

Investigation the Effect of Intratesticular Autologous Platelet Rich Plasma Injection on Follicle Stimulating Hormone and Inhibin B Levels

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ABSTRACT: Abstract Background: Platelet rich plasma becomes widely used in reproductive medicine, recent use in male infertile, injection of platelet rich plasma within testes showed functional and structural enhancement of impaired testes especially in non-obstructive azoospermia, furthermore it increases the chance for successful sperms retrieval in testicular sperm extraction techniques. Objective: To investigate the effect of autologous platelet rich plasma intratesticular injection on Sertoli cells activity, follicle stimulating hormone, testosterone and inhibin b levels. Methods: Thirty four Semen specimen analyzed and blood sample for hormonal assay taken from each participant came to High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University from August 2024 to May 2025. Who decided to receive intratesticular platelet rich plasma injection course which long approximately 4 months, intervals between injection take a month then another semen sample analyzed and blood sample taken after course complete for hormonal analysis using mini Vidas (FSH and testosterone), seminal fluid analysis examined and Inhibin B levels assay by ELISA kit using serum before and after platelet rich plasma injection (intratesticular injection). Results: Changes following PRP treatment (n= 34), in seminal analysis azoospermia decreasing by 8.9%, cryptozoospermia increasing by 5.9%, and severe OAT increase by 3%. Inhibin B demonstrated considerable inter-individual variability. Median rose from 31.69 pg/mL to 80.96 pg/mL representing 155.5% increase but did not achieve statistical significance (p=0.437). Testosterone (ng/ml) showed a significant improvement represent: +1.46 or (+36.6%). And significant increase for FSH (mIU/ml) represent: +6.08 or (+38.7%). Conclusion: Markable shift in spermatogenesis outcomes, follicle stimulating hormone and testosterone increased significantly and Inhibin B levels increased but not reach statistically significant.



Keywords: Platelet Rich Plasma, Seminal fluid Analysis, Inhibin B, Sertoli Cells, reproductive hormones (FSH and Testosterone)

1. INTRODUCTION

Infertility defined lastly as disease which can affect reproductive system of both males and females known by failure to attain pregnancy following a year or more of regular, unprotected sexual coitus. which may happen owing to male, female or even unexplained factors. Some infertility causes can be preventable. infertility handling often includes in vitro fertilization technique (IVF) and further types of therapeutically assisted reproductive techniques. It may be of primary type in which females of couples not became pregnant at all or of secondary type where females cannot become pregnant following successful birth [1]. Male fertility can be assessed by performing a laboratory test for semen specimens [2]. Inhibin B consider as one of growth factors that mediate among the Sertoli cells and the germ cells [3]. Studies about inhibin B influence on the concentration and the spermatogenic activity confirm, that inhibin B

could be use as marker for Sertoli cells function estimation [3] [4]. It had been demonstrated that levels of serum inhibin B within adult men positively correlate with sperms concentration [3]. Inhibin B could also be used to diagnosis and monitoring many spermatogenesis disturbances like azoospermia with different etiology and primary testicular failure [3]. In males, Inhibin B is synthesized exclusively by testicular Sertoli cells and acts to suppress follicle-stimulating hormone (FSH) secretion through a negative feedback loop [5] [6] [7], and appears to be significant marker of seminiferous tubules function [5] [6].

In retrospective longitudinal study, researchers have previously identified Inhibin B as a significant biomarker with the potential to predict fertility outcomes within young males [8]. FSH and Inhibin B jointly appear to be further sensitive and specific spermatogenesis marker than either separately [9]. Levels of inhibin B of the infertile patients might offer helpful evidence about spermatogenesis and might serve as more straight spermatogenesis marker than the FSH [10]. Conclusion of previous study mentioned that inhibin B might be marker for exocrine function of testis and can suggestion better diagnosis and therapy modes for infertility of men [11].

In clinical assessments of male infertility, increased FSH concentrations are recognized as a highly reliable marker for the presence of germinal epithelial damage. This hormonal profile is consistently observed in clinical scenarios involving severe oligozoospermia and azoospermia [12].

Non-obstructive azoospermia (NOA) was defined when no sperms found in ejaculate owing to failure in spermatogenic process represent most severe male infertility forms. NOA etiology either intrinsic impairment of testes or insufficient gonadotropin production. Therefore, high-magnification microscopic sperm retrieval from testicular tissue (micro-TESE) had been the primary line treatment for those infertile patients. It is crucial to observed that at minimal examination of two semen specimens should be done to accurate the assessment. Additionally, the lack of sperms must be checked by semen specimen centrifugation. Conducting accurate microscopic examination for numerous sediment droplets of centrifuged semen informed to result in revealed of sperms in approximately up to 35 % of patient who were diagnosed initially as NOA.

Once found few sperm after the centrifugation, this condition defined as cryptozoospermia. TESE probably unnecessary in like this condition for ICSI performing within those patients, despite better rates of the implantation had been reported when testicular sperm used compare with sperms from the ejaculate [13]. In infertile males diagnosed with non-obstructive azoospermia (NOA) who experienced a prior unsuccessful microdissection testicular sperm extraction (mTESE), intra-testicular injection with Platelet-Rich Plasma (PRP) facilitated sperm identification in 21.8% (12/55) of participants.

This group also achieved a sustained implantation and live birth rate of 10.9% (6/55). In contrast, the efficacy of PRP was notably lower in patients with a history of two or more failed mTESE procedures; sperm cells were identified in only 8.3% (3/36) of cases, with the sustained implantation and live birth rate reaching 2.8% (1/36) [14]. Research indicates that Platelet-Rich Plasma (PRP) has the capacity to ameliorate both structural and functional impairments within the testes of infertile males diagnosed with non-obstructive azoospermia [15].

2. MATERIALS AND METHODS

2.1 ENROLLED PATIENTS AND STUDY DESIGN

Prospective observational study consists of 34 participants with mean age 31.8 ± 5.6 concentrated mostly under 37 years within High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University from August 2024 to May 2025. Study approval was by local Ethics Committee with approval code:(0702-MF-2025168-C2) from High Institute for Infertility Diagnosis and Assisted Reproductive Technologies.

Each patient received written informed agreement before enrollment. Data collection was based on the institute's lab results and written inquiry form. Study norm was consisting of participants with previous record of medical therapy without any assessable change within semen parameters. Men with diverse semen characteristics such as non-obstructive azoospermia, severe oligoasthenoteratozoospermia, and cryptozoospermia were criteria of study inclusion, while patients with chronic diseases, diabetes mellitus, identified genetic agents, previous chemotherapy record, and liquor drinker were excluded.

2.2 SEMEN ANALYSIS

Routinely collection started following abstinence period from 2 to 7 days by masturbation in special room near lab using labeled sterile wide mouth clean cub, then macroscopical examination of specimen done like assessment of color, volume, and PH after fully liquefaction of specimen which may take 30 or up to 60 minutes at room temperature or at 37 °C incubator Figure (1) after that twice 10 microliter volume following well mixing of sample taken to make two drops for microscopic examination that consists of concentration, count, morphology, motility and round cell assessments according to WHO (2021) criteria [16]. This test done before and after intra testicular injection with platelet rich plasma.



FIGURE 1. – Incubator device

2.3 PLATELET RICH PLASMA PREPARATION

Preparation of platelet rich plasma required 10 ml of whole blood placed in special tube then centrifuged for 8 min at 3600 RPM, formed buffy coat with plasma Figure (2) which then transferred to conical tube and centrifuged again for 3 minutes at 3600 to obtain plasma rich with platelet 2 ml needed where 1ml for each testis injected after local anesthesia, therapy course long 4 months with one month interval between each injection.

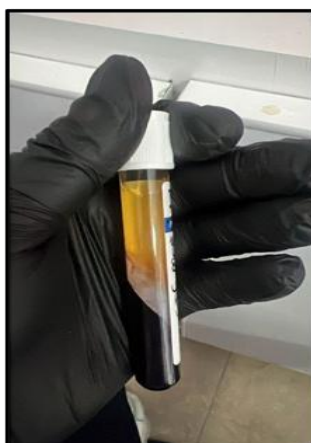


FIGURE 2. - Special laboratory tube for PRP separation

2.4 HORMONAL ANALYSIS

Blood specimen taken from each patient 5 ml before and after therapy and centrifuged in gel tube to form serum that used for testosterone and follicle stimulating hormone measurement Figure (3), remained serum preserved as aliquots in -20 °C Figure (4) for measuring Inhibin B later by ELISA kit. Figure (5).



FIGURE 3. - Mini Vidas for Hormonal assessment



FIGURE 4. - Patient's serum aliquots



FIGURE 5. - ELISA kit (Inhibin B)

2.5 BIOSTATISTICAL ANALYSIS

All statistical evaluations were accomplished using R software (R program, Austria) with significance set at $p < 0.05$. Continuous variables were presented for normally distributed data as mean $\pm$ standard deviation (SD) or for non-normally distributed data as median with interquartile range (IQR, 25th -75th percentile). Categorical data (variables) were expressed as frequencies and percentages. To evaluate the efficacy of PRP treatment, paired comparisons among pre- and post-treatment values were directed using Wilcoxon signed-rank test due to the non-normal distribution of most parameters.

3. RESULT

Present study consists of 34 patients with mean age 31.8 ± 5.6 and mean Body Mass Index 28.6 ± 4.17 , 35% were overweight, 38% were obese and 27% were normal weight. The response of Inhibin B demonstrated considerable inter-individual variability. Although median levels rose from 31.69 pg/mL to 80.96 pg/mL representing a substantial 155.5% increase in specific instances this improvement did not achieve statistical significance ($p = 0.437$, Figure 6). The combination of a high percentage change and elevated variance suggests a varying response, in which certain patients exhibited a robust clinical reaction while others showed negligible physiological change.

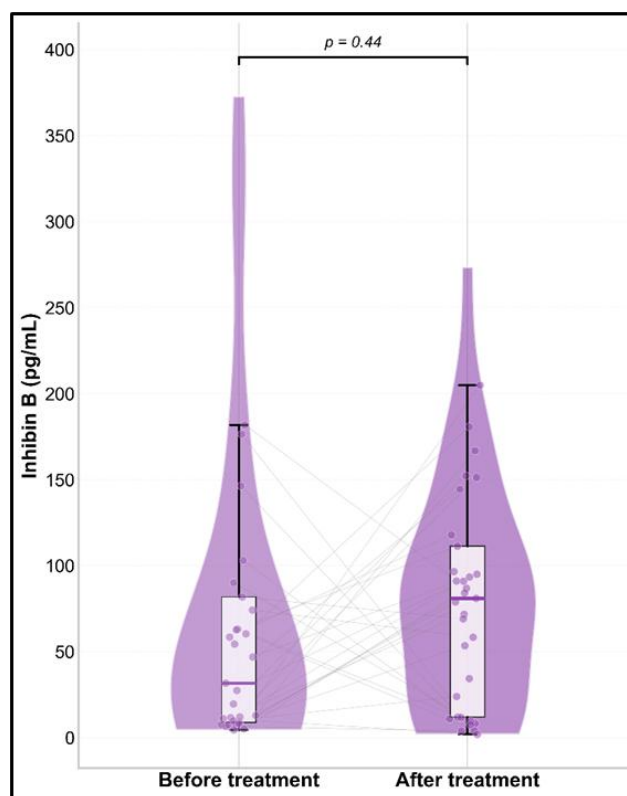


FIGURE 6. - Distribution of Inhibin B levels prior to and following PRP treatment

Illustrated via violin plots, which characterize the data spread alongside embedded box plots representing median and interquartile range. Each patient trajectories are depicted as distinct data points, with gray lines delineating the connection between paired samples. Statistically, Inhibin B concentrations rose from a median of 31.69 pg/mL (IQR: 8.84–81.83) to 80.96 pg/mL (IQR: 12.07–111.36). Despite a calculated change of 155.5%, the results of Wilcoxon signed-rank test showed that this rise was not statistically significant ($p = 0.437$; $n = 34$)

Changes in seminal analysis characteristics following PRP treatment ($n = 34$): Distribution of patients across SFA categories before and after treatment respectively, showing percentages for azoospermia (76.5% to 67.6%) improve toward sperm production, cryptozoospermia (14.7% to 20.6%) improved in sperm production, and severe OAT (8.8% to 11.8%); percentage alteration (Right side) in each category, with azoospermia decreasing by 8.9%, cryptozoospermia increasing by 5.9%, and severe OAT increase by 3%. Other hormones measured illustrated in Table (1), Figure (7) and (8).

Table 1. - Comparison between hormonal levels before and after PRP treatment

Parameter	n	Pre-treatment Mean $\pm$ SD	Pre-treatment Median (IQR)	Post-treatment Mean $\pm$ SD	Post-treatment Median (IQR)	p-value	sig
Testosterone (ng/ml)	3	3.99 $\pm$	3.30	5.45 $\pm$	5.06	<0.001	*
	4	2.18	(2.30-5.11)	2.21	(4.25-6.71)		
FSH (mIU/ml)	3	15.70 $\pm$	14.37	21.78 $\pm$	16.12	0.012	*
	4	10.09	(7.63-18.70)	18.82	(8.67-29.67)		

Data is presented as mean $\pm$ SD and Median (IQR) Abbreviations: n= patient number; Standard Deviation =SD; Interquartile Range (25th -75th percentile) =IQR. Significant improvements for Testosterone (ng/ml): +1.46 (+36.6%) and FSH (mIU/ml): +6.08 (+38.7%).

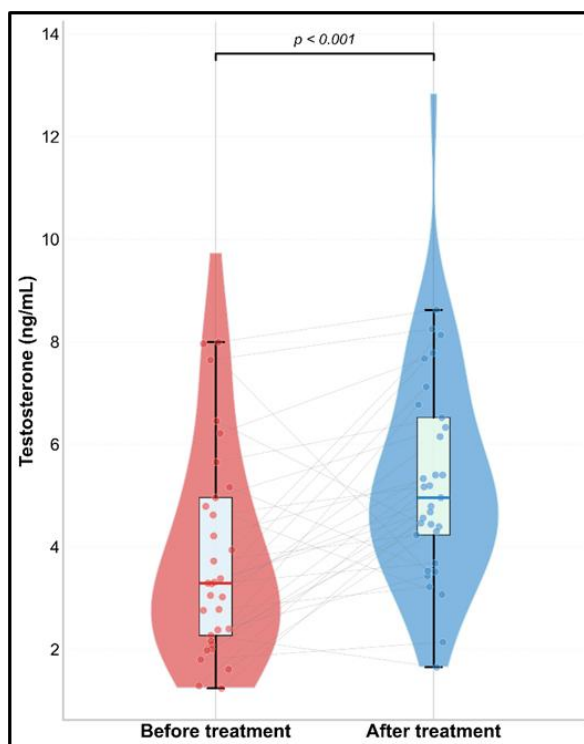


FIGURE 7. - Testosterone Levels Before and After PRP Treatment

Violin plots demonstrate value distributions, supplemented by nested box plots showing median and interquartile statistics. (range). Individual patient values are shown as dots with gray lines connecting paired samples. Testosterone increased from a median of 3.30 (2.30-5.11) to 5.06 (4.25-6.71) ng/mL (53.3% change, Wilcoxon signed-rank test, $p = 0.001$, (n= 34).

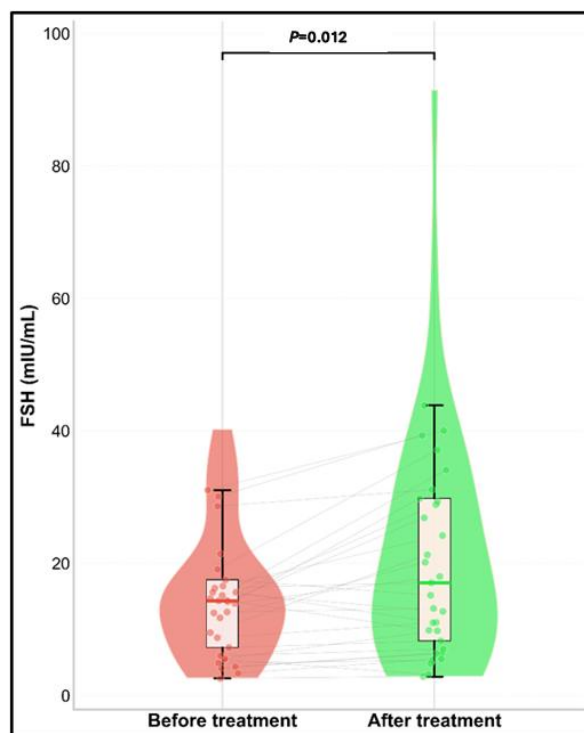


FIGURE 8. - FSH Levels Before and After PRP Treatment

Violin plots illustrate data distribution, featuring embedded box plots that show median and interquartile range (IQR). Separate patient values are shown as dots with gray lines connecting paired samples. FSH increased from a median of 14.37 (7.63-18.70) to 16.12 (8.67-29.67) mIU/mL (12.17% change, Wilcoxon signed-rank test, $p = 0.012$, $n = 34$).

4. DISCUSSION

Following PRP injection Inhibin B demonstrated considerable inter-individual variability. Although median levels rose from 31.69 pg/mL to 80.96 pg/mL representing a substantial 155.5% increase in specific instances this improvement did not achieve statistical significance. The combination of a high percentage change and elevated variance suggests a varying response, in which certain patients exhibited a robust clinical reaction while others showed negligible physiological change. Represent first study worldwide that evaluate Inhibin B levels before and after PRP therapy. Present study findings pointed toward spermatogenesis enhancement supported by another study findings which reported significant positive correlation that detected between inhibin B levels in serum and sperms count [17].

Distribution of patients across SFA categories before and after treatment respectively, showing percentage change in each category, where azoospermia decreasing by 8.9%, cryptozoospermia increasing by 5.9%, and severe OAT increase by 3%. Present study findings suggested that testes function enhanced following PRP injection supported by previous study which indicated that PRP has the capacity to ameliorate both structural and functional impairments within the testes of infertile males diagnosed with non-obstructive azoospermia [15].

Testosterone (ng/ml) showed a significant improvement represent: +1.46 or (+36.6%) following intratesticular PRP injection. Present study findings pointed toward testes function enhancement after PRP injection. Which supported by previous study conclusion while differ in testosterone levels findings [15].

And significant increase for FSH (mIU/ml) represent: +6.08 or (+38.7%) following intratesticular PRP injection. Present findings differ from previous study that showed slight rise of Follicle Stimulation Hormone detected after Platelet Rich Plasma therapy within all participants, but not reach statistically significant [18].

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CONFLICTS OF INTEREST

No potential conflict of interest was reported by the authors.

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