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The Positive Effect of Autologus Platelets Rich Plasma Testicle Therapy in Infertile Men with Non-obstructive Azoospermia

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Abstract

Platelets rich plasma (PRP) therapy proposed to be a promising option to treat some ceases of Non-Obstructive Azoospermia (NOA) due to its regenerative potential. The aim was To investigate the effectiveness of PRP intra-testicular therapy for male with NOA.This clinical trial included 100 men with NOA. attended to High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University. The diagnosis of azoospermia based on more than two semen analyses performed at least 15 days apart, testicular volume measure, and sex hormones profile study. Mean age was (34.34 ± 7. 49). First, PRP was prepared by centrifuging the patient's own blood in the anticoagulant- tubes containing Acid Citrate Dextrose olution A (ACD-A), Second, PRP testicle injection, repeated at least 3 times for each patient, one mouth apart. Third, micro testicular sperm extraction (TESE) was done and the presence of spermatozoa assessed under microscope. Results show Thirty men (30%) had positive sperms retrieval. In these cases; LH and FSH levels were significantly decreased and testosterone level was significantly increased after PRP injection. Patients with history of testicular biopsies had showed significantly higher positive spermatogenesis rate (86.7 % versus 13.3%, *p* value ~ 0.007). the Conclusions reveal Autologous PRP therapy has been successful rate in the treatment of some casas male infertility with NOA.

Keywords: Non Obstructive Azoospermia, Platelet Rich Plasma, Testicular Biopsy

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التأثير الإيجابي لعلاج الخصية بالبلازما الغنية بالصفائح الدموية لدى الرجال المصابين بالعقم والذين يعانون من فقد النطاف غير الانسدادي

الاستاذ المساعد رنا السعدي[1] ، الاستاذ الدكتور علا محمد الكواز[2] ، الاستاذ المساعد علي ابراهيم[3]
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المستخلص

- يُقترح أن يكون العلاج بالبلازما الغنية بالصفائح الدموية لحالات فقدان النطاف غير الانسدادي نظرًا لقدرته على التجدد. كان الهدف هو التحقق من فعالية العلاج بالبلازما الغنية بالصفائح الدموية داخل الخصية للذكور المصابين بـ NOA. وشملت هذه التجربة السريرية 100 رجل مصاب بـ NOA. التحقت بالمعهد العالي لتشخيص العقم وتقنيات الإنجاب المساعدة/جامعة النهرين. يتم تشخيص فقد النطاف بناءً على أكثر من تحليلين للسائل المنوي يتم إجراؤهما بفارق 15 يومًا على الأقل، وقياس حجم الخصية، ودراسة ملف الهرمونات الجنسية. متوسط العمر كان (34.34 ± 7.49). أولاً، تم تحضير البلازما الغنية بالصفائح الدموية (PRP) عن طريق الطرد المركزي لدم المريض نفسه في أنابيب مضادة للتخثر تحتوي على محلول حمض السترات ودكستروز A (ACD-A)، ثانياً، حقن الخصية بالبلازما الغنية بالصفائح الدموية(PRP) ، تكرر 3 مرات على الأقل لكل مريض، مع فصل فم واحد. ثالثاً، تم إجراء استخراج الحيوانات المنوية الدقيقة من الخصية (TESE) وتقييم وجود الحيوانات المنوية تحت المجهر. أظهرت النتائج أن ثلاثين رجلاً (30٪) لديهم نتائج إيجابية لاسترجاع الحيوانات المنوية. في هذه الحالات؛ انخفضت مستويات LH و FSHبشكل ملحوظ، كما ارتفع مستوى هرمون التستوستيرون بشكل ملحوظ بعد حقن PRP. أظهر المرضى الذين لديهم تاريخ لأخذ خزعات من الخصية معدل تكوين الحيوانات المنوية الإيجابي أعلى بكثير (86.7% مقابل 13.3%، قيمة p ~ 0.007). تكشف الاستنتاجات أن العلاج الذاتي بالبلازما الغنية بالصفائح الدموية كان ناجحًا في علاج بعض حالات العقم عند الذكور باستخدام NOA.

انتساب الباحثين

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الكلمات المفتاحية: البلازما الغنية بالصفائح الدموية، فقدان النطاف غير الانسدادي، استخراج الحيوانات المنوية الدقيقة من الخصية

Introduction

Male factor causes about 30% to 50% of infertile couples. The failure of spermatogenesis within the testis called Non-Obstructive Azoospermia (NOA), 10% of infertile men in world have non-obstructive azoospermia .Occur due to either problem within the testis tissue or insufficient gonadotropin stimulation [1,2]

NOA can be linked to various medical conditions, such as varicocele,cryptorchidism, hypogonadotropic hypogonadism, chromosomal defects, and Klinefelter's syndrome, chronic diseases and systemic inflammation such as atherosclerosis due to their damaging impact on sperm quality, Oxidative stress of these inflammatory processes has been associated with sperm DNA and membrane damage, resulting in azoospermia [3]

Yong Tao ,(2022) reported that hypothalamic–pituitary–gonadal axis (HPGA) plays a vital role in human spermatogenesis and Any disorder of HPGA could result in abnormal spermatogenesis and even azoospermia [4]. Several studies had suggested about hormonal therapies and stem cell therapy for NOA [2] .

PRP is nowadays used in various clinical scenarios that required improved tissue regeneration in maxillofacial surgery, neurobiology, orthopedics, sports medicine and ophthalmology [5]

PRP reduces oxidative stress and reactive oxygen species that help decrease degenerations. Platelet-derived growth factor (PDGF) has an important role in the regulation of autocrine/paracrine and germinal cell function. Also, VEGF regulates micro environmental changes in testicular tissue through a paracrine mechanism. VEGF helps to stimulate the testicular epithelial cells' proliferation and maintain testicular microcirculation permeability [6].In Assisted Reproductive Technology, PRP have been studied in field of gynecology; a study revealed PRP can increase endometrial thickness and improve the pregnancy outcome with thin endometrium [7]. According to Pantos et al. [8] more than 800 types of protein molecules, cytokines, hormones and chemo-attractants are carried by the platelets Furthermore,platelet-rich plasma (PRP) may give chance to treat males with non-obstructive azoospermic infertility [9]. In Iraq , till date, no article was published to identify potential benefits of PRP in NOA treatment.

Testicular Sperm Extraction (TESE), is surgical procedures that have been established to retrieve sperm cells in men with NOA with less harm to the testes tissue [6, 9].

Surgical sperm retrieval in azoospermic patients can be affected by several factors, including age, testicular volume, reproductive hormone levels, and preoperative DTB ,However, not all these factors have been found to significantly impact sperm acquisition in patients undergoing mTESE[10].

Kaltsas, ,2022 reported that TB repair and contribute in the reappearance of spermatozoa in semen, reduce the need for testosterone replacement therapy in cases of late-onset hypogonadism[11].

Subject, Materials and Methods:

One hundred infertile male were enrolled in the present study, included infertile men with non-

obstractive azoospermia a mean age of (34.34 ± 7.49). They attended to the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/IVF Department at Al-Nahrian University ,Baghdad/Iraq. The inclusion criteria, of this study include Non abstractive azoospermic infertile men while the exclusion criteria were :patient of age >55 years, ,Obstructive Azospermia ,Anatomical abnormalities in the genital tract, other genetic chromosomal disease like Klinefelter syndrome.

The diagnosis of male with Non-obstructive azoospermia based on more than two semen analyses performed at least 15 days apart, testicular volume measure ,All of patients participate in this study did not exhibit any improvement with continuing treatment by hormonal therapy for a further period,

TESE performed on the 100 men who had been considered pre-mTESE as NOA-men using following examinations:

○Studying medical and sexual history, physical examination results;

○Evaluating peripheral serum hormonal levels (FSH, LH, TT), which may influence the spermatogenesis, These hormone have been estimated before PRP injection, testicular volume (TV).

○Performing scrotal ultrasonography, transrectal ultrasonography,

Males with high FSH or small testicular volume/atrophic testes were considered NOA-men ,To distinguish between NOA and obstructive azoospermia in males with normal FSH and normal testicular volume, we were assisted by the findings of the physical examination (i.e., presence or absence of dilated epididymis, presence or absence of vas deferens, the scrotal ultrasound, According to the European Association of Urology Guidelines on Sexual and Reproductive Health 2023 [12].

PRP was prepared by collecting 3 mL of blood from each patient using a sterile syringe placed in a Jel tube containing the anticoagulant. The citrated blood tube was centrifuged at 4000 rpm for 6 min and the supernatant, which contains the buffy coat with platelets and leucocytes, was aspirated by a micropipette, and then it was placed in another sterile falcon tube for a second centrifugation at 4000 rpm for 6 min. Platelet pellets appeared in the bottom of the tube, which represents PRP. The platelet pellets were resuspended in 1 mL of the supernatant, representing the platelet-poor plasma (PPP). Finally, under light anesthesia, 1 mL of autologous PRP was immediately injected into each testis by an insulin syringe.

The final concentration of platelets in the obtained sample was 950.000 – 1.250 000 cells in 1 ml. PRP testicle injection, repeated at least 3 times for each patient, one mouth apart, PRP injected through seminiferous tubule or interstitial space of each testis (by the specialist doctor).

Negative patient response to PRP, demonstrated through Semen analysis and hormonal level , in this case patient prepared for Testicular biopsy done by the specialist doctor and autologous PRP adding during the operation, few or one sperm may found in biopsy after lab examination of testes tissue under inverted microscope. Finally , the sperms may be stored using a cryopreservation technique for IVF or ICSI .

- **Technique of mTESE**

The mTESE procedure was conducted under general anesthesia by a consistent urologist surgical team, Both scrotal skin ,dartos muscle

and tunica vaginalis were uncovered revealing the tunica albuginea. About 4 millimeter of tunica albuginea were removed close to the epididymal head .the tissue was carefully removed using sharp scissors then put it in clean petridish. Sample taken from the testis each testicular sample averaged 50 mg in weight size , parenchyma were examined under an dissecting microscope ,after procedure was complete the tunica was closed using vicryl 3-1 sutures , and scrotal layers were closed independently

- **Testicular Histopathology**

All testes' samples were sent to the same pathologist for histological analysis. A small piece of subcapsular parenchyma was taken, preserved in Bouin's solution, and sent to the pathologist for analysis.

- **Statistical analysis**

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft office 2010. The descriptive statistics including frequency, mean and standard deviation were measured to describe the data .The groups were compared by applying analysis of variance (ANOVA between more than two groups), independent sample t test (Comparison between two different groups), paired samples t-test (Before and after PRP), chi square test (Comparison between non continuous variables and percentages) and repeated measures ANOVA (Test for the difference in means across different groups). The results were considered statistically significant when p value was equal or less than 0.05.

Results

One hundred infertile male were enrolled in the present interventional study, 30 (30%) males had a positive spermatogenesis response at testicular biopsies after multiple PRP injection and 70 patients (70%) were had a negative spermatogenesis response as shown in Figure (1).

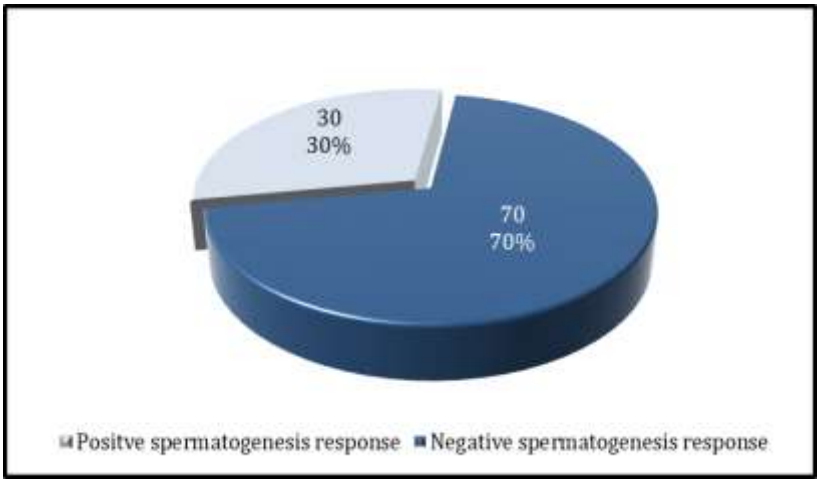

Figure (1): Positive and negative spermatogenesis response rates after multiple testicular PRP injection

- **Comparison of mean age, duration of infertility, serum LH, FSH and testosterone levels between positive and negative spermatogenesis responders**

There were no significant differences regarding mean patients age, duration of infertility and LH levels between positive and negative spermatogenesis responders (33.40 ± 1.72 vs. 34.74 ± 0.77; *p*=0.412), (9.36 ± 0.72 vs. 9.62 ± 0.65; *p*=0.817) and (10.56 ± 1.06 vs. 9.02 ± 0.42; *p*=0.104) respectively, on the other hand there were significantly lower FSH (26.17 ± 0.90 vs. 29.81 ± 0.83; *p*=0.01) and testosterone levels (1.94 ± 0.15 vs. 3.54 ± 0.19; *p*< 0.001) in positive spermatogenesis responders as show in Table (1) and figure(2).

Table (1): Comparison of mean age, duration of infertility, serum LH, FSH and testosterone levels between patients with positive and negative spermatogenesis response

Parameters (Mean±SE)	Positive spermatogenesis n.=30	Negative spermatogenesis n.=70	p value
Age (years)	33.40 ± 1.72	34.74 ± 0.77	0.412 Ŧ NS
Duration of infertility (years)	9.36 ± 0.72	9.62 ± 0.65	0.817 Ŧ NS
LH (mIU/ml)	10.56 ± 1.06	9.02 ± 0.42	0.104 Ŧ NS
FSH (mIU/ml)	26.17 ± 0.90	29.81 ± 0.83	0.01 Ŧ S
Testosterone (ng/ml)	1.94 ± 0.15	3.54 ± 0.19	< 0.001 Ŧ S

SE: Standard Error; NS: Not significant (*p* value > 0.05); S: Significant (p≤ 0.05); Ŧ: Independent sample t test.

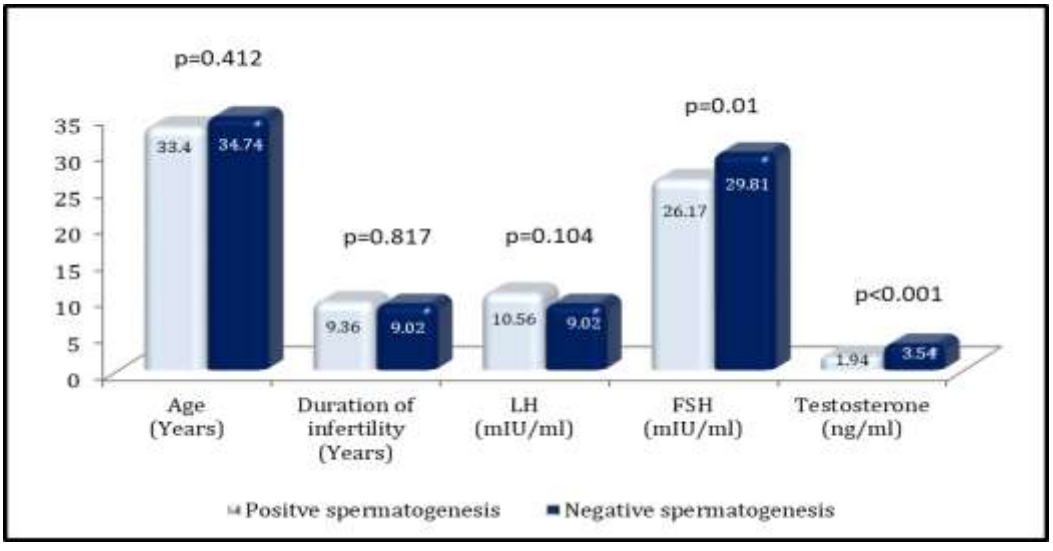

Figure (2): Comparison of mean age, duration of infertility, serum LH, FSH and testosterone levels between patients with positive and negative spermatogenesis response

- **Comparison of hormonal levels between positive and negative spermatogenesis responders before and after PRP injections**

In positive responder infertile males; LH and FSH levels were significantly decreased after PRP injection (10.56 ± 1.06 vs. 5.36 ± 0.52; *p*< 0.001) and (26.17 ± 0.90 vs. 13.69 ± 0.21; *p*< 0.001) respectively; on the contrary there was significant increase in testosterone levels after PRP injections (1.94 ± 0.15 vs. 3.21 ± 0.24; *p* < 0.001).

In negative spermatogenesis responders there was only significantly lower FSH levels after PRP injections (30.01 ± 0.84 vs. 27.45 ± 1.05; *p*< 0.001) without significant differences in LH and testosterone levels (8.91 ± 0.42 vs. 8.59 ± 0.46; *p*=0.336) and (3.54 ± 0.19 vs. 3.50 ± 0.17; *p*=0.595) as show in table(2), figure(3) and figure (4).

Table (2): Comparison of hormonal levels in positive and negative spermatogenesis responders before and after PRP injections

Positive spermatogenesis responders			
Hormone (Mean±SE)	**Pre PRP injections n.=30**	**Post PRP injections n.=30**	***p* value**
LH (mIU/ml)	**10.56 ± 1.06**	**5.63 ± 0.52**	**< 0.001 Ť S**
FSH (mIU/ml)	**26.17 ± 0.90**	**13.69 ± 0.21**	**< 0.001 Ť S**
Testosterone (ng/ml)	**1.94 ± 0.15**	**3.21 ± 0.24**	**< 0.001 Ť S**
Negative spermatogenesis responders			
Hormone (Mean±SE)	**Pre PRP injections n.=70**	**Post PRP injections n.=70**	***p* value**
LH (mIU/ml)	**8.91 ± 0.42**	**8.59 ± 0.46**	**0.336 Ť NS**
FSH (mIU/ml)	**30.01 ± 0.84**	**27.45 ± 1.05**	**< 0.001 Ť S**
Testosterone (ng/ml)	**3.54 ± 0.19**	**3.50 ± 0.17**	**0.595 Ť NS**

SE: Standard error; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; S: Significant (*p* value ≤ 0.05); NS: Not significant (p> 0.05); Ť: Paired sample t test.

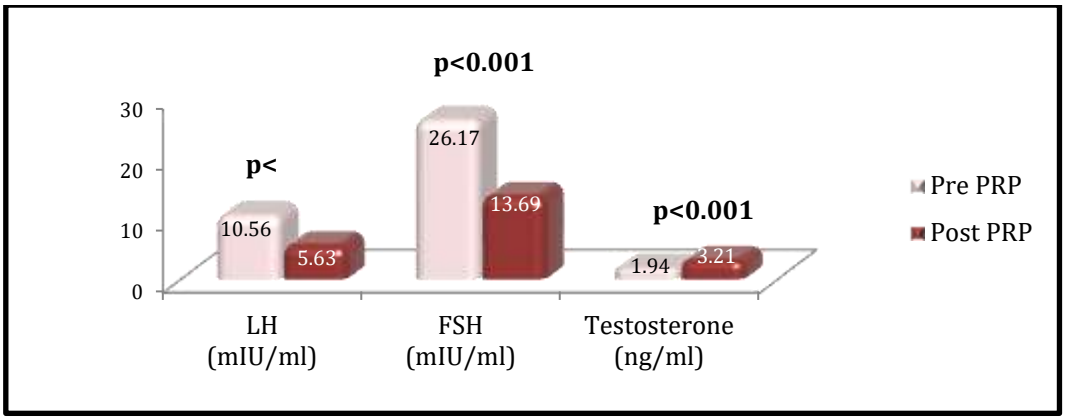

Figure (3): Comparison of hormonal levels in positive spermatogenesis responders before and after PRP injections

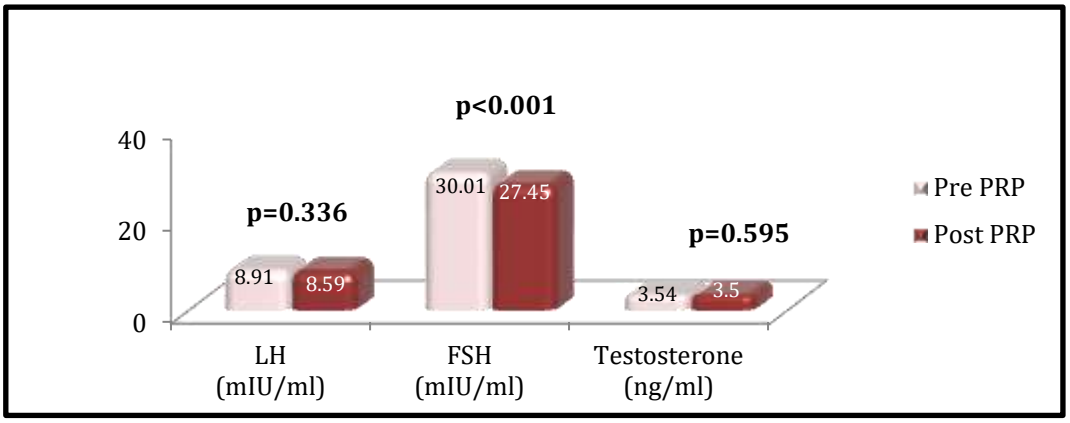

Figure (4): Comparison of hormonal levels in negative spermatogenesis responders before and after PRP injections

Effects of previous testicular biopsies on total numbers of positive spermatogenesis responders

Patients with history of testicular biopsies had showed significantly higher positive spermatogenesis rate (86.7 % versus 13.3%) with *p* value equal to 0.007 and this indicates an association between previous testicular biopsies and positive spermatogenesis response after PRP injections as show in table (3) and figure (5).

Table (3): Effects of previous testicular biopsies on the total numbers of patients with positive and negative spermatogenesis response

History of testicular biopsies	Positive spermatogenesis n=30	Negative spermatogenesis n=70	*p* value
Positive history of testicular biopsy	26 (86.7%)	32 (45.7%)	0.007 € S
Negative history of testicular biopsy	4 (13.3%)	38 (54.3%)	

S: Significant (*p* value ≤ 0.05); €: Chi square.

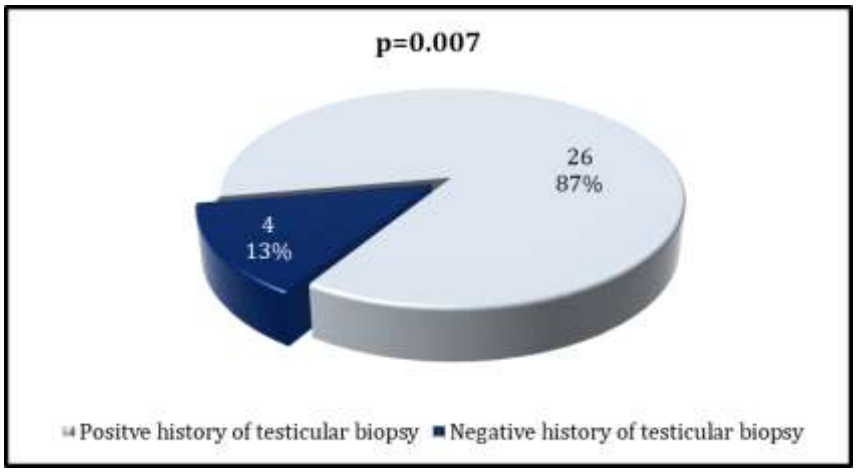

Figure (5): Effects of previous testicular biopsies on total numbers of patients with positive and negative spermatogenesis response

Discussion

In this work used PRP in Assisted Reproductive Technology in treated male with NOA, we observed 30 males had a positive spermatogenesis response at testicular biopsies after multiple PRP injection. the idea of this work came from many previous studied found the beneficial effect of PRP in medical field and in ART.

Chang Y observed using PRP in the field of gynecology, cause increase endometrial thickness and implantation rate then improve the pregnancy outcome with thin endometrium [7].Also, observed that PRP has potential to reduce the implantation failures by increasing the expression of adhesion molecules, ovarian rejuvenation and folliculogenesis reactivation in peri-menopausal women.

Many studies support the effectiveness role of PRP therapy in the treatment of male infertility and sexual dysfunction,by improve the structural and functional impairment of the testis.

The Article published by Pochini A, describe that PRP contains a unique composition of cytokines and growth factors, including fibroblast growth factor (FGF) vascular endothelial growth factor (VEGF), platelet-derived growth factor, interleukin-1B, interleukin-10, insulin-like growth factor-1(13).According to Somova, found that PRP, containing many biological active molecules, realizes its effect through the tissue regeneration and cell proliferation.[14].

According to Kutluhan , who revealed Epidermal growth factor (EGF) has a good rule on spermatogenesis. Platelet-derived growth factor (PDGF) has a positive role with the germinal cells. These growth factors help in the maintenance of germinal epithelium, as well as in regulating Sertoli and Leydig cells' function. It seems that the combination of these growth factors might have a positive effect on spermatogenesis and fertility [15].

Al-Nasser , concluded the role of PRP in improvement of hormonal level and spermatogenesis in Men with Non-Abstractive Azoospermia, also Al-Nasser did not observe any deteriorating effect of PRP therapy. The results of all these study agreement and give the support for the results of present study[16].

Kumar, define the Non-obstructive azoospermia as a failure of spermatogenesis within the testis

due to either a lack of appropriate stimulation by gonadotropins or an intrinsic testicular impairment. The medical management of these men is to improve quantity and quality of sperm retrieved from their testis for in vitro fertilization[1].

According to Kumar.revealed that an elevated follicle-stimulating hormone presence of azoospermia is generally considered sufficient evidence of a non-obstructive etiology. however, identify category of NAO either hypogonadotropic(low FSH), hypogonadism or hypergonadotropic hypogonadism . This observation agreement with present work ,that show positive responder infertile males; LH and FSH levels were significantly decreased after PRP injection respectively; on the contrary there was significant increase in testosterone levels after PRP injections.

All cases in this study have non-responders with continuing treatment with Gonadotropin therapy or GnRH therapy for a further period and all of them did not exhibit any improvement. So because PRP is prepared from patient's own blood , the therapy doesn't cause any major complications ,various studies that mentioned above explain the excellent regenerative potential of PRP .According to McLachlan, show for distinguishing between obstructive and non-obstructive azoospermia , testicular biopsy serves as a crucial diagnostic tool, predicting mature spermatid recovery [17] .

Age was also investigated as a potential factor influencing the success of sperm retrieval in our study. We aimed the impact of paternal age on the outcomes of mTESE in men with NOA. However, our findings did not demonstrate a statistically significant correlation between age and positive and negative spermatogenesis responders these results agreement with study done by Rohayem[18]

In the current study, Table 3 show positive responder infertile males; LH and FSH levels were significantly decreased after PRP injection on the contrary there was significant increase in levels of testosterone, we observed substantial effect of PRP on the maintenance of reproductive hormones in patients with non-obstructive azoospermia, such as FSH and Testosterone . This finding agree with Plant [19] who explanation the impact of FSH on Sertoli cell FSH receptors (an indirect effect of PRP through its effect on Sertoli cells), according to which the Sertoli cells are prompted by FSH to produce ABP(Androgen binding proteins)are glycoproteins produced by the sertoli cells in the seminiferous tubules. These proteins specifically bind to the testosterone and other androgens increasing its concentration inside the testes. This accumulation stimulates spermatogenesis .

Kraemer [1] explained that ABP plays a critical role in concentrating testicular testosterone at the proper levels, which are crucial for the process of spermatogenesis and maintaining semen quality.

Yong Tao[20], find that FSH and LH play pivotal roles in human spermatogenesis. Specifically, the testicular target cells of FSH are Sortoli cell present in seminiferous tubules, FSH improves the proliferation, maturation, and function of Sortoli cell , which produce regulatory molecules and nutrients needed for spermatogenesis, The testicular target cells of LH are Leydig cells in the interstitial space, and LH stimulates Leydig cell to produce Teststeron. This information consistent with finding of present study.

According to the research of Plant [19], proposed that the secretion of FSH is tightly controlled via a negative feedback loop, notably by Sertoli cell peptides, inhibin B, and by limiting the activin activation of FSH gene expression.

The current study found that PRP therapy improved hormone levels and spermatogenesis, bringing the FSH levels back into the normal range. This finding is similar to that of Al-Nasser[16], and the statistical difference between the initial FSH and post procedure FSH for patients was based on the male infertile observed improvement in terms of hormonal level and spermatogenesis in the initial TESE reports.

This research showed that multiple intratesticular PRP injections yielded better results than single injections with PRP this result agree with Chouhan [21] who suggested different results between single and multiple injections of PRP because most growth factors contained in platelets are short-lived .

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