



# EFFECT OF INTRA OVARIAN PRP ON THE QUALITY AND QUANTITY OF OOCYTES, AND FERTILIZATION RATE

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## Abstract

Infertility is on a rise in the current scenario. There are various factors that contribute to infertility. One of them is ovarian dysfunction. Ovarian dysfunction includes PCOS, ovarian aging, etc. Ovarian aging is a physiological process wherein the antral follicle count decreases thereby leading to decrease in the quantity and quality of the oocytes and causing menopause in females. Premature ovarian ageing is increasing in females who are below the menopausal age. In order to restore the ovarian reserve, one of the therapeutic treatments that is being used is autologous platelet rich plasma therapy. The platelets are rich in growth factors, integrins, chemokines and cytokines which promote cell division and differentiation, angiogenesis, cell migration, tissue repair and restoration. The platelets are extracted from the whole blood and are then injected into ovaries. Following the injection, the stimulation is started from the next cycle and the IVF procedure is performed. The objective of our study is to see the effect of PRP on the quality and quantity of oocytes, fertilization rate and implantation rate and to compare it with IVF cycle done prior to PRP. It was observed that there was an increase in the quality and quantity of oocytes and fertilization rate in post PRP IVF cycles compared to Pre cycles. The significance level of our study is above 95% hence proving the data statistically significant.

**Keywords:** Infertility, IntraOvarian, Autologous Platelet Rich Plasma Therapy, Premature Ovarian failure.

## INTRODUCTION

Infertility is defined as the inability to conceive even after trying for 1 year without the use of protection and is currently a global issue affecting 8 - 10% of total global population, whereas the infertility prevalence rate in India itself is 25%. There are various factors that are contributing to infertility in both males and females. In couples struggling to infertility, Assisted Reproductive Techniques (ART) can come to rescue. There are different methods of ART which can be helpful in conceiving.

One of the common causes in females that is leading to infertility is the diminish in the ovarian reserve. Ideally, there are a number of follicles present in the ovaries which decrease over the time and are usually diminished by the time a female reaches menopausal age, but this decrease in ovarian reserve is being observed in females in the fertility age group that is 21 to 35 years old and is known as Poor Ovarian Reserve (POR). The exact cause of POR is not known although there could be possible reasons such as genetic factors, environmental factors, lifestyle factors etc.

[1]

The females diagnosed with POR usually have low Antral Follicle Count (AFC's) and low AMH levels. In order, to classify any female with POR Posedion classification is used. According to



Posedion, there are four groups of POR. The first group consists of females who are less than the age of 35 years with AFC of more than or equal to 5, AMH levels more than or equal to 1.2 ng/mL and who have retrieved less than 4 oocytes in previous stimulated cycles. The second group consists of females who are more than the age of 35 years with AFC of more than or equal to 5, AMH levels more than or equal to 1.2 ng/mL and who have retrieved less than 4 oocytes in previous stimulated cycles. The third group consists of females who are less than the age of 35 years with AFC less than 5 and AMH levels less than 1.2 ng/mL. The last fourth group consists of females who are more than the age of 35 years with AFC less than 5 and AMH levels less than 1.2 ng/mL [2].

In patient's struggling with POR, Platelet Rich Plasma (PRP) therapy is found to be very much fruitful. The PRP is derived from blood via centrifugation separating it from the rest of the blood components. These platelets contain alpha granules within them which when activated releases plenty of factors which contribute in the growth, proliferation and angiogenesis. The growth factors present in the platelets are found to be an important key player in many tissue level processes such as enhancing the collagen synthesis, proliferating bone cells, chemotaxis of immune cells, macrophage activation, angiogenesis, cytokine secretion by mesenchymal and epithelial cells etc. PRP treatment is used as an adjunct along with the Assisted Reproductive Treatments such as Endometrial PRP prior to embryo transfer in order to increase the thickness of endometrium thus increasing the chances of implantation and as Intraovarian PRP in females with POR to increase the follicle count and the number of oocytes [3,4,10].

Our paper mainly aims in studying the effect of Intraovarian PRP by comparing the oocyte quality, number of oocytes and embryo fragmentation in the patients who have undergone 1st IVF cycle, followed by intraovarian PRP treatment and then again with 2nd IVF cycle. The inclusion criteria for our study were - women with POR between the age group of 20 - 35 years, male with normal semen parameters and previous IVF failure. This comparison will help in having a clear understanding about the Intraovarian PRP role and its effectiveness thereby offering valuable insights for patients facing infertility.

## METHODOLOGY

A total of 10 females with poor ovarian reserves underwent the first IVF cycle with an all-freeze cycle. Following the outcomes of the first cycle, all the females underwent the ovarian PRP treatment along with diagnostic laparoscopy.

Pre PRP treatment stimulation:

For the first IVF cycle; all the patients were stimulated with Inj HMG 300 IU from the 2<sup>nd</sup> day of menses for 5 days. Following the 5 days, all the patients were screened and an antagonist was added for another 5 days. On the 10<sup>th</sup> day of the cycle, the patients were screened again for checking the follicle sizes. If the follicles were found of the size 20 mm or more, Inj. Decapeptyl 0.2 mg SC trigger was given and in patients whose follicle did not reach size were stimulated for 2 more days and then they were given the same trigger.

Oocyte pickup was done using a single lumen needle under general anaesthesia. The follicular fluid was scanned for oocyte cumulus complex. Once the scanning was completed; all the OCCs were transferred in 1 ml of culture media and incubated for 3 hours. Fresh semen sample was collected, and prepared using swim up technique. After 3 hours, the OCCs were denuded and ICSI was performed. The injected oocytes were transferred in the culture dish containing culture media with an oil overlay and the dish was transferred in trigas dry incubator. Fertilization check was performed the next day at 18-20 hours post injection. The embryos were subjected to fresh media again on day 3 and were cultured till day 5. On the 5th day, when the embryos were in the blastocyst stage, they were graded along with embryo fragmentation and then they were frozen.

PRP Treatment:

All the females underwent diagnostic laparoscopy along with Intra ovarian PRP treatment between days 7 to 10 of menses. For the PRP treatment, 16 mL of intravenous blood was collected in ACD vacutainer tubes. The tubes were labelled with patient's name and then were centrifuged at 1000 rpm for 10 mins to separate the plasma from the rest of the blood. Once the run was completed, the plasma was collected with the help of a sterile 3 mL Pasteur pipette and transferred into a 15



mL conical tube. The tube was again centrifuged at 2200 rpm for 20 min. After 20 mins, the platelets formed a pellet at the bottom of the conical tube. The plasma supernatant was discarded keeping 1 mL in the tube. The pellet was then suspended into the remaining plasma. This prepared sample was injected into the ovaries at different points in the cortex. The patients were given a break for 2 months before starting for another stimulation cycle so that the PRP injection can also stimulate the development of the follicles.

Post PRP stimulation:

After the break of 3 months, the patients were again stimulated with injection HMG 300 IU from day 2 of menses for 5 days. On day 6th, the patients were scanned for follicular growth and then antagonist was

added for 5 days. On day 10th of menses, the females were scanned again. If the follicles were of the size 20 mm or more, Inj. Decapeptyl 0.2 mg Sc trigger was given.

Oocyte pickup was done using a single lumen needle under general anaesthesia. The follicular fluid was scanned for oocyte cumulus complex. Once the scanning was completed; all the OCCs were transferred in 1 ml of culture media and incubated for 3 hours. Fresh semen sample was collected, and prepared using swim up technique. After 3 hours, the OCCs were denuded and ICSI was performed. The injected oocytes were transferred in the culture dish containing culture media with an oil overlay and the dish was transferred in trigas dry incubator. Fertilization check was performed the next day at 18-20 hours post injection. The embryos were subjected to fresh media again on day 3 and were cultured till day 5. On the 5th day, when the embryos were in the blastocyst stage, they were graded and they were frozen.

## STATISTICAL ANALYSIS

Since our data was not normally distributed, we have performed a non- parametric test that is Wilcoxon Signed Rank test.

## RESULT

In our study we observed a significant change in the quality of oocytes when comparing the oocytes from pre PRP treatment and Post PRP treatment. There has been a decrease in the abnormal cytoplasmic or extracytoplasmic oocytes.

The number of OCC retrieved in the Pre PRP cycle and Post PRP cycle has also been changed. There is an increase in the total number of oocytes. The average mean of the OCC prior to PRP treatment was  $4.2 \pm$

$2.5$  while that in post PRP treatment was  $9.1$

$\pm 4.1$  The Z value is  $2.5$ .

The number of mature oocytes retrieved has also been increased. The mean mature oocyte in Pre PRP treatment was  $2.66 \pm$

$2.12$  while in the Post PRP treatment was

$7.4 \pm 3.8$ . The Z value for the same is  $2.37$ .

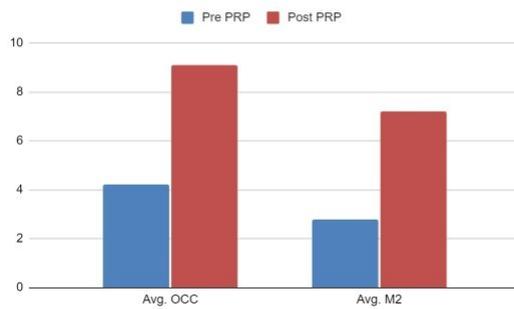
We have also observed an increase in the number of blastocysts formed in the Post PRP treatment cycle as compared to the Pre PRP-treated cycle. The average mean of blastocysts  $1.3 \pm 1.05$  while the average mean of post PRP treatment cycle was  $4.1$

$\pm 2.5$ . The Z value is  $2.67$

The embryo fragmentation is found to be decreased in the Post PRP treatment IVF cycle while it was higher in the Pre PRP treatment IVF cycle.

Based on the Z values we can interpret that our Alternate Hypothesis stating that Autologous PRP treatment is found to be useful in the treatment for the patients suffering from POR.

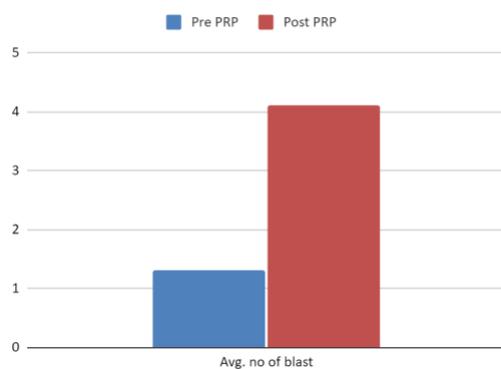
Average Oocyte Cummuls Complex & Mature Oocyte  
Comparing Pre PRP vs Post PRP



**Fig. Bar chart showing comparison in the Total no of OCC in IVF cycle**

Comparison of Blastocyst Formed

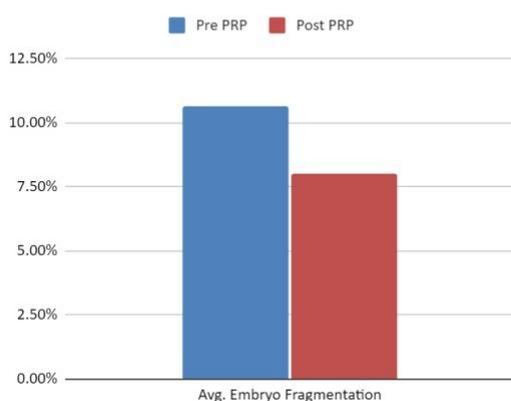
In Pre vs Post PRP IVF Cycle



**Fig.: Bar chart showing comparison in the M2 oocytes retrieved in IVF cycle**

Comparison of Embryo Fragmentation

in Pre PRP vs Post PRP



**Fig.: Bar graph showing comparison in the embryo fragmentation in IVF cycle**

## DISCUSSION

In our study we evaluated the effect of Intraovarian PRP on the oocyte quality, quantity and embryo fragmentation rate in women who underwent IVF treatment. The results have indicated that there is a significant change observed by an increase in the quality and quantity of oocytes and a decrease in embryo fragmentation. These findings suggest that intraovarian PRP can be a promising treatment in patients suffering from infertility due to POR.

There has been an increase in the oocyte quality, quantity and number of blastocyst and a decrease in the embryo



fragmentation. There has also been an increase in the M2 oocytes retrieved. One major limitation of our study is absence of a control group where some patients would have undergone multiple IVF cycles without PRP treatment.

Many studies have also shown an increase in embryo count [5,6,7, 12, 13].

A study by Melo et.al in 2020, revealed that women with POR, who had undergone intraovarian PRP showed an increase in higher antral follicle count and embryo quality compared to those women who did not receive any treatment [14]. Various authors have shown an increase in the total number of OCC and M2 oocytes as well as the quality of oocytes in post PRP treatment compared to pre PRP treatment [16,17, 18,17].

Studies have found an increase in serum AMH level and a decrease in FSH levels. The FSH is inversely proportional with the quality of oocytes. A study by Santos et al., found that women with increased FSH had poor quality of oocytes characterized with granular cytoplasm and presence of vacuoles [10].

A study has revealed that the most effective therapeutic treatment of PRP can only be observed when the cytokines and growth factor are injected into the ovaries directly. The possible mechanism behind this can be cytokine signalling between the oocyte, granulosa cells and theca cells. Another possible mechanism can be the communication between the plasma proteins and platelets which leads to the reservoir of growth factors. Out of the 7 main growth factors associated with platelets, the Transforming growth factor  $\beta$  isoform 1 is of major importance as it is involved in cell proliferation, angiogenesis and the deposition of extracellular matrix [7, 8,13,14,15].

There has also been an increase in the implantation rate and live birth rate in patients post PRP treatment [13, 14].

The PRP treatment has been also found to be beneficial in increasing the endometrial thickness prior to embryo transfer [12].

PRP treatment has also been found to resume the menstrual cycle in 8 perimenopausal women [9, 11].

Intraovarian PRP has also been associated with an increase in euploid embryos with an explanation that there is a probability that the PRP treatment can improve the quality of oocyte by altering the mitochondrial damage, thereby leading to normal mitosis and hence a greater number of euploid embryos [17].

## CONCLUSION

Our study consists of a small sample size. Despite the small size, we have observed significant changes in the treatment. The future prospects could be in analyzing the exact mechanism of how the Intraovarian PRP decreases embryo fragmentation. Moreover, a customized volume preparation for the patients can be helpful as it can maximize the result. The role of PRP has been found to be beneficial in women with POR, so it can further be used in treating male infertility due to Non obstructive Azoospermia.

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