INTERNATIONAL JOURNAL OF CLINICAL EMBRYOLOGY & REPRODUCTIVE BIOLOGY

IJCERB
Department of Biochemistry & Forensic Science

ISSN: In Process

EFFECT OF INTRAOVARIAN 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. Ovarianagingisaphysiologicalprocesswhereintheantralfolliclecountdecreases 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. Inordertorestoretheovarianreserve,oneofthetherapeutictreatmentsthatisbeingused 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 compareitwithIVFcycledonepriortoPRP.Itwasobservedthattherewasanincreaseinthe 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,AutologousPlateletRichPlasmaTherapy,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 currentlyaglobalissueaffecting8-10%of total global population, whereas the infertility prevalence rate in India itself is 25%. There are various factors that are contributingtoinfertilityinbothmalesand females.Incouplesstrugglingtoinfertility, 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 isleadingtoinfertilityisthediminishinthe ovarianreserve.Ideally,thereareanumber 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 OvarianReserve(POR).Theexactcauseof POR is not known although there could be possible reasons such as genetic factors, environmentalfactors,lifestylefactorsetc.
[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. Thefirstgroupconsistsoffemaleswhoare 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

retrievedlessthan4oocytesinprevious
stimulated cycles. The second group consists of females who are more than the age of 35 years with AFC of more than or equalto5,AMH levels morethanor equal to 1.2 ng/mL and who have retrieved less than 4 oocytes in previous stimulated cycles. The thirdgroup consistsoffemales who are less than the age of 35 years with AFClessthan5andAMHlevelslessthan
1.2ng/mL.Thelastfourthgroupconsistsof females who are more than the age of 35 yearswithAFClessthan5andAMHlevels 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 withinthemwhichwhen activated releases plenty of factors which contribute in the growth,proliferationandangiogenesis.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 theAssistedReproductiveTreatmentssuch as Endometrial PRP prior to embryo transferinordertoincreasethethicknessof endometriumthusincreasingthechancesof 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 theoocytequality,numberofoocytesand
embryo fragmentation in the patients who haveundergone1stIVFcycle,followedby 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 previousIVFfailure.This comparisonwill 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 reservesunderwentthefirstIVFcyclewith anall-freezecycle.Followingtheoutcomes ofthefirstcycle,allthefemalesunderwent the ovarian PRP treatment along with diagnostic laparoscopy.
PrePRPtreatmentstimulation:
ForthefirstIVFcycle;allthepatientswere stimulated with Inj HMG 300 IU from the 2nddayofmensesfor5days.Followingthe 5 days, all the patients were screened and anantagonistwasaddedforanother5days. On the 10th day of the cycle, the patients were screened again for checking the follicle sizes. If the follicles were found of thesize20mmormore,Inj.Decapeptyl0.2 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. Thefollicularfluidwasscannedforoocyte cumulus complex. Once the scanning was completed;alltheOCCsweretransferredin 1 ml of culture media and incubated for 3 hours. Fresh semen sample was collected, and prepared using swim up technique. After3hours, theOCCsweredenuded 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 trigasdryincubator. Fertilizationcheckwas performed thenextdayat18-20hourspost injection. The embryos were subjected to fresh media again on day 3 and were culturedtillday5.Onthe5thday,whenthe embryos were in the blastocyst stage, they were graded along with embryo fragmentation and then they were frozen.
PRPTreatment:
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 sterile3mLPasteurpipetteandtransferred into a 15 mL conical tube. The tube was again centrifuged at 2200 rpm for 20 min. After20mins,theplatelets formedapellet at the bottom of the conical tube. The plasmasupernatantwasdiscardedkeeping
1 mL in the tube. The pellet was then suspended into the remaining plasma. This prepared sample was injected into the ovariesatdifferentpointsinthecortex.The patients were given a break for 2 months

beforestartingfor another stimulationcycle sothatthePRPinjectioncanalsostimulate the development of the follicles.

PostPRPstimulation:

After the break of 3 months, the patients were again stimulated with injection HMG 300IUfromday2ofmensesfor5days.On day 6th, the patients were scanned for follicular growthandthenantagonistwas

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. Thefollicularfluidwasscannedforoocyte cumulus complex. Once the scanning was completed;alltheOCCsweretransferredin 1 ml of culture media and incubated for 3 hours. Fresh semen sample was collected, and prepared using swim up technique. After3hours,theOCCsweredenudedand 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 trigasdryincubator.Fertilizationcheckwas performedthenextdayat18-20hourspost injection. The embryos were subjected to fresh media again on day 3 and were culturedtillday5.Onthe5thday,whenthe 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 PRPcycleandPostPRPcyclehasalsobeen changed. There is an increase in the total number of oocytes. The average mean of theOCCpriortoPRPtreatmentwas4.2± 2.5whilethatinpostPRPtreatmentwas9.1
±4.1TheZvalueis2.5.

Thenumberofmatureoocytesretrievedhas also been increased. The mean mature oocyteinPrePRPtreatmentwas2.66± 2.12whileinthePostPRPtreatmentwas
7.4±3.8.TheZvalueforthesameis2.37.

We have also observed an increase in the number of blastocysts formed in the Post PRPtreatmentcycleascomparedtothePre PRP-treated cycle. The average mean of blastocysts 1.3 ± 1.05 while the average meanofpostPRPtreatmentcyclewas4.1
±2.5.TheZvalueis2.67

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.

BasedontheZvalueswecaninterpretthat our Alternate Hypothesis stating that Autologous PRP treatment is found to be useful in the treatment for the patients suffering from POR.

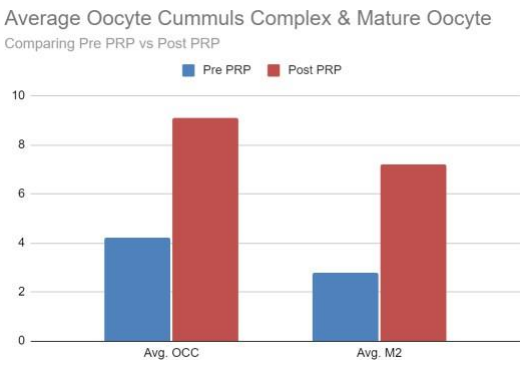

Fig. Barchartshowingcomparisoninthe Total no of OCC in IVF cycle

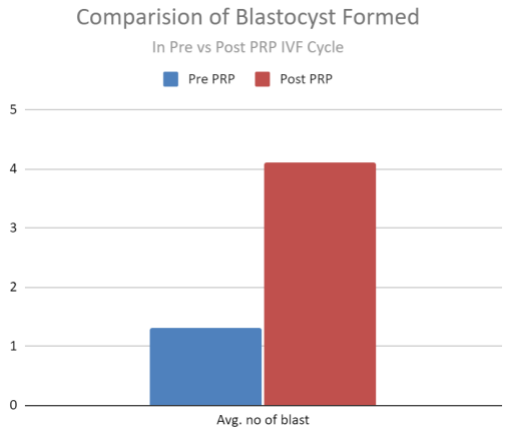

Fig.:Barchartshowingcomparisonin the M2 oocytes retrieved in IVF cycle

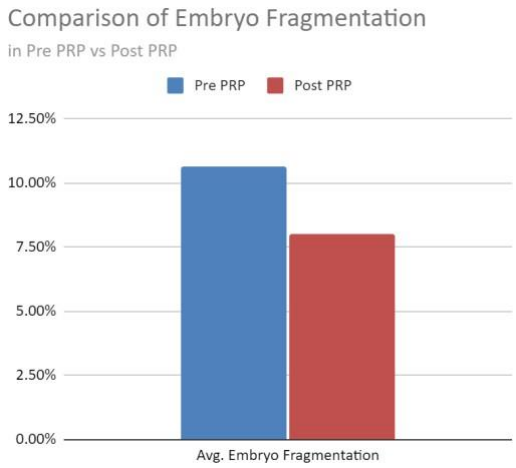

Fig.:Bargraphshowingcomparisoninthe embryo fragmentation in IVF cycle

DISCUSSION

In our study we evaluated the effect of Intraovarian PRP on the oocyte quality, quantity and embryo fragmentation rate in womenwhounderwentIVFtreatment.The results have indicated that there is a significantchangeobservedbyanincrease inthequalityandquantityofoocytesanda 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 andadecreaseintheembryo

fragmentation. There has also been an increase in the M2 oocytes retrieved. One major limitation of our study is absence of acontrolgroupwheresomepatientswould have undergone multiple IVF cycles without PRP treatment.

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

AstudybyMeloet.atin2020,revealedthat 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 haveshownanincreaseinthetotal numberof OCCand M2oocytesaswellas thequalityofoocytesinpostPRPtreatment compared to pre PRP treatment [16,17,18,17].

Studies have found an increase in serum AMH level and a decrease in FSH levels. TheFSH is inverselyproportionalwiththe qualityofoocytes.AstudybySantosetal., found that women with increased FSH had poor quality of oocytes characterized with granular cytoplasm and presence of vacuoles [10].

Astudyhasrevealedthatthemosteffective therapeutic treatment of PRP can only be observed when the

cytokines and growth factorare injectedintotheovaries directly. Thepossiblemechanismbehindthiscanbe 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,theTransforminggrowth factorβ isoform 1 is of major importance as it is involvedin cellproliferation, angiogenesis andthedepositionofextracellularmatrix[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].

Introvarian PRP has also been associated withanincreaseineuploidembryoswithan 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 mechansim 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 womenwithPOR,soitcanfurther beused in treating male infertility due to Non obstructive Azoospermia.

ACKNOWLEDGMENT

We authors acknowledge Dr. Krupa Shah MS Gynec, Ayuh FertilityCentre forallowingustousetheirdatatostudy and publish.

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