EFFECT OF PLATELET ACTIVATING FACTOR MEDIUM FOR IN VITRO SPERM ACTIVATION OF ASTHENOZOOSPERMIA MEN ON INTRAUTERINE INSEMINATION OUTCOME

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ABSTRACT

Aim of the study: To determine the effect of platelet activating factor (PAF) into the culture medium to prepare and activate the semen samples of male factor infertility.

Materials and Methods: This study was carried out in the Infertility Unit, AL-Hussein Teaching Hospital, Al-Nassirya City, through the period from 30th October 2020 till 17th April 2021. The study included thirty couples who are complaining from infertility.

Results: The frequency distribution of enrolled infertile men with asthenozoospermia. Baseline characteristics of seminal fluid analysis of males with asthenozoospermia those with PAF medium were Semen volume ml, pH, Sperm concentration before million/ml, Sperm motility %, morphologically normal sperm( %), were mean ±SD (2.80 ±1.03, 7.37 ±0.07, 51.67 ±8.80, 8.67 ±6.11, and 40.00 ±0.00, respectively).

Conclusion: Certain sperm function characters were highly improved after in vitro activation by a medium containing PAF of asthenozoospermic samples resulted in improvement of IUI outcome.

Keywords: platelet-Activating Factor, Asthenozoospermia, Infertility, intrauterine insemination.

I. INTRODUCTION

It has been suggested that infertility is considered as a major clinical and a social problem, affecting one out of six couples 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 women more than 35 years old (Mol, et al. 2018). Usually the couple complaining from infertility problem must examined together, and a full general, family, surgical, drug and medical history taken from both of them. 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 primary semen result is abnormal. In most couples, the initial history and examination will not give the exact cause of their infertility, thus a full investigation will be required (Flyckt et al., 2019).

It has been reported that semen sample prior to IUI must be first processed by one of in vitro preparation techniques e.g. wash and spin, density gradient separation, or swim-up (Lemmens, 2017, Al-Dujaily et al., 2016). This processing of spermatazoa aims to ensure that the most normal motile sperms are used to help facilitate conception. Therefore, the 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, removal of seminal plasma also help the sperm to enhancing capacitating and acrosome reaction resulting in the enhancement of sperm motility (Al-Dujaily, 2021).

The exact mechanism of Platelet-activating factor is uncertain, yet its importance in normal fertility is clear. Naturally PAF present in human sperm and its endogenous content has a significant and positive relationship...
with motility and pregnancy rate. Exogenous PAF have been used to stimulate human sperm motility (Agarwal, and Sengupta, 2020).

It has been found that PAF present in the endometrial tissue and appears to be associated with implantation window as its concentration within the uterus change dramatically during the implantation period while the PAF antagonists inhibit implantation. During the final stages of pregnancy, the concentrations of PAF will be increased too, possibly as a result of the fetus lung (Yang et al., 2019). The objective of this study is to enhance the IUI outcome by in vitro activation the sperm of asthenozoospermic men with motility stimulant using PAF as new treatment for this purpose.

**Subjects and Methods:** This study was carried out in the Infertility Unit, AL-Hussein Teaching Hospital, Al-Nassirya City, through the period from 30th October 2020 till 17th April 2021. The study included thirty couples who are complaining from male infertility (they failed to conceive after one year at least of regular unprotected intercourse. The 30 couples were divided into two groups 15 prepared by swim up method (SWM) without PAF, and other 15 semen samples washed with PAF medium.

**Culture Media:** FertiCult Flushing medium (FertiCult Company, Belgium) is a chemically balanced salt solution 4-(2-hydroxyethyl)-1-piperazineethanesulphonic 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. This medium contains physiologic salt solution (HEPES buffered), 2-Hydroxypropanoic acid (lactate), and human serum albumin (HSA) (4.00g/liter), with Gentamicin Sulphate added upon request (10mg/liter).

**Platelet activating factor (PAF)** is a Synthetic water-soluble platelet activating factor and potent mediator of inflammation that increases vascular permeability. Packaging in 1 mg in 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 C°.

### II. 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 number and percentage, whereas, quantitative (numeric) variables were first evaluated for normality distribution using 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).

### III. RESULTS:

Baseline characteristics of seminal fluid analysis of males with asthenozoospermia 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 = 0.073). There was highly significant difference in mean pH between PAF and non-PAF groups (p < 0.001). There was highly significant difference in mean sperm concentration between PAF and non-PAF groups (p = 0.007); in favor of PAF group.

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 30)</th>
<th>PAF (n = 15)</th>
<th>No PAF (n = 15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>3.17 ±1.12</td>
<td>2.80 ±1.03</td>
<td>3.53 ±1.13</td>
<td>0.073 I NS</td>
</tr>
<tr>
<td>Range</td>
<td>1 -5.5</td>
<td>1 -4.5</td>
<td>1.5 -5.5</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Characteristic</td>
<td>Total  n = 30</td>
<td>PAF  n = 15</td>
<td>No PAF n = 15</td>
<td>p</td>
</tr>
<tr>
<td>-------------------------------</td>
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<tr>
<td>Sperm concentration before million/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>54.00 ±14.99</td>
<td>61.67 ±13.32</td>
<td>46.33 ±12.74</td>
<td>0.003 I HS</td>
</tr>
<tr>
<td>Range</td>
<td>25 -80</td>
<td>40 -80</td>
<td>25 -65</td>
<td></td>
</tr>
<tr>
<td>Grade A %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>27.50 ±8.98</td>
<td>31.00 ±7.61</td>
<td>24.00 ±9.10</td>
<td>0.030 I S</td>
</tr>
<tr>
<td>Range</td>
<td>10 -40</td>
<td>20 -40</td>
<td>10 -40</td>
<td></td>
</tr>
<tr>
<td>Grade B %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>46.67 ±11.24</td>
<td>51.33 ±5.81</td>
<td>42.00 ±13.47</td>
<td>0.020 I S</td>
</tr>
</tbody>
</table>
Post activation sperm characteristics of males with asthenozoospermia contrasted between those with PAF and those with no PAF are shown in table (2).

There was highly significant \( (p = 0.003) \) difference in mean sperm concentration between PAF and non-PAF groups; the level being higher in PAF group. Grade A sperm motility % was higher in PAF group in comparison with non-PAF group and the difference was significant \( (p = 0.030) \), in addition, grade B sperm motility % was higher in PAF group in comparison with non-PAF group and the difference was significant \( (p = 0.020) \), thus, Grade A+B sperm motility % was higher in PAF group in comparison with non-PAF group and the difference was highly significant \( (p < 0.001) \).

Both grade C and grade D immotile sperm % were lower in PAF group in comparison with non-PAF group and the difference was significant \( (p < 0.05) \). Moreover, there was no significant difference in normal morphology sperm % \( (p = 0.064) \).

Figure (1): Comparison of changes in mean sperm concentration before and after activation contrasted between those with PAF and those with no PAF.
Table (3): Comparison of successful IUI outcome between males whom seminal fluid was treated with PAF and males whom seminal fluid was not treated with PAF

<table>
<thead>
<tr>
<th>Group</th>
<th>Present IUI</th>
<th>Total n = 30</th>
<th>PAF n = 15</th>
<th>No PAF n = 15</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthenozoospermia</td>
<td>Success, n (%)</td>
<td>9 (30.0 %)</td>
<td>6 (40.0 %)</td>
<td>3 (20.0 %)</td>
<td>0.426 Y NS</td>
</tr>
<tr>
<td></td>
<td>Failure, n (%)</td>
<td>21 (70.0 %)</td>
<td>9 (60.0 %)</td>
<td>12 (80.0 %)</td>
<td></td>
</tr>
</tbody>
</table>

IV. DISCUSSION.

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 genital organs of the male and there is the link between semen quality and fertility state. (Andrade, 2017)

In vitro activation of the semen:

The main aim of many ART researches 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)

There are many types of these preparation techniques available to select a more progressive motile spermatozoa. 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 factor to the media like PAF will promote the spermatozoa hyper activation and improved outcome of fertilization as stated by Strzelecki et al in 2017.

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 statistical significant increase in grade B 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; Ahmed et al.,2020; Hailat, et al.,2016) 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 increases their movement”. That may explain a significant improvement in sperm motility percentage in both treated and non-treated groups in asthenospermic men.
In 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, and Eidan, 2021)

It is concluded from this study that certain sperm parameters were highly improved after in vitro activation by PAF in asthenozoospermic samples. Therefore, IUI outcome was improved .as in table (3)

**REFERENCE:**