



In Vitro Studying the Effect of Adding Autologous Platelet Rich Plasma (PRP) to the Human Semen on the Sperm DNA Integrity

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ABSTRACT

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For conception and the development of healthy embryos, sperm DNA integrity is crucial. According to a growing body of studies, there is a strong correlation between sperm DNA damage and male infertility. Among the new medicines being developed in the medical field, the application of Platelet Rich Plasma (PRP) in human reproduction has yet to be examined. A total of 100 semen samples were used in the current experimental investigation. From November 2020 to June 2021, the research was conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies. Masturbation was used to get an ejaculated semen sample. After semen analysis, the samples were separated into two equal parts, one without autologous PRP and the other with 2% autologous PRP, with the DNA fragmentation assessed using the Sperm Chromatin Dispersion Test. There was highly significant reduction in DNA fragmentation index ($p < 0.001$). The mean sperm DNA integrity was reduced after adding PRP (33.85 ± 16.73 vs 38.55 ± 16.64), Mean ($\pm$ SE). PRP has been shown to improve human sperm DNA integrity.

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PRP, DNA, Sperm Preparation, DFI, Sperm DNA Fragmentation

1. Introduction

After one year of regular, unprotected sexual contact, infertility is defined as a couple's inability to conceive (**Zegers-Hochschild, et al.** ^[1]). Infertility affects around 15 % of couples globally, with 50 % of instances attributed to male factors alone or in combination with female factors. (**Choy and Eisenberg** ^[2]). Traditional semen analysis is still used to assess infertile men, despite the fact that it does not accurately predict male fertility or the success of assisted reproductive technology (ART), it is widely used. (**Wang and Swerdloff** ^[3]). In reality, a normal semen analysis is found in roughly 15 % of infertile patients (**Agarwal and Allamaneni** ^[4]). On the other hand, Sperm concentration, motility and morphology, might not adequately reflect sperm DNA integrity (**Guzick, et al.** ^[5]), which is harmful to embryo development, normal fertilization and ART success (**Simon, et al.** ^[6]). Platelets are megakaryocyte derivatives with α granules containing many secretory proteins. They are members of the growth factor, cytokine, and chemokine families, through growth factors, and their dense

granules are involved in the acceleration and control of wound healing processes. (**Carmona, et al.** ^[7]). According to previous studies (**Anitua, et al.** ^[8], **Rodriguez, et al.** ^[9]), Platelets Rich Plasma (PRP) is a concentrated platelet fraction that is higher than baseline values. It is recommended that the platelet concentration in the PRP be 3 to 5 times higher than normal. PRP is a one-of-a-kind therapeutic option used in a variety of medical settings, such as orthopedics and dermatology (**Lubkowska, et al.** ^[10]). PRP's positive effects are mostly due to its different bioactive components. (**Magalo, et al.** ^[11]).

2. Materials and Methods

The study was approved by the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies' ethical committee, which took 100 sperm samples from males who visited the infertility clinic at Al Nahrain University. Each participant had given informed consent to use the remainder of their sample before being included in the validation trial. The research began in November 2020 and ended in February 2021.

2.1. Sample Collection and Processing

Masturbation was performed to collect samples, which were then placed in sterile containers. Only one seminal sample was taken from each patient. Following receipt of the samples, they were placed directly in a 37 °C incubator to complete liquefaction in preparing for semen analysis. After multiple trials and Pilot study for using different concentrations of PRP 2 %, 4 %, and 7 % it has been found that the 2 % was the best percentage for PRP preparation and yielding significant results (**Bader, et al.** ^[12]). Each semen sample was divided into two equal parts. The first was administered PRP at a concentration of 2 %, while the second was not. After incubation for 15 min., the Sperm Chromatin Dispersion (SCD) test was used to determine the DNA integrity of the sperm. The SCD tests are depended on the concept that when sperm are treated with an acid solution prior to lysis buffer, the DNA dispersion halos visible following the removal of nuclear proteins in non-fragmented DNA sperm, as shown in **Figure 1**, nuclei which are either marginally

present or not present at all in fragmented DNA sperm nuclei, by using light microscopy (**Agarwal, et al.** ^[13]).

3. Statistical Analysis

The statistical package for the social sciences (SPSS version 23) computer software application was used to analyze the data. The degree of significance was determined using P-values of 0.05 or less. P-values of less than or equal to 0.01 were considered highly significant.

4. Results

Here we compare the sperm DNA fragmentation prior to and following the addition of PRP in infertile men with normozoospermia $p < 0.001$, asthenozoospermia $p < 0.001$, asthenoteratozoospermia $p < 0.001$, oligozoospermia $P = 0.012$, oligoasthenozoospermia $P = 0.002$, oligoteratozoospermia $P = 0.135$ and oligoasthenoteratozoospermia $P = 0.044$, as shown in **Table 1**. The significant reduction in DFI (%) after than before adding PRP

Table (1): Demographic parameters of patients enrolled in the study

Groups	Before adding PRP	After adding PRP	P
Normozoospermia (N31)	37.32 ±18.05	33.00 ±18.88	< 0.001 P HS
Asthenozoospermia (N24)	42.38 ±19.56	36.46 ±18.85	< 0.001 P HS
Asthenoteratozoospermia (N4)	39.50 ±11.39	30.50 ±12.12	< 0.001 P HS
Oligozoospermia (N17)	35.88 ±16.07	32.47 ±15.80	0.012 P S
Oligoasthenozoospermia (N12)	37.67 ±8.08	32.42 ±9.14	0.002 P HS
Oligoteratozoospermia (N6)	37.67 ±23.76	35.00 ±23.53	0.135 P NS
Oligoasthenoteratozoospermia (N6)	39.17 ±6.79	35.67 ±8.94	0.044 P S

S: significant, HS: highly significant, NS: no significant, P: paired t-test

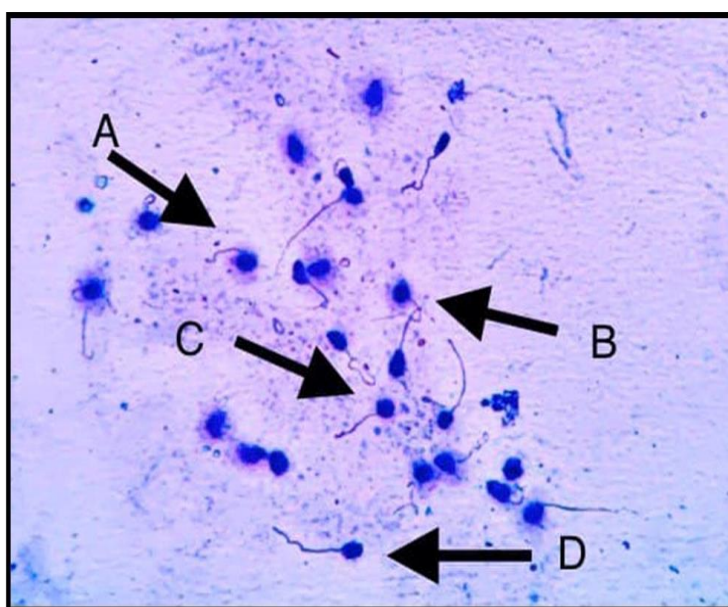


Figure (1): (A) spermatozoa with big halo (Normal sperms), (B) medium sized halo (Normal sperms), (C) small halo and (D) no halo (Abnormal sperms)

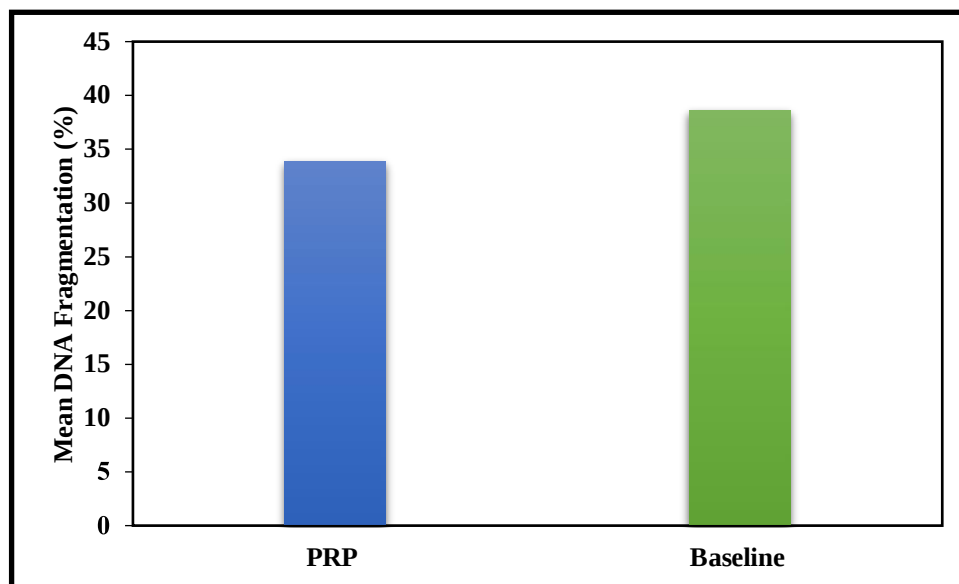


Figure (2): Comparison of mean DNA fragmentation (%) between PRP and baseline seminal fluid

(85 ± 16.73 vs 38.55 ± 16.64), Mean ($\pm$ SE), as shown in **Figure 2**.

5. Discussion

Around half of all reproductive problems are thought to be caused by male factor infertility. These problems either prevent sperm from being produced or impact sperm function after they have been produced. According to new medical therapies, in human reproduction, the use of autologous PRP looks to be a safe therapy option with a variety of potential benefits. (Lubkowska, et al. ^[10]). The current study's aims -in this regard- were to be one of the few that looked at the effects of PRP on a

variety of sperm functionality characteristics before looking into the possible influence of PRP on human sperm DNA fragmentation for the first time. Although there have been no previous human research or papers on this topic (to the best of our knowledge), animal experimental investigations have revealed that PRP can assist to increase the sperm quality. PRP outperforms other treatments in terms of minimizing the fraction of sperm DNA fragmentation, according to the findings of this study, which support those of some experimental investigations that have indicated the influence of PRP in sperm activation. The biological function of autologous PRP in male

infertility treatment was assessed by comparing DNA fragmentation of nontreated spermatozoa to those treated with 2 % PRP. Surprisingly, the second group's DNA fragmentation was reduced. **Figure 2** shows a comparison of sperm characteristics before and after PRP addition in all involved samples. The DNA fragmentation index decreased by a significant amount. Agreeing with (**Bader, et al. [12]**) who observed that PRP inhibits ROS by antioxidant and antiapoptotic action, resulting in a highly significant reduction in DNA fragmentation index. Because of the wide range of growth factors found in his alpha granules. The antioxidant Zn/Cu/SOD enzyme, a significant component of PRP, has been discovered to serve a protective effect in sperm motility, which is linked to sperm membrane integrity, in accordance with its mechanism of action. In fact, as a critical component of the Reactive Oxygen Species (ROS) scavenger system, its mode of action is based on decreasing lipid peroxidation (LPO) in human spermatozoa, which reduces DNA fragmentation caused by Hydrogen Peroxide (H₂O₂) exposure (**Perumal, et al. [14]**, **Lee, et al. [15]**, **Zhao, et al.**

[16]). The use of Insulin growth factor (IGF1) to incubate sperm has been found to result in a significant reduction in DNA fragmentation (**Susilowati, et al. [17]**). In patients undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (**Obermair, et al. [18]**) established a relationship between seminal plasma Vascular endothelial growth factor (VEGF) concentrations and pregnancy rates. Also, the results for this patient's show reduction in DNA fragmentation, Zinc supplementation has also been proven to boost the production of metallothioneins, which help to keep seminal fluids consistent and prevent sperm from harm (**Di Leo, et al. [19]**). Metallothioneins have the ability to protect biological issues from oxidative stress damage by trapping harmful oxidant species including hydroxyl radicals and superoxide (**Suriya, et al. [20]**). Zinc in seminal plasma contributes to the stability of sperm chromatin (**Björndahl and Kvist [21]**)

6. Conclusions

Autologous PRP has been shown to improve human sperm DNA integrity. In patients who

have been diagnosed, a highly significant reduction in DNA fragmentation index was noticed in the oligoasthenozoospermia, asthenoteratozoo-spermia and asthenozoospermia, respectively. On the other hand, a significant reduction in DNA fragmentation index in infertile men with oligoasthenoteratozoospermia and oligozoospermia was realized. Additionally, there was no significant in DNA fragmentation index in men with oligoteratozoospermia.

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Author Contribution

Hamdan, DA, performed the study, examined and reviewed results, and manuscript writing with the help and supervision of Rahim, AI, and Al-Kawaz, UMR.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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