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The efficiency of using plasma rich platelets as activation media to assessment of human sperm parameters and chromatin integrity

Hayder Abdullah Kadhim Al-Sarray[1], Muhanad Sachit Al-Delphi[2], Zahraa Zuhair Alsaidi[3]

[1,2,3] Middle Technical University, Kut Technical Institute, Iraq

Abstract

Background: Plasma rich platelets (PRP) are blood-derived product with platelet concentrations exceeding five times those of whole blood. PRP factors have beneficial effects including the enhancement of sperm motility and morphology. Methods: The study included 40 participant men were attendance to Al-Batool hospital in wasit province in Iraq; from the period February to June 2025. Each semen sample was assessed according to WHO 2010 and for chromatin integrity and considered as before activation group. Afterward, each sample of semen was split into two sections. one of the two parts the sperms suspended with (1ml) FertiCult™ medium and considered as (FertiCult group) and the second part the sperms suspended with (1ml) PRP only and considered as (PRP group) then each sample assessed for sperm parameters and chromatin integrity after 30 and 60 minutes. Results: The result showed rising in percentage of progressive motility, normal morphology and chromatin integrity in PRP group when comparing with before activation and FertiCult groups. The results also appeared that there was a noteworthy improvement in normal sperm morphology ($P<0.05$) in PRP media group after incubation of sperms for 30 and 60 minute. The result suggest that the chromatin integrity had improved significantly ($P<0.05$) for the Ferticult media and PRP media groups by comparing with before activation groups. Conclusions: This study are concluded the use of plasma rich platelets (PRP) as media for activation of sperms play a main role for efficient in enhancing sperm parameters and improve the DNA sperm integrity.

Keywords: Plasma rich platelets (PRP), FertiCult media, Sperm parameters, DNA fragmentation, chromatin integrity.

Introduction

Infertility is characterized by a couples fail to conceive after engaging in unprotected sexual intercourse for a one year (1). The prevalence of infertility ranges from (8 to 15 %) with the male factors accounting to approximately half of these cases (2). The various factors that cause male infertility may stem including sperm abnormalities parameters like low concentration, minimize motility or abnormal in morphology can reduce the rates of successive of assisted reproductive technology (ART) treatment and is thought to be a critical component in infertility of male. (3,4).

Plasma rich platelets (PRP) are substance made from blood that has high concentration of platelet five times more than the amount found in fresh blood (5). PRP includes around 800 protein molecules such as hormones, chemo-attractants; cytokines (6) and contain growth factors, like insulin_like growth factor_1,, platelet_derived growth factor,, tumor necrosis factor-B, interleukin_1B; fibroblast growth factor (FGF), interleukin-10, and vascular endothelial growth factors (VEGF). (7). Also contain superoxide dismutase (SOD), serotonin, histamine, calcium as well zinc ions then ATP are also present in PRP (8).

Due to its high efficacy and safety, sperm samples incubated for five minutes with PRP media showed notable increases in motility and morphology, according to a current study investigating the function of PRP in sperm physiology (9).

Sperm motility has been shown to be improved by PRP factors like VEGF and TGF,, capacitation and acrosomal reaction to the sperms are supported by some ions such as calcium and zinc ions sperms are protected from damage during and post-cryopreservation quality is improved by NGF,, IGF_1,, ATP,, zinc ions, SOD and platelet_activating factors (8).

As a result the aim of this study is to explain the rate of concentration,, motility, morphology and chromatin integrity of human sperm parameters following 30 and 60 minutes of PRP incubation.

Correspondence: Hayder Abdullah Kadhim Al-Sarray, Middle Technical University, Kut Technical Institute, Iraq, **e-mail:** hayder-abdullah@mtu.edu.iq;,
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Materials and Methods

This study included 40 participant men with the range of age between 22 to 38 years old were had a normal semen analysis based on the assessment of semen fluid analysis during their attendance to Al-Batool hospital in wasit province in Iraq; from the period February to June 2025.

Semen collection

A sterile wide-mouth plastic container was used to collect the semen sample after being obtained through masturbation. The sample incubated at 37°C for 30 minute to allow the liquefaction of the semen. The sperm parameters assessed accordance with the guidelines established by the World Health Organization (WHO) in 2010 (10).

Study groups

The sperm concentrations; motility, morphology and chromatin integrity of each semen sample were assessed and considered as (before activation group) then every semen sample was split into two sections each one was centrifuged in six minutes at 2300 rpm.

The supernatant removed the pellet that contains the sperms suspended as the following; one of the two parts the sperms suspended with (1ml) FertiCult™ Flushing HEPES buffered medium and considered as (FertiCult group) and the second part the sperms suspended with (1 ml) PRP only and considered as (PRP group) then each sample assessed for sperm parameters and chromatin integrity after 30 and 60 minutes.

Preparation of Plasma Rich Platelets (PRP)

For every participant, five milliliters of blood were extracted. Two centrifugation processes were used to prepare autologous PRP. To divide the blood into three layers, the centrifugation firstly was performed at a speed 380 g to fifty min. Then second centrifugation was performed at a speed 1300 g to eight min. in order to obtain PRP. After that, a filtration procedure was carried out to eliminate WBCs using WBC filtration filters (11).

Chromatin integrity

After 30 minutes of fixing in 3% glutaraldehyde, semen slides were stained for five minutes with acidic aniline blue. Under 40×, the stained slides were visible. The dark blue stained sperm was regarded as aberrant or containing DNA fragmented, but sperm with unstained or faintly stained the nuclei were seen normally. One hundred sperm were counted, and the percentage of chromatin integrity was noted (12,13).

Statistical analysis

SPSS software (version 21, Chicago, USA) was utilized. An independent-samples T-test with a complete randomized design (CRD) was used to examine the sperm parameters. The mean ± SD is used to express the data.

Ethical approval

This study was authorized by a Scientific Ethical Committee at middle technical university (MEC 132).

Results

Table 1: Comparison of sperm properties following a 30-minute incubation period

Sperm parameter		**Before activation**	**FertiCult Group**	**PRP Group**
Sperm concentration (millions/mL)		51.50 a ±1.68	27.50 b ±1.02	41.50 c ±2.79
Sperm motility (%)		61.00 a ±1.22	78.00 b ±0.81	79.50 b ±0.83
Sperm grade activity (%)	Progressive sperm motility (%)	32.50 a ±1.48	50.50 b ±0.83	60.00 b ±1.07
	Non Progressive sperm motility (%)	28.50 a ±0.95	27.50 a ±0.73	19.50 b ±0.43

	Immotile sperm (%)	39.00 a ±1.22	24.00 b ±0.59	20.50 b ±0.83
Normal sperm morphology (%)		78.10 a ±0.95	87.40 b ±0.64	90.20 c ± 0.42

The means with distinct letters within each row differ significantly (P<0.05).
There is non significant differences between means with comparable letters in each row (P>0.05).
The data is mean ±. S.E.
Table (2) appeared indicated the rise was significant (P<0.05) in sperms motility and normal morphology whereas significant fall (P<0.05) was seen in immotile sperms in PRP group when comparing with before activation and FertiCult group after incubation of sperms in PP for 60 minute.

Table 2: Comparison of sperm parameters following a 60-minute incubation period

Sperm parameter		**Before activation**	**FertiCult Group**	**PRP Group**
Sperm concentration (millions/mL)		51.50 ab ±1.68	35.00 a ±1.38	47.00 ab ±2.35
Sperm motility (%)		61.00 a ±1.22	80.50 b ±0.66	85.00 c ±0.50
Sperm grade activity (%)	Progressive sperm motility (%)	32.50 a ±1.48	55.50 b ±1.10	66.50 b ±1.19
	Non Progressive sperm motility (%)	28.50 a ±0.95	25.00 b ±0.62	18.50 b ±0.72
	Immotile sperm (%)	39.00 a ±1.22	19.50 b ±0.66	15.00 c ±0.50
Normal sperm morphology (%)		78.10 a ±0.95	87.40 b ±0.64	90.30 c ±0.41

There is non significant difference between means with comparable letters in each row (P>0.05). The data is mean ±. S.E.

The result is presented in table (3) illustrated the comparison between sperm parameters in the PRP group when incubation of sperms for 30 and 60 minute.

The results showed that a significantly increase (P<0.05) in motility also there was grow in percentage (%) of progressive sperm motility when incubation of sperms for 60 minute.

There was non-significant difference (P>0.05) when comparing the concentration and normal morphology to the sperm.

Table 3: Comparison between sperm parameters in 30 and 60 minutes of PRP incubation

Sperm parameter		**PRP Group**	
		After 30 min.	**After 60 min.**
Sperm concentration (millions/mL)		41.5 a ±2.79	47.00 a ±2.35
Sperm motility (%)		79.50 a ±0.83	85.00 b ±0.50
Sperm grade activity (%)	Progressive sperm motility (%)	60.00 a ±1.07	66.50 a ±1.19
	Non Progressive sperm motility (%)	19.50 a ±0.43	18.50 a ±0.72
	Immotile sperm (%)	20.50 a ±0.83	15.00 b ±0.50
Normal sperm morphology (%)		90.20 a ±0.42	90.30 a ±0.41

There is a significantly difference (P<0.05) between means with the different letters within each row.

There is no significant difference between means with comparable letters in each row (P>0.05).

Data are mean ± S.E

Following 30 and 60 minutes of sperm incubation, Figure 1 showed the chromatin integrity between the research groups. According to the data, the Ferticult and PRP groups' normal chromatin integrity grow significantly (P<0.05) for both 30- and 60-minute incubation periods as compared to the pre-activation group. Additionally, these results demonstrated that the chromatin integrity of the PRP and FertiCult groups at varying incubation times did not differ significantly (P>0.05).

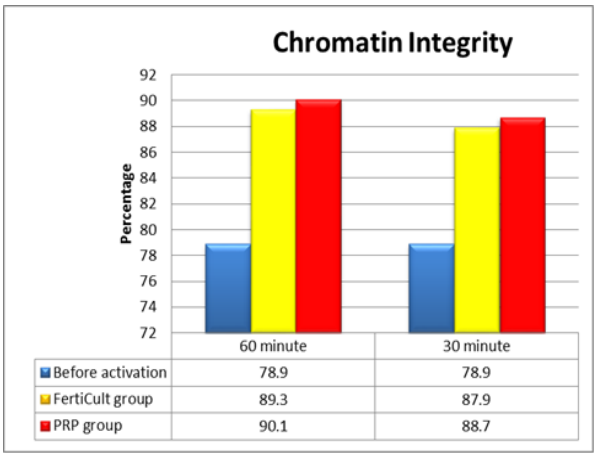

Figure (1): Comparison of sperm chromatin integrity among PRP group, Before activation and FertiCult groups

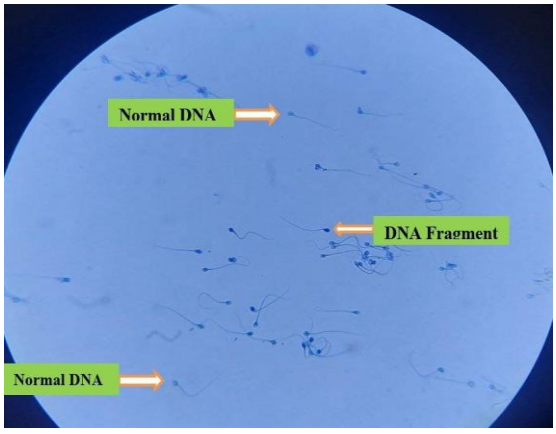

Figure (2): Chromatin integrity for sperms by using Acidic Aniline blue.

Discussion

The presented study demonstrated that the use of autologous plasma rich platelets (PRP) as activation media for human sperm showed increasing in proportion (%) of normal sperm morphology, increasing sperm motility, and sperm motility in PRP group when comparing with before activation and FertiCult groups. The presented study agrees with a According to a recent study by Yan B et al. (2021), autologous PRP enhanced the progressive motility and viability of sperm (8) . According to (Bader et al. 2020) determined that PRP concentration at a 2% has demonstrated as protective effect in some specific sperm characteristics, including sperm DNA integrity and then progressive mobility (14).

According to this findings, there was a noteworthy growth (P<0.05) in motility and moreover, the percentage (%) of progressive motility to the sperm increased when incubation for 60 minute when comparing with 30 minute of incubation. This findings suggested that normal chromatin integrity had significantly increase (P<0.05) for PRP media groups when comparing with before activation group for both incubation periods (30 min. and 60 min.). This finding is consistent with another study that suggested PRP might help lower the reactive oxygen species and DNA fragmentation index percentage in semen samples.

According to studies, poor implantation rates its related with broken DNA sperm and linked to fertilization and embryo development, as well as an increased risk of miscarriages or repeated pregnancy loss (15). One of the primary components of PRP is superoxide dismutase (SOD) that can lower oxygen free radicals. Studies have demonstrated that by lowering lipid peroxidation in spermatozoa, SOD can stop the DNA fragmentation during sperm storage. Additionally, this enzyme lowers the quantity of dead sperm (16).

Conclusions

This study concluded that can use of plasma rich platelets (PRP) as media for activation of sperms was helpful in enhancing sperm parameters including motility, progressive sperm motility and normal motility. This study shown that incubation of sperms for 1 hr. give best sperm parameters that for 30 minute. Also the study concluded that incubation of sperms with PRP media can improve the DNA

integrity for sperms.

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