follicle count and serum levels of AMH and FSH. Nevertheless, further studies are needed to evaluate the impact of PRP on pregnancy outcomes in women undergoing ART.

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