



***In Vivo* Effects of PRP Intratesticle Injection on Spermatogenesis and Reproductive Hormonal Level in non Obstructive Azoospermic Infertile men**

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Abstract

Background: Non Obstructive Azoospermia (NOA) is the absence of sperm in the ejaculate of infertile men resulting from severe deficits in spermatogenesis or hormonal dysfunction .

Objective: To evaluate the therapeutic effect of PRP outologus intratesticle injection in non obstructive azoospermic infertile men on spermatogenesis and some reproductive hormonal level .

Materials and Methods: The current study has involved of 50 non obstructive azoospermia infertile men, mean age (34.34 ± 7. 49) the study was established in high institute for infertility diagnosis and assisted reproductive technologies/ART Department/Iraq-Baghdad, during 6 months .At First, Autologous platelet rich plasma prepared by collected the fresh blood from peripheral vein of the patient and put the blood into the Jel tube containing Acid Citrate Dextrose solution A (ACD-A) anticoagulant. Second, under light anesthesia , by the specialist doctor ,patient had been undergone intra-testicular injection with 1 ml of platelet rich plasma in each testicle using 1 ml syringe fine needle which injected directly into the seminiferous tubule or through interstitial space, this procedure have been repeated at least 3 times, one month between each injection ,Seminal fluid analysis was done , FSH , Testosterone hormonal level were estimated, after one month from each platelet rich plasma injection ,If there was no sperm count in SFA ,then patients should be prepared for Testicular biopsy by the specialist doctor with platelet rich plasma adding during the operation, Third, few sperm may found in biopsy during lab examination of testes tissue under dissecting microscope, Furthermore, these sperms can storage at cryopreservation technique at future using in IVF or ICSI giving more chance for getting baby .

Results: The results of 50 infertile men enrolled in this current study and revealed the therapeutic effect of platelet rich plasma, the effect was observed in 15 patients during 3-4 months from the beginning using of autologous platelet rich plasma , either nor the procedure that have been followed by the specialist doctor for using the autologous platelet rich plasma. Results was divided according to estimation the blood serum hormonal level include Testosterone and FSH level and sperm count , the result show there was highly significant difference of FSH hormonal level after PRP injection P(<0.001) than before PRP adding , also there was highly significant differences of



S. Testosterone level after PRP injection P (<0.004) than before, Whereas there was significant differences in sperm count in men after PRP using at the same period.

Conclusion : PRP can improve the structural and functional impairment of the testis of non obstructive azoospermic infertile male .

Key words: Azoospermia, Platelet Rich Plasma, Non Obstructive Azoospermia.

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Introduction

A person who is infertile is unable to complete their biological requirement to bear children, both men and women can have infertility, and both have an impact on the patients who experience it, approximately 30% to 50% of infertile marriages have a male component (Kumar, 2013), about 10% of infertile men are found to have non-obstructive azoospermia, it is the inability of spermatogenesis due to either insufficient gonadotropin stimulation or a fundamental problem within the testis tissue (Kumar 2013; Hendriks et al. 2014). Males with Non-Obstructive Azoospermia (NOA) previously, had no further treatment choices for conceiving a biological child in ART, then, various restricted publications had recommended hormone medications and stem cell therapy for NOA .

Today, In Assisted Reproductive Technology, (Chang et al ,2015). Potential benefits of PRP have also been studied in the field of gynecology; a study revealed PRP can increase endometrial thickness and improve the pregnancy outcome with thin endometrium [9].

Platelet Rich Plasma (PRP), also known as autologous platelet gel, Plasma Rich in Growth Factors (PRGF) and Platelet Concentrate (PC), is a high concentration of autologous platelets suspended in a small volume of plasma after centrifugation (Wang HL, Avila G (2007). More than 800 types of protein molecules, cytokines, hormones and chemo-attractants are carried by the platelets Pantos et al. (2016). Furthermore, in this study injections of platelet-rich plasma (PRP) may give chance to treat males with non-obstructive azoospermic infertility (Gudelci et al. 2021). Testicular sperm extraction (TESE), a successful approach to

recover spermatozoal cells in patients with NOA, is one of the surgical treatments that have been developed to retrieve sperm cells in men with NOA (Wang et al. 2007; Gardner et al. 2004) because of its less damage to the testes tissue.

No human investigation had been conducted in Iraq to determine the possible advantages of PRP in the NOA therapy, not much is reported about the advantage use of PRP in testis.

Materials and Methods:

The current study has involved of 50 non obstructive azoospermia infertile men, mean age (34.34 ± 7.49), the study was established in High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/IVF Department Al.Nahrian University , Iraq. All the patient enrolled in this study have the same Inclusion and Exclusion criteria ,the inclusion criteria include non obstructive azoospermic infertile men ,and the exclusion criteria was 1.age <18 yrs and >55 yrs, 2. primary hypogonadism or associated testicular diseases, 3.residual adenoma, 4. smoking , 5.diabetes mellitus, 6. previous androgen replacement therapy. FSH and Testosterone level was measured before the procedure. All the required data for the study was collected retrospectively from the institute lab. Steps of these study include :

- First, autologous PRP prepared by collected the 10 ml fresh blood from peripheral vein and put the blood into the Jell tube containing Acid Citrate Dextrose solution A (ACD-A) anticoagulant, using PRP preparation technique (Chang Y,2015)
- Second, under light anesthesia men had been undergone PRP intra-testicular injection with 1 ml in each testicle using 1 ml syringe fine needle, platelet rich plasma injected into the seminiferous tubule



or into interstitial space, this procedure has been repeated at least 3 times for 3-4 month , just one month between each injection, FSH ,and Testosterone hormonal levels were analyzed, after one month from each platelet rich plasma injection ,if there was any response there may be few sperm found in FSA, while if there was no change in sperm count, the patients should take a date from the urologist doctor for doing Testicular biopsy, and adding outologus PRP during the operation.

- Third ,sometime the biopsy specimen may contains sperms when examined by dissecting microscope, these sperms were storage in cryopreservations technique at freezing tank , for future using at IVF or ICSI technique.

Statistical analysis :The categorical variables were presented as frequencies and percentages and continuous variables were presented as mean \pm standard deviations. The statistical difference between the initial FSH and post procedure FSH for all were calculated by paired samples t- tests. The analysis was performed in 95% confidence interval using Statistical Package for Social Science (SPSS), version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as number and percentage; and comparison of these variables between groups were done using Fisher exact test and Yates chi square test. Whereas, quantitative (numeric) variables expressed as mean (an index of central tendency) and standard deviation (an index of dispersion); and comparison of these variables within same group was done using paired ttest, between different two groups using unpaired ttest, and ANOVA (analysis of variance) were used when comparison done among 3 groups. The level of significance was

considered at P-value of equal or less than 0.05.

Results

The results of 50 infertile men enrolled in the current study revealed the therapeutic effect of platelet rich plasma in male infertility , and was observed in 15 patients during 3-4 months from the beginning using of autologous PRP, the procedure that have been followed by the specialist doctor for using the autologous PRP method , the results of hormonal level estimation in blood and the sperm count in seminal fluid or in the testis, the hormonal results show there were highly significant differences of FSH hormonal level after PRP injection $P(<0.001)$ than before PRP adding, while there were highly significant differences of S.Testosterone level after PRP injection $P(<0.004)$ than before, also, there was a significant difference in sperm count in men after PRP using at the same period.

In the current study FSH reach the normal level after 3 times of PRP, FSH which may play important role improvement hormonal level and spermatogenesis activation.

• **Statistical Analysis**

Comparison of hormones before and after PRP injection, as show in Table (1) and figure (1) and (2).

- The mean of S.FSH hormones was (28.96 ± 6.72) before PRP injection while the mean of S.FSH after PRP injection was (23.39 ± 9.54) .
- The p value of S.FSH hormones was
- The mean of S.Testosterone hormone before PRP injection was (5.00 ± 2.91) , and the mean of S. Testosterone hormone after PRP injection was $(3.952. \pm 28)$.
- The p value of S.Testosterone hormone before PRP injection and S.T difference $(P=0.004)$.

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Table (1): Comparison of hormones before and after PRP injection

Hormones	Before PRP Injection Mean \pm SD	After PRP Injection Mean \pm SD	PRPP Value
S. FSH (m iu/ml)	28.96 \pm 6.72	23.37 \pm 9.54	<0.001HS
S. Testosterone (ng/ml)	5.09 \pm 2.91	3.95 \pm 2.28	0.004 HS



n=50 Total Number of Patient Paired T- Test.
 HS: highly significant

- The result show the effect of intratesticular injection of autologous platelet rich plasma (PRP) on sperm retrieval rates mTESE procedure was 4 case , while the number of cases after 2 or 3 PRP intratesticular injection was 5.as show in table (2)
- The P value according to sperm count parameter was show none significant change in (P=0.729) in all protocol use in this study.

Table (2) Protocol Type and Positive Cases of Sperm Cont

Parameter	3 PRP Inj then	2 nd	PRP	3 rd PRP Inj.SFA(+)	P value*
	Biopsy (+) (mix) Mean±SD	Inj.SFA(+) Mean±SD		Mean±SD	
Sperm count mean	10.25±7.76	14.5±5.96		13.2±10.66	0.729 NS
	n (%)	n (%)		n (%)	P value**
Mode of PRP inj.	4(8%)	6 (12%)		3(6%) After direct PRP Inj	0.039 S
				2(4%) After Biopsy PRP Inj	

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n=15 number of positive cases.
 * P Value By ANOVA.
 ** P Value By Yates Chi Square. S:significant.
 NS: not significant at p>0.05.

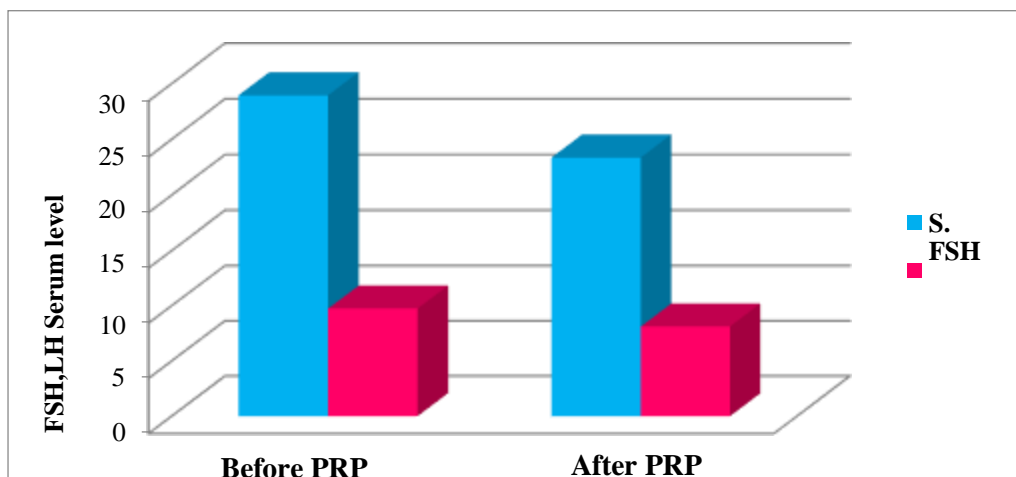
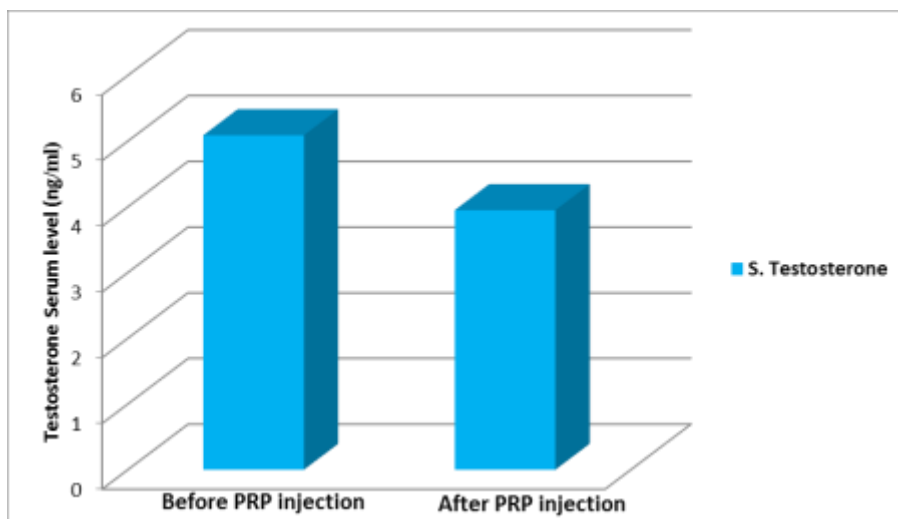


Figure (1): Serum level of follicle stimulating hormone and luteinizing hormone before and after PRP injection





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Figure (2): Serum level of Testosterone before and after PRP injection



Discussion

Table (1) show a significant impact of PRP on the maintenance of reproductive hormones in patient with non obstructive azoospermia, such as FSH, and T, in men with non-obstructive azoospermia. after 3 or 4 times of PRP intratesticular injection , this finding agree with Plant et al.(2001) who explained effect of FSH on Sertoli cell FSH receptors(indirect effect of PRP by it effect on sertoli cell) the Sertoli cells are stimulated by FSH to generate ABP. According to Kraemer *et al.* (2005) explain ABP, play a role in the development of the blood-testis barrier, found that T presumably is exclusively responsible for its continuation and ABP play a key role to concentrate the testicular testosterone in appropriate levels, which are important in the process of spermatogenesis and preserve semen quality; however, higher spermatozoa production has been linked to higher FSH levels. The findings by Plant et al. (2001) suggested that the FSH secretion is stimulated by pulsatile GnRH release and is sensitive to hypothalamic frequency modulations. They also suggested that the secretion of FSH is tightly controlled via a negative feedback loop, primarily by Sertoli cell peptides, inhibin B, and by inhibiting the activin stimulation of FSH gene expression, all of these observations are consistent with the findings of the current study. The current study founding that PRP therapy improved hormone levels and spermatogenesis, access the FSH levels to the normal range, this conclusion in this study similarity with research by Al-Nasser et al.(2018) and the statistical difference between the initial FSH and post procedure FSH for patients the primary & secondary spermatocytes in their initial FNA reports were as per the male infertile observed improvement in terms of hormonal level and spermatogenesis in the study. The great regenerating potential of PRP has been reported by numerous research, and the therapy does not cause any

severe issues because PRP is prepared from the patient's own blood, accordingly, there was no evidence of any deterioration effects of PRP therapy in this study. There were many studies which evaluate the influence of PRP in the treatment of non-obstructive azoospermia infertile men who have demonstrated statistically significant levels of spermatogenesis activation,for instant, Al-Nasser et al. (2018).

The current study contributes important knowledge to the initial assessment of infertile men receiving PRP therapy, preparing management strategies for the future. The level of FSH before PRP therapy was higher than it was after, with major effects, as shown in Table (4.7), the PRP can be controlled close to its baseline value (WHO,2010).

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