International Journal of Reproductive BioMedicine
Volume 24, Issue no. 5, https://doi.org/10.18502/ijrm.v24i5.21291
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Original Article

The effect of intratesticular autologous platelet-rich plasma injection on sperm DNA fragmentation in men with oligoasthenoteratozoospermia: An RCT

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Received: 17 June 2025
Revised: 1 December 2025
Accepted: 15 April 2026

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Editor-in-Chief:
Aflatoonian Abbas M.D.

Abstract

Background: Oligoasthenoteratozoospermia (OAT) is one of the most common multifactorial causes of male infertility. Platelet-rich plasma (PRP), a biological product, has emerged as a promising regenerative therapy in various fields of medicine, including reproductive health. Therefore, we investigated the effect of PRP administration on sperm DNA fragmentation in OAT men.

Objective: The aim was to evaluate the effect of intratesticular injection of PRP on DNA fragmentation index (DFI) in men with idiopathic OAT and assess its potential as a novel therapeutic intervention to enhance sperm DNA integrity.

Materials and Methods: This randomized controlled trial study involved 160 infertile men diagnosed with OAT without underlying diseases who were referred to the infertility center of Fatemieh Infertility Center, Hamadan, Iran, from July 2023 to July 2024. The participants were randomly assigned to intervention and control groups using a block randomization method. The intervention group received 1–2 cc of PRP in each testicle. Sperm parameters and DFI were measured; a hormonal study was also performed before and after the procedure.

Results: The results revealed a significant difference in concentration ($p = 0.030$), progressive motility ($p < 0.001$), DNA fragmentation ($p < 0.001$), normal morphology ($p < 0.001$), and also testosterone levels ($p < 0.001$) between groups after PRP injection.

Conclusion: PRP Injection into the testis appears to be a safe, minimally invasive, and potentially effective therapeutic approach in enhancing sperm quality and reducing DFI in men with OAT.

Keywords: *Male infertility, Oligoasthenoteratozoospermia, Platelet-rich plasma, Sperm DNA fragmentation.*

Registration ID in IRCT: IRCT20220317054318N4

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How to cite this article: Pilehvari Sh, Ghorbani M, Babalhavaeji H, Talebi-Ghane E, Fazli F. "The effect of intratesticular autologous platelet-rich plasma injection on sperm DNA fragmentation in men with oligoasthenoteratozoospermia: An RCT," *Int J Reprod BioMed* 2026; 24: 421–432. https://doi.org/10.18502/ijrm.v24i5.21291

1. Introduction

About 50% of infertility cases have male causes, and there are several factors involved in male infertility that are related to problems with sperm production or transport (1). Sexual and reproductive dysfunction is the result of some male infertility problems that are related to quantitative and qualitative problems in spermatogenesis. The most common infertility disorder in men is oligoasthenoteratozoospermia (OAT) (2). OAT syndrome is characterized by abnormal sperm counts and motility, aberrant sperm morphology, and immature sperm in infertile men, in which environmental, nutritional, behavioral, and genetic factors play a role in the development of this syndrome (3). Sperm DNA fragmentation (SDF) is also an etiology of OAT. Fragmentation of the sperm nucleus leads to miscarriage, low-quality embryos, and failed implantation. Although damaged sperm can fertilize oocytes, they may cause abnormalities and defects in the development of the embryos (4).

Given that sperm production depends on testicular activity and hormones, growth factors, and genetics, the treatment of these participants can be modified by these factors (5). Several treatments are available for this group of men, including hormone therapy, which may increase spermatogenesis in men with OAT; testosterone, estradiol, dopamine agonists can be used to increase spermatogenesis in infertile men with hyperprolactinemia (6).

Recently, platelet-rich plasma (PRP) has been used for numerous treatments. PRP is a regenerative autologous medicine containing concentrated platelets that have 3–7 times higher mean platelet concentration compared to whole blood. Growth factors are naturally found in a person's blood. PRP is involved in the release of growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor, insulin-like growth factor-1 (IGF-1), and epidermal growth factor (EGF) (7). These platelet-derived products have shown promise in a variety of medical fields, including dermatology, ophthalmology, maxillofacial surgery, orthopedics, and increasingly, female reproductive medicine (8). However, its role in male reproductive health is not well defined. Recent studies have indicated the use of PRP in reproductive and therapeutic aspects in medical fields as a reliable treatment option. At the reproductive level, many studies have shown that intratesticular PRP injection has resulted in the treatment of infertility (9). PRP increases testicular function in rats by reducing the production of malondialdehyde and interleukin-6 levels. Also, the use of PRP has been shown to repair the gonads of male rats under toxic conditions (10).

The aim of this study was to examine the effect of PRP injection on improving sperm parameter such as sperm DNA damage in infertile men with severe oligoasthenozoospermia who had referred to Fatemieh Infertility Center, Hamadan, Iran.

2. Materials and Methods

2.1. Experimental design

In this study, a triple-blinding randomized controlled trial was conducted at Fatemieh Infertility Center, Hamadan, Iran, from July 2023-July 2024. For this trial, blinding was maintained for the physician, laboratory technician, and data analyst with respect to allocation. Blinding for participants was not

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possible because PRP injections were given only to the intervention group.

Men aged between 20 and 45 yr with sperm count 16 ≥ million/ml, motility < 42%, progressive motility < 30%, and normal morphology < 4% were included in the study. People who were undergoing other treatments such as hormone therapy, had other diseases such as diabetes, cancer, and kidney or liver disease, drug and alcohol addiction, and, in addition, had chemotherapy, or had signs of infection in the semen, or had abnormal levels of follicle-stimulating hormones (FSH) and testosterone, or had untreated varicocele were excluded from the study (exclusion criteria). Assisted reproductive technology (ART) history, and type of infertility as basic characteristics were primary data obtained from medical records.

The eligible male participants (N = 160) who enrolled in the study were randomly assigned to intervention (n = 80) and control groups (n = 80) utilizing a block randomization method with a block size of 4. For this purpose, the intervention and control groups were randomly assigned to letters "A" and "B", respectively. This study was conducted in accordance with CONSORT 2010.

2.2. Sample size

According to the study by Hamadan et al. the mean ± SD of DFI was presented 39.17 ± 6.79 and 35.67 ± 8.94 before and after intervention (11); so, the 160 sample size (80 sample in each group) was estimated considering the 80% power and a type I error level of 5% based on following sample size formula:

$$n = \frac{\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2 \times \left[\sigma_1^2 + \sigma_2^2\right]}{\left(\mu_1 - \mu_2\right)^2}$$

2.3. Outcomes, data collection, and preparation of sperm samples

Study participants were asked to collect a sperm sample in a sterile plastic or glass container by masturbation after 3 days of sexual abstinence. To further ensure the accuracy of the semen sample and being an OAT, before the start of the study, 2 semen samples were taken from individuals on different days (3 days of sexual abstinence). Samples were placed in a 37°C incubator for approximately 30–60 min to complete liquefaction. Samples and semen parameters were then routinely analyzed according to the World Health Organization 2021 standards (12). Macroscopic characteristics, such as liquefaction time, volume, appearance, and viscosity, and microscopic characteristics, including sperm count, motility, morphology, and DFI were assessed by a single technician. Primary outcomes sperm analysis, DFI and secondary outcomes, sperm analysis and DFI 3 months after PRP injection in both groups. Common treatment recommendations in cases of high DFI include the use of antioxidant supplements; therefore, supplements are prescribed in both groups, but in the study group, PRP is injected to observe greater effectiveness. Due to the sensitivity of testicular tissue, it is unethical to inject placebo to the control group, and only routine treatment, which is supplement intake, was performed for them, and the test group received the injection in addition to routine treatment.

2.4. Preparation of PRP and injection into the testis

Following standardized protocols, PRP was processed from autologous blood samples (1). 10 cc of whole blood was collected in tubes

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containing anticoagulant. Then, it was centrifuged at 3000 rpm for 5 min, and the red blood cells, buffy coat, platelets, and plasma were separated into separate layers. Using a pipette, the upper layer (plasma) was removed and centrifuged again for 15 min at 3500 rpm. Finally, the upper 2/3rd of the plasma, which contained platelet-poor plasma, was removed, leaving the lower one-third enriched with PRP. To activate the PRP, 10% calcium chloride was added. In the intervention group, a urologist injected PRP into the testicular tissue under local anesthesia, with the injected volume ranging from 1–2 cc depending on testicular size. After 3 months, semen samples were collected again from the same participants for sperm analysis and DNA fragmentation index assessment. No intervention was performed in the control group (13).

2.5. Evaluation of DNA fragmentation

The evaluation of DNA fragmentation proportion was conducted using the Halo Sperm kit (Halo kit, Idehvarzan Farda Co., Tehran, Iran) following established protocols (14). Evaluation of DNA fragmentation was performed before and after PRP injection in both groups.

2.6. Evaluation of hormone levels of FSH, luteinizing hormone (LH), and testosterone

Hormonal assays such as serum FSH, LH, and testosterone levels were measured using chemiluminescent immunoassays (Ideal Tashkhis Atieh kit, Iran) before and after PRP injection in both groups. Reference ranges were as follows: LH (follicular phase) 2–10 IU/L; FSH (follicular phase) 1.5–8.0 IU/L; and testosterone 10 = 30 nmol/L.

2.7. Ethical Considerations

This study was recorded with the Iranian registry of clinical trial (IRCT20220317054318N4) and was approved by the ethics committee of Hamadan University of Medical Sciences, Hamadan, Iran (Code: IR.UMSHA.REC.1402.227). The date of registration was July 05, 2023, and date of updating information was July 05, 2023. All participants signed a research consent form, and data were kept confidential.

2.8. Statistical Analysis

In the present study, quantitative and qualitative characteristics were described as mean ± standard deviation (SD) and number (%), respectively. In this study, clinical characteristics were measured before and after the intervention. Chi-square test was used to examine the association between 2 qualitative variables. The independent *t* test was used to compare the quantitative characteristics of control and intervention groups. To examine the before and after results in each group, a paired *t* test was used. Moreover, we utilized the analysis of covariance (ANCOVA) to compare the results in intervention and control groups by adjusting for the effect of initial values. The significance level was set at $p < 0.05$, and data description and analysis were performed using SPSS software for Windows (version 26.0, Chicago, SPSS Inc.).

3. Results

In this study, 160 infertile men with severe OAT, aged 20–45 yr were randomly assigned to intervention and control groups. No men declined follow-up (Figure 1).

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The results for the participants' demographic variables are shown in table I. The mean (SD) age in the intervention and control groups did not differ significantly and were 34.31 ± 5.08 and 34.66 ± 4.69, respectively. Also, no significant difference was observed in the frequency distribution of these 2 groups in terms of education, occupation, previous ART cycle, and type of infertility. The findings in table I show that approximately one-fifth of the participants had been married for more than 10 yr. Furthermore, approximately 80% of the volunteers in this study were undergoing ART for the first time.

In table II, the mean ± SD of sperm parameter analyses before and after the intervention are compared between 2 groups. In addition, ANCOVA was used to adjust baseline values in order to enable a more accurate comparison of post-intervention outcomes. It was observed that, after adjusting for baseline values, the mean ± SD sperm count in the intervention group was significantly higher than in control group. The post-intervention mean in the intervention group was 13.93 ± 10.82, while in the control group it was 9.44 ± 5.64 ($p = 0.001$). Although no significant difference between the intervention and control groups was observed in the sperm morphology variable using ANCOVA, the mean value of this variable in the PRP group was significantly higher than the pre-intervention value. The mean after the intervention was 1.40 ± 0.95, compared to 0.80 ± 0.67 before the intervention ($p < 0.001$). Additionally, for the progressive motility variable, based on the significant ANCOVA results, after adjusting for baseline effects, the mean progressive motility value showed a significant increase in the PRP group after intervention ($p = 0.001$). The adjusted mean ± SD of DFI after the intervention was significantly lower in the intervention group compared to the control group. The post-intervention mean in the intervention group was 21.84 ± 9.29, while in the control group it was 29.72 ± 11.30 ($p < 0.001$). Finally, for the FSH variable, no significant difference between the intervention and control groups was detected in the ANCOVA analysis. However, in the PRP group, the post-intervention mean was significantly lower than the pre-intervention value; the post-intervention mean was 4.96 ± 3.66 compared to 5.81 ± 5.34 before the intervention ($p = 0.020$). No statistically significant difference was observed in other variables.

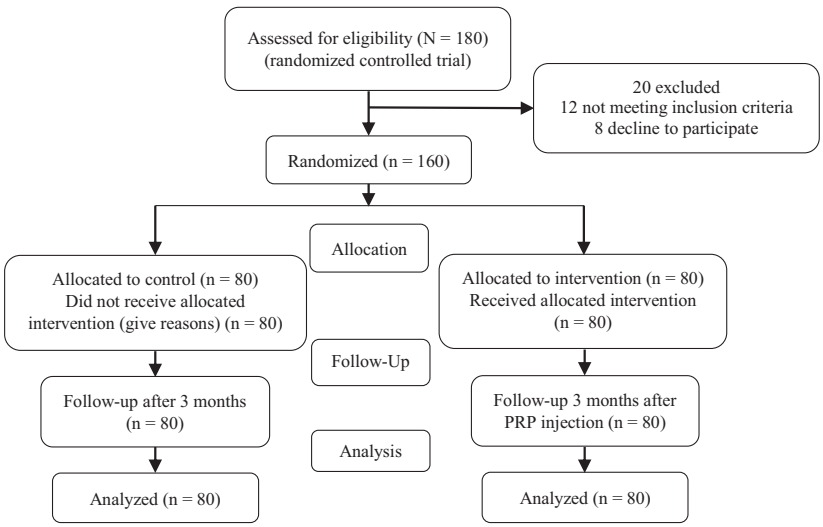

Figure 1. CONSORT flowchart for the triple-blinding randomized controlled trials.

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Table I. Demographic characteristics in control and intervention groups

Variables	Intervention group	Control group	P-value
Age (yr)*	34.31 ± 5.08 34 (30–38)	34.66 ± 4.69 35 (31.5–38)	0.652
Education**			
Under diploma	32 (40)	36 (45)	0.771
Diploma	30 (37.5)	26 (32.5)	
University	18 (22.5)	18 (22.5)	
Duration of marriage (yr)**			
< 5	31 (38.75)	26 (32.5)	0.704
5–10	32 (40)	36 (45)	
> 10	17 (21.25)	18 (22.5)	
Job**			
High risk	9 (11.25)	7 (8.75)	0.663
Moderate	24 (30)	29 (36.25)	
Low	47 (58.75)	44 (55)	
Previous ART cycle**			
1.00	46 (57.5)	43 (53.75)	0.631
2.00	26 (32.5)	25 (31.25)	
3.00	8 (10)	12 (15)	
Kind of infertility**			
Primary	64 (80)	63 (78.75)	0.845
Secondary	16 (20)	17 (21.25)	

*Data presented as mean ± SD. **Data presented as n (%). ANCOVA test. ART: Assisted reproductive technology

Table II. Sperm parameters and hormonal status between groups

Variables	Intervention	Control	P-value*	P-value**
Semen concentration (mil/ml)				
Before	7.62 ± 3.28	6.52 ± 3.05	0.031	0.004
After	13.93 ± 10.82	9.44 ± 5.64	0.001	
P-value***	< 0.001	< 0.001		
Morphology sperm (%)				
Before	0.80 ± 0.67	1.25 ± 0.81	< 0.001	0.387
After	1.40 ± 0.95	1.56 ± 1.35	0.387	
P-value	< 0.001	0.072		
Total motility (%)				
Before	16.7 ± 6.12	15.95 ± 3.21	< 0.001	0.001
After	25.33 ± 11.21	16.66 ± 4.78	0.052	
Progressive motility (%)				
Before median (interquartile range)	4.73 ± 5.10 3.21 (9.24)	7.99 ± 5.77	< 0.001	0.001
After	10.68 ± 10.33	7.98 ± 6.78	0.054	
P-value	< 0.001	0.993		

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Table II. Continued

Variables	Intervention	Control	P-value*	P-value**
Volume (ml)				
Before	2.22 ± 1.03	2.08 ± 0.72	0.326	0.939
After	2.19 ± 1.17	2.13 ± 0.78	0.734	
P-value	0.766	0.655		
DFI				
Before	38.30 ± 13.47	36.73 ± 13.38	0.461	< 0.001
After	21.84 ± 9.29	29.72 ± 11.30	< 0.001	
P-value	< 0.001	< 0.001		
FSH				
Before	5.81 ± 5.34	4.63 ± 4.19	0.121	0.232
After	4.96 ± 3.66	4.50 ± 3.85	0.442	
P-value	0.020	0.261		
LH				
Before	5.88 ± 4.70	5.05 ± 3.47	0.207	0.305
After	5.78 ± 4.49	4.80 ± 2.92	0.104	
P-value	0.801	0.111		
Prolactin				
Before	12.27 ± 8.10	13.27 ± 7.46	0.418	0.682
After	10.09 ± 3.86	10.72 ± 4.24	0.329	
P-value	0.002	< 0.001		
Testosterone				
Before	4.00 ± 1.73	4.00 ± 1.54	0.990	< 0.001
After	6.06 ± 2.39	4.53 ± 1.69	< 0.001	
P-value	< 0.001	< 0.001		

Data presented as mean ± SD, *Independent *t* test, **ANCOVA test, ***Paired *t* test, DFI: DNA fragmentation index, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone

4. Discussion

This study aimed to investigate the therapeutic potential of intratesticular autologous PRP injection on SDF in men with OAT. PRP exhibited the most generalized effects, being associated with improvements in sperm concentration, progressive motility, and DFI in OAT men. Additionally, testosterone after adjusting for the initial effect in the intervention group was significantly higher than in the control group. SDF has been increasingly recognized as a critical factor in male infertility, affecting not only fertilization but also embryo quality, implantation rates, and miscarriage (15). Elevated DFI levels have been associated with oxidative stress, defective chromatin packaging, apoptosis, and unresolved DNA strand breaks during spermatogenesis (16). In participants with OAT, these mechanisms are often exacerbated, contributing to poor reproductive outcomes. Our findings revealed a statistically significant reduction in sperm DFI following PRP administration, supporting the hypothesis that local injection of autologous PRP may contribute to the enhancement of the

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testicular microenvironment and spermatogenic function. In line with our study, PRP treatment has been shown to increase sperm concentration and motility while reducing SDF (13). Also, the positive effect of PRP on antioxidant factors such as total antioxidant capacity (TAC), superoxide dismutase, and catalase has been proven in the follicular fluid of women with diminished ovarian reserve (17).

PRP has been studied as a possible therapeutic approach for testicular toxicity and dysfunction because of its rich content of bioactive molecules and its role in supporting tissue regeneration, whose beneficial effects are exerted through antioxidant defense, tissue structure modification, increased spermatogenesis, and anti-inflammatory properties (18). The internal contents of platelet-derived products, such as growth factors, lead to improved viability, acrosome and plasma membrane integrity, mitochondrial membrane potential, and DNA integrity, and these factors help in maintaining the integrity of the acrosome protein (19). Platelet-rich products have been reported to increase the levels of antioxidant enzymes, such as glutathione, TAC, glutathione peroxidase, and superoxide dismutase, to reduce lipid peroxidation MDA (20). This antioxidant capacity can be attributed to the presence of antioxidant molecules and essential trace elements such as zinc, alongside growth factors like VEGF and IGF-1. VEGF, specifically, has been shown to mitigate oxidative damage through activation of the nuclear factor erythroid 2-related factor-2 pathway (21).

PRP is a concentrated autologous biological product taken from the participant's blood and once activated, releases a series of growth factors and cytokines. These include platelet-derived growth factor, VEGF, transforming growth factor, fibroblast growth factor, EGF, etc. (22). These bioactive molecules are known to influence cell survival, stem cell proliferation, and tissue remodeling, promote tissue repair, stimulate angiogenesis, and support stem cell proliferation and differentiation (23). Several factors explain the positive effect of PRP on stimulating sperm production; PRP may improve Leydig cell proliferation and testicular hormone production (24). In addition, PRP can activate growth factors such as VEGF and IGF-1, which lead to the activation of several biological proteins that lead to increased proliferation, growth, cell differentiation, and angiogenesis (25). The intratesticular administration of PRP likely enhances the microvascular network within the testes, improves oxygenation, reduces oxidative stress, and supports the function of Sertoli and Leydig cells, which are vital for proper spermatogenesis and maintenance of germ cell integrity.

Also, PRP improves sperm DNA damage due to its anti-apoptotic and antioxidant properties, which play a key role in maintaining the DNA of germ cells. As it has been shown, oxidative stress leads to the production of reactive oxygen species and can cause single-strand and double-strand DNA breaks in sperm cells, which is reduced by PRP (26).

By potentially enhancing the local antioxidant capacity and supporting cellular homeostasis, PRP may reduce reactive oxygen species-induced DNA damage. In our study, participants exhibited improved sperm DNA integrity following treatment, indicating that PRP may shift the testicular microenvironment toward a more favorable state for germ cell development and

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chromatin remodeling. The novelty of our study is the intratesticular route of PRP injection, which allows for direct interaction with testicular tissue, potentially maximizing the local bioavailability of growth factors. While other systemic treatments for male infertility often face limitations in effectively delivering drugs to target tissues, the direct injection approach overcomes these obstacles. Although intratesticular injection was somewhat invasive, no major side effects were reported, indicating that this treatment strategy is safe and feasible, and offers hope for infertile men who have had many difficulties along the way.

Our findings are consistent with previous limited reports investigating the role of PRP in male reproductive function (7). In animal models, PRP has been shown to improve spermatogenesis and testicular tissue structure (27). A few studies have reported enhanced semen parameters following PRP treatment, though most of them have focused on ejaculatory duct or seminal vesicle applications (28).

The control of spermatogenesis, a multifaceted and precisely regulated biological sequence that includes the proliferation, differentiation, and apoptosis of germ cells, is attributed to various hormonal factors such as FSH, LH, and testosterone, as well as genetic and environmental factors. Testosterone is essential for the proper development of spermatogenesis and the proliferation of spermatogonia until the maturation of germ cells (29). In our study, the mean testosterone level in the PRP group was significantly higher than that in the control group, indicating that PRP may positively affect Leydig cells and testicular steroidogenesis may be restored (30).

It should be noted that control group was not given injections because intratesticular injection is an invasive procedure that, even with a neutral solution, carries known risks of pain, bleeding, infection, and trauma (however small), and that the participants in the study were infertile individuals with the hope of recovery. Exposing half of them to these risks without any possibility of direct benefit (even theoretical) was not defensible to our ethics committee. Our main criterion was “no harm”. Also, due to ethical issues, both groups were given complementary treatment, so that the control group was not left without specific treatment during the research period.

4.1. Strengths and Limitations

A limited number of participants with total OAT, follow-up of participants for 3 months, and inability to eliminate environmental factors affecting DFI, such as smoking and other factors, were the limitations of our research. Placebo injections were not ethically permissible for the study group. This is because our target population consisted of individuals who had sought infertility treatment. Performing an invasive procedure (injection) without a therapeutic effect and not providing necessary care during the 3-month study period was contrary to ethical and humane standards.

The strengths of the design were spontaneous pregnancy, sperm with intact DNA and chromatin were used for intracytoplasmic sperm injection. However, most of these treatments are known as “unproven” because they originate from scientific research but have not been approved by health authorities for commercial use due to insufficient evidence of effectiveness or safety.

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5. Conclusion

This study suggests that intratesticular PRP injection may increase sperm concentration and progressive motility, as well as reduce sperm DNA fragility index in oligospermic males, and a significant difference was observed in testosterone levels after injection, leading to increased testosterone levels in infertile men. PRP shows potential as a safe and effective treatment for male infertility based on these findings. Crucial next steps involve validating these outcomes and gaining a complete understanding of the operational mechanisms through which PRP modulates sperm parameters.

Data Availability

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Author Contributions

Sh. Pilehvari, M. Ghorbani, and H. Babalhavaeji designed the study and drafted the paper. H. Babalhavaeji, F. Fazli, and Sh. Pilehvari contributed to sample collection. E. Talebi-Ghane and F. Fazli contributed to performing experiments and evaluating the results. All authors approved the final manuscript and take responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgments

The authors express their gratitude for the generous permission granted by the participants, allowing the utilization of their medical information in this study. The authors declare that they did not use artificial intelligence to write this article.

Conflict of Interest

The authors declare that there is no conflict of interest.

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