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Study the effect of mixing platelet-activating factor with sperm preparation medium of normozoospermic men on intra uterine insemination outcome.

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ABSTRACT

Intrauterine insemination (IUI), it is a type of artificial insemination, used to treat infertility by which the Sperm that have been prepared and placed directly into the woman's uterus around the time of ovulation. The main purpose of this procedure is to enable the sperm to swim into the fallopian tubes and fertilize a ready egg in order to lead to a natural pregnancy. IUI can be coordinated with the normal cycle with fertility or medications depending on the reasons for infertility The IUI outcome still low about 20-25% so adding number of biochemical factors to the culture medium which are essential to regulate the fertility potential of spermatozoa, for example, platelet-activating factor (PAF) which is a unique and novel signaling phospholipid that has multiple biologic properties have been tried. To determine the effect of adding the PAF into the culture medium to prepare and activate the semen samples of non-male factor infertility (normal semen analyses). This study was carried out in the Infertility Unit, AL-Hussein Teaching Hospital, Al-Nassirya City, through the period from 30th of October 2020 till 17th of April 2021. The study included thirty couples who are complaining from infertility (they failed to conceive after one year at least of regular unprotected intercourse. The frequency distribution of enrolled infertile men with normozoospermia Baseline characteristics of seminal fluid analysis of males with normozoospermia those with PAF are Semen volume ml, pH, Sperm concentration before million/ml, Sperm motility %, morphologically normal sperm %, were Mean ±SD (3.10 ±1.23, 7.39 ±0.03, 46.67 ±15.31, 1.33 ±3.52, 36.00 ±5.07) respectively. Certain sperm parameters were highly improved after in vitro activation by PAF medium of Normozoospermic samples.

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KEYWORD

Platelet-Activating Factor, Normozoospermia, Infertility, Intrauterine Insemination (IUI)

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1. Introduction

Infertility consider as a major clinical and social problem, affecting one couple in six in the whole world. The evaluation usually starts after complete 12 months; however, it may be indicated earlier (after 6 months) if the age of the mother is more than 35 years old **(MOL Ben W. et al., 2018[1])**. The couple with infertility problem should be seen together, and a full general, family, surgical, drug, and medical history should be taken from both of them as well as the examination of both is necessary, especially for woman, while the examination of man is not usually helpful unless indicated by his medical history (such as previous orchidopexy, inguinal hernia repair, or testicular torsion) or if his initial semen analysis result is abnormal.

(Karamahmutoglu et al.,2014[2]). In most couples, the initial history and examination will not give the exact cause of their infertility, so that a full investigation will be needed. (**Flyckt et al.,2019[3])**.

Spermatozoa prior to IUI must be first processed by one of the in vitro preparation techniques, e.g., density gradient separation, wash, or swim-up **(Lemmens, L. et al.,2017[4]).**

This processing of spermatozoa aims to ensure that the most normal motile sperm are used to help facilitate conception. Therefore, semen washing is important as it removes prostaglandins, white blood cells, and debris, as well as for reducing the number of non-motile and morphologically abnormal sperm cells. Additionally, the removal of seminal plasma also helps the sperm to enhancing capacitating and acrosome reaction, resulting in the enhancement of sperm motility **(Karamahmutoglu et al.,2014[2])**.

The exact mechanism of the Platelet-activating factor is uncertain, yet its importance in normal fertility is clear. It is naturally present in human

spermatozoa and its endogenous content have a significant and positive relationship with motility and pregnancy rate; Exogenous PAF have been used to stimulate human sperm motility (**Agarwal et al.,2020[5]**).

Also, PAF is present in the endometrial tissue and appears to be associated with the implantation process as in the concentration within the uterus changes dramatically during the implantation period while the PAF antagonists inhibit implantation.

2. Patients and Methods

This study was carried out in the Infertility Unit, AL-Hussein Teaching Hospital, Al-Nassirya City, through the period from 30th of October 2020 till 17th April 2021. The study included thirty couples who are complaining from infertility (they failed to conceive after one year at least of regular unprotected intercourse). Women included in this study was between 20 years and 43 years old; they have a normal, regular, and ovulatory cycle and normal pelvic U/S. Hormonal assay within normal range, as well as have at least one patent tube in Hysterosalpingogram. The husbands have Normozoospermic seminal fluid analysis. The 30 couples were divided into two groups: 15 were prepared by the swim wash-up method (SWM) without adding PAF, and the other 15 semen samples were washed with a medium containing PAF.

2.1Culture Media

FertiCult Flushing medium is a chemically balanced salt solution 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES buffered) with 0.4% human serum albumin that can be used for washing of human ovum, spermatozoa, and embryos. FertiCult flushing medium may also be used for swim-up techniques of human spermatozoa.

2.2 Platelet-activating factor (PAF)

It is a Synthetic water-soluble platelet activating factor and potent mediator of inflammation that increases vascular

permeability. Activates MAP kinase and MAP kinase (MEK) in CHO cells, Packaging in 1 mg in a Plastic ampoule. Sperms in one aliquot were treated with an exogenous mixture of PAF (final concentration, (10 -7 moll/L) in SWM. Synthetic PAF (Calbiochem-Novabiochem, La Jolla, CA, USA) was stored in a stock solution (1x10 -6 moll/L) of chloroform and methanol. Before use, 0.1 ml of stock PAF was dried and dissolved in 1 ml of SWM. The sperm was incubated in the solution at 37 o C. Other group (SWM) Sperms in other aliquots were incubated in Gamete buffer in a similar as for other groups.

2.3 Statistical analysis

Data were collected, summarized, analyzed, and presented using Statistical Package for Social Sciences (SPSS) version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as numbers and percentages, whereas quantitative (numeric) variables were first evaluated for normality distribution using the Kolmogorov-Smirnov test, and then accordingly, normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion).

3. Results

Baseline characteristics of seminal fluid analysis of males with normozoospermia contrasted between those with PAF and those with no PAF are shown in **(Table 1)**. There was no significant difference in mean semen volume between PAF and non-PAF groups ($p = 0.604$).

There was a highly significant difference in mean pH between PAF and non-PAF groups ($p < 0.001$). There was no significant difference in mean sperm concentration between PAF and non-PAF groups ($p = 0.748$). Grade A sperm motility % was higher in the non-PAF group in comparison with the PAF group, and the difference was

highly significant ($p < 0.001$). In addition, Grade B sperm motility % was higher in the non-PAF group in comparison with the PAF group, and the difference was highly significant ($p < 0.001$). Thus, Grade A+B sperm motility % was higher in the non-PAF group in comparison with the PAF group, and the difference was highly significant ($p < 0.001$).

There was no significant difference in grade C motile sperm % ($p = 0.426$), but Grade D immotile sperm % was lower in the non-PAF group in comparison with the PAF group, and the difference was highly significant ($p < 0.001$). Moreover, normal morphology sperm motility was higher in the non-PAF group in comparison with the PAF group, and the difference was highly significant ($p = 0.002$).

Post-activation sperm characteristics of males with normozoospermia contrasted between those with PAF and those with no PAF are shown in **(Table 2)**.

There was a highly significant difference in mean sperm concentration between PAF and non-PAF groups ($p < 0.001$), the level being higher in the non-PAF group. Grade A sperm motility % was higher in the non-PAF group in comparison with the PAF group, and the difference was highly significant ($p < 0.001$), but there was no significant difference in mean grade B sperm motility % between PAF and non-PAF groups ($p = 0.341$), thus, Grade A+B sperm motility % was higher in the non-PAF group in comparison with PAF group and the difference was significant ($p = 0.001$).

There was no significant difference in grade C motile sperm % ($p = 0.125$), but Grade D immotile sperm % was lower in the non-PAF group in comparison with the PAF group, and the difference was highly significant ($p < 0.001$).

Both grade C and grade D immotile sperm % were lower in the PAF group in comparison with the non-PAF group,

and the difference was significant ($p < 0.05$). Moreover, there was no significant difference in normal morphology sperm % ($p = 0.247$).

Comparison of changes in mean sperm characteristics before and after activation contrasted between those with PAF and those with no PAF are shown in **(Table 2)**. There was no significant difference in mean change in sperm concentration between PAF and non-PAF groups ($p = 0.05$), as shown in **(Figure 1).**

There was no significant difference in mean change in grade A sperm motility % between PAF and non-PAF groups ($p = 0.529$), but the mean change in grade B sperm motility % was higher in the PAF group in comparison with the non-PAF group and the difference was significant ($p = 0.015$). Thus, the mean change in grade A+B motile sperm % was higher in the PAF group in comparison with the non-PAF group, and the difference was significant ($p = 0.028$).

There was no significant difference in mean changes in grade C and grade D motile sperm % between PAF and non-PAF groups ($p > 0.05$). Moreover, there was no significant difference in mean change in normal morphology sperm % ($p = 0.051$), as shown in **(Figure 1). The** comparison of successful IUI outcomes between males whom seminal fluid was treated with PAF and males whom seminal fluid was not treated with PAF is shown in **(Table 3).**

Table (1): Baseline (pre-activation) characteristics of seminal fluid analysis in of males with normozoospermia contrasted between those with PAF and those with no PAF.

Characteristic	Total n = 30		PAF n = 15	P - Value
Semen volume ml				
Mean ±SD	3.20 ±1.03	3.10 ±1.23	3.30 ±0.82	0.604 I
Range	1.5 -5	1.5 -5	2 - 4.5	NS
Ph				
Mean ±SD	7.31 ±0.12	7.39 ±0.03	7.23 ±0.12	< 0.001 I
Range	7 -7.4	7.3 -7.4	7 -7.4	HS
Sperm concentration before (million/ml)				
Mean ±SD	45.83 ±13.84	46.67 ±15.31	45.00 ±12.68	0.748 I NS
Range	20 -70	20 -70		
Sperm motility %				
Grade A %				
Mean ±SD	6.00 ±7.12	1.33 ±3.52	10.67 ±6.78	
Range	0 -25	0 -10	0 -25	
Grade B %				
Mean ±SD	35.67 ±11.58	27.67 ±9.42	43.67 ±7.19	
Range	15 -50	15 -45	30 -50	
Grade A+B %				
Mean ±SD	41.67 ±15.16	29.00 ±10.39	54.33 ±4.95	
Range	15 -65	15 -45	45 -65	
Grade C%				
Mean ±SD	7.67 ±4.50	8.33 ±3.09	7.00 ±5.61	
Range	0 -20	0 -10	0 -20	
Grade D %				
Mean ±SD	50.67 ±14.37	62.67 ±9.80	38.67 ±4.81	
Range	30 -80	50 -80	30 -45	

Normal morphology %			
Mean ±SD	39.17 ±6.03	36.00 ±5.07	42.33 ±5.30
Range	30 -55	30 -45	35 -55
n: number of cases; **SD**: standard deviation; **I**: independent samples t-test; **NS**: not significant at $p > 0.05$; **HS**: highly significant at $p \leq 0.01$			

Table (2): Post-activation sperm characteristics of males with normozoospermia contrasted between those with PAF and those with no PAF

Characteristic	Total n = 30	PAF n = 15	No PAF n = 15
Sperm motility %			
Grade A %			
Mean ±SD	25.17 ±9.14	19.67 ±8.55	30.67 ±5.94
Range	10 -40	10 -35	15 -40
Grade B %			
Mean ±SD	45.83 ±10.35	44.00 ±8.28	47.67 ±12.08
Range	15 -60	30 -60	15 -60
Grade A+B %			
Mean ±SD	70.00 ±15.43	63.67 ±14.70	76.33 ±13.82
Range	40 -90	40 -90	50 -90
Grade C%			
Mean ±SD	0.83 ±2.96	0.00 ±0.00	1.67 ±4.08
Range	0 -15	0 -0	0 -15
Grade D %			
Mean ±SD	27.33 ±14.96	36.33 ±14.70	18.33 ±8.59
Range	10 -60	10 -60	10 -40
Normal morphology %			
Mean ±SD	36.00 ±6.38	35.33 ±5.16	36.67 ±7.19
Range	30 -50	30 -50	35 -50

Table (3): The comparison in Doppler study findings according to pregnancy outcome

Group	Present IUI	Total *n* = 30	PAF *n* = 15	No PAF *n* = 15	
Normozoospermia	Success, n (%)	8 (26.7 %)	6 (40.0 %)	2 (13.3 %)	0.21 Y 5 NS
	Failure, n (%)	22 (73.3 %)	9 (60.0 %)	13 (86.7 %)	

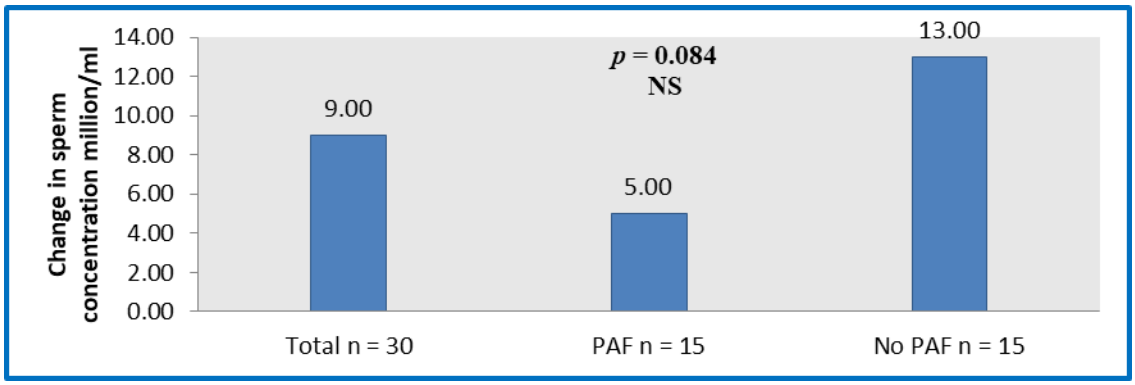

Figure (1): Comparison of changes in mean sperm characteristics before and after activation contrasted between those with PAF and those with no PAF

4. Discussion:

4.1 The Seminal Fluid Analysis and its Functional Parameters

Male infertility evaluation depends basically on semen analysis. It provides essential information of the clinical status of the individual in addition to any problems in the genital organs of the male, and there is the link between semen quality and fertility state (**Andrade-Rocha & Fernando Tadeu,2017 [6]**). Concerning the results of this study, the Normozoospermia showed that all the sperm's function parameters were higher than that of Asthenozoospermia. These results were in accordance with the results reported by WHO1999.

4.2 In vitro activation of the semen:

The main aim of many ART research is to improve the ability of a sperm to fertilize a mature oocyte; this can be achieved by any one sperm preparation technique, which is important to give a good quality and fully matured sperm to reach a successful outcome. (**Haddad et al.,2021[7]**).

There are many types of these preparation techniques available to select a more progressive motile spermatozoon. In the present study, the swim-up washing technique (SWM) was applied, which was achieved by washing sperm by a free FertiCult flushing medium. Adding of some exogenous factors to the media, like PAF, will promote spermatozoa hyperactivation and improved the outcome of fertilization, as stated by (**Lecewicz, M et al., 2017 [8]**).

4.3 Motility of sperm

Regarding sperm motility, there was a significant increase in sperm motility after activation in PAF and non-PAF-treated groups compared with before activation .

A high statistically significant increase in grade A motile sperms in both treated and non-treated groups was observed compared to results before activation, as well as the total progressive motility (A+B) have been increased significantly in both groups following the activation compared to before activation. This is in agreement with previous studies of authors.

(**Ibis et al.,2021[9]**); (**Ahmed S. M et al.,202[10]); (Hailat et al., 2016 [11]**) who suggested that "The flushing culture media contains many ions such as sodium, potassium, calcium, magnesium, phosphate, pyruvate, and lactate and These ions works as a source of energy to activate sperms that increase their movement." That may explain a significant improvement in sperm motility percentage in both treated and non-treated groups in

normospermic men.

In the contrary, both grade C and D sperm motility have been reduced after semen processing in both groups, that means only the motile sperms swim up to the upper layer while dead and immotile sperms remain in the pellet of the medium (**Hassan, M. S et al.,2021[12]**).

5. Conclusion

that certain sperm parameters were highly improved after in vitro activation by PAF in normospermic samples. A statistically significant increase in the sperm concentration of normospermic samples under the effect of PAF addition

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Author Contribution Anaam H. Shahed performed the study, and Saad S. Al-Dujaily, and Mufeda A. AL-Amar, supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

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