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Role of Exogenous Platelet Activating Factor on Sperm Capacitation and Acrosome Reaction in Male-Factor Infertility

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ABSTRACT

Aim: To evaluate the effect of exogenous platelet activating Factor on capacitation and acrosome reaction in the sperms of infertile subjects.
Methods: Forty samples of semen were collected, twenty each from non-male factor infertile subjects (controls) and twenty from male factor infertile subjects (patients). The sperms were separated from seminal fluid by using Sydney IVF sperm gradient kit. The sperms were incubated in both sperm wash medium (SWM) and platelet activating factor (PAF). A comparatively new assay, chlortetracycline (CTC) was used to assess the incapacitation, capacitation and acrosome reaction of sperms from both groups.
Results: The results were obtained as percentage of sperms stained with chlortetracycline for the specific immunofluoroscent pattern. A significant difference was observed between SWM and PAF groups within normospermic subjects while no statistically difference was found in male-factor infertile subjects. When SWM & PAF groups of controls and patients were compared significant differences were found.

Keywords: Spermatozoa, Capacitation, Acrosome Reaction, SWM, PAF, CTC.

INTRODUCTION

If a couple is unable to conceive a spontaneous pregnancy even after a year of unprotected intercourse, they are labeled as infertile. Surveys reveal that almost 15% couples worldwide are infertile and both sexes have been found to be involved evenly[1,2,3,4,5].

Male factor infertility (MFI) usually present because of either no sperms in semen (azospermia), less number of sperms in semen (oligispermia) or sperms are unable to move swiftly (low motility, asthenopermia)[6,7,8,9].

After being in the female reproductive tract for sometimes, the sperms get hyperactivated. There, though no physical but some biochemical and physiological changes occur in the sperms which are collectively termed as capacitation. The changes in capacitation are efflux of cholesterol, decrease in the net negatives surface charge, loss of receptor masking components thus exposing receptors, change in membrane permeability, rise in intracellular pH and increased influx of calcium ions. The last two changes cause acrosome reaction. Sperm-zona binding triggers acrosome reaction (AR). There are zona pellucida penetration and oocyte fusion by virtue of release of proteolytic enzyme and hydrolases. AR is irreversible phenomenon. Only those sperms attain acrosome reaction which are already capacitated[10,11].

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There are many factors which affect the functional capability of the sperms. One of these factors is platelet activating factor which is a unique and potent signaling phospholipid[12]. Platelet activating factor not only activate platelets but also has many effects on reproduction like sperm motility[13,14,15,16], sperm capacitation[17,18], sperm acrosome reaction, ovulation, fertilization, pre-implantation, implantation, embryo development[21,22] and parturition[23,24]. There is second messenger system involved as inositol triphosphate and diacylglyceral are formed intracellularly once PAF binds with the sperm surface receptors. This promotes release of calcium from intracellular stores as well as entry of calcium from outside the cell[25,26]. This high level of calcium causes acrosome reaction[27].

Medical literature show beneficial effects of exogenous PAF on penetration of zona free hamster oocyte and penetration index of asthenospermic and normospermic specimens.[28] A significant increase in human pregnancy outcomes has also been reported when sperms were exposed to exogenous PAF before intrauterine insemination[29,30]. It is postulated that treatment of sperms with PAF may increase their fertilizing capacity by stimulating AR[31,32]. However in another study no increase in pregnancy outcome was found when male factor sperms were treated with PAF[33]. The reports available on PAF effects on motility and acrosome reaction of fresh and frozen sperms are controversial[34].

In Pakistan no study was conducted to determine the role of exogenous PAF on sperm capacitation and acrosome reaction.

MATERIALS AND METHODS

This analytical cross-sectional study was carried out in the Department of Physiology and Cell Biology, University of Health Sciences, Lahore. Forty semen samples, twenty of normospermic subjects (controls) and twenty of male factor infertile subjects (patients) as per WHO criteria were collected from Conception Unit, Services Hospital Lahore.

Semen samples were obtained from the subjects by masturbation after minimum 48 hours and no longer than 7 days of sexual abstinence[35,36] Semen specimens were collected in wide mouthed and clean containers.

First, the specimens were allowed to liquefy for 30-60 minutes at room temperature (37°C). Sperm concentration and motility were evaluated by using Howell chamber. The sperms were separated from seminal fluid by using Sydney IVF Sperm Gradient Kit, (Cook, Sydney, Australia)[34]. The sperm pellet was re-suspended in 1 ml of sperm-washing medium (SWM) (Sydney IVF Sperm Medium, Cook, Sydney, Australia). A portion of the sperms was stained by Chlortetracycline (CTC). Various types of CTC patterns were obtained as the basal values (0 hour). The rest of the sperms were divided into two aliquots. Group A (PAF) Sperms in one aliquot were treated with an exogenous mixture of PAF (final concentration, (10^{-7}mol/L) in SWM. Synthetic PAF (Calbiochem-Novabiochem, La Jolla, CA, USA) was stored in a stock solution ($1x10^{-6}$mol/L) of chloroform and methanol (1:4). 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. Group B (SWM) Sperms in other aliquot were incubated in Gamete buffer in similar conditions as for other groups. This group (SWM) served as control. At 1, 2, 3, and 4 hours of incubation, part of the sperms from the two groups were removed and placed separately in two 0.5 ml centrifuge tubes. The sperms from the two groups were stained with CTC for determination of capacitation and acrosomal status. The data on the percent capacitated and acrosome-reacted sperms were compared in the above-mentioned two groups.

Determination of capacitation and acrosomal status: Chlortetracycline (CTC) fluorescence assay has been introduced to assess capacitation and acrosomal status of sperms[37].

The different fluorescence patterns with CTC staining are as follows[38].

F pattern, characteristic of incapacitated sperm with intact acrosome, displaying a uniform fluorescence in the whole head of the sperm and in the middle piece.

B pattern, characteristic of capacitated sperm with intact acrosome, showing fluorescence in the middle piece and in the acrosome.

AR pattern, characteristic of acrosome-reacted sperm showing fluorescence restricted mainly in the middle piece.

Chlortetracycline (CTC) staining: A stock solution was prepared (20 mM Tris, 130 mM NaCl, 5 mM L-Cysteine [Sigma-Aldrich Canada, Mississauga, ON]) and stored at 5°C. On the day of assessment, 2.5 mg of CTC powder [Sigma-Aldrich Canada, Mississauga, ON] was added to 10 ml of the stock solution and mixed using a stirring bar. 20µl of sperm suspension removed from each group was mixed rapidly with 20µL of CTC staining solution by vortexing in a 0.5ml light-protected centrifuge tube. 2µl of fixing solution (12.5% glutaraldehyde in Tris buffer) was added to the tube and mixed thoroughly. 5µl of the sperm sample was placed on a clean glass slide and covered with a cover slip. The capacitation and acrosomal status of 100 sperms per sample was evaluated with a fluorescence microscope. CTC staining pattern was observed using a filter set consisting of an excitation filter (BP 450-490), a chromatic beam splitter (FT510), and a barrier filter (LP520). In our study, the nomenclatures for different immunofluorescent patterns of CTC staining used are as follows

- F pattern as CTC1
- B pattern as CTC2
- AR pattern as CTC3

RESULTS

The results were collected as percentage of sperms for the different patterns of CTC staining. The data was analysed by using SPSS 17.0. Mean±SD were taken for quantitative variabls and Two-independent Sample t-Test was applied to observe group-mean differences between two groups. A p-value of <0.05 was considered statistically significant.

Comparison of results between SWM and PAF Control Groups: On analysing the data about the values of both groups (SWM & PAF) in controls it was revealed that incapacitation (CTC1) were significantly less in PAF group than in SWM group at 1 hour, 2 hour, 3 hour and 4 hour of incubation time ($p < 0.001$). Furthermore, the values of capacitation (CTC2) were significantly more in PAF group than in SWM group as incubation time progressed. The acrosome reaction (CTC3) showed the same pattern (Table 1).

Comparison of Results between SWM and PAF Patients Groups: In patients, when values of SWM and PAF groups were compared regarding CTC patterns, no significant differences were found (Table 2).

Comparison of SWM Groups of Controls and Patients: Significant differences were found when values of SWM groups in controls and patients were compared. Capacitation and acrosome reaction appeared to be greater in controls than patients (Table 3).

Comparison of PAF Groups of controls and Patients: On comparing PAF groups of controls and patients, it was found that PAF facilitated capacitation and its effect on acrosome reaction in controls was more profound than in patients (Table 4).

Table 1: Comparison between SWM and PAF groups in Controls

CTC Incubation period	Group	Mean	Mean Difference	P-Value
CTC1_0	SWM PAF	68.15 68.15	.00000	1.000
CTC2_0	SWM PAF	27.35 27.35	.00000	1.000
CTC3_0	SWM PAF	4.85 4.85	.00000	1.000
CTC1_1	SWM PAF	53.75 44.10	9.65	.000*
CTC2_1	SWM PAF	37.35 45.75	-8.40000	.001*
CTC3_1	SWM PAF	8.40 11.50	-3.10000	.002*
CTC1_2	SWM PAF	45.75 27.80	10.30000	.000*
CTC2_2	SWM PAF	42.45 48.45	-6.00000	.006*
CTC3_2	SWM PAF	13.90 16.10	-2.20000	.198
CTC1_3	SWM PAF	38.50 27.80	10.70000	.000*
CTC2_3	SWM PAF	43.30 48.75	-5.450000	.090
CTC3_3	SWM PAF	17.75 21.00	-3.25000	.000*
CTC1_4	SWM PAF	33.00 22.25	10.75000	.000*
CTC2_4	SWM PAF	46.85 51.35	-4.50000	.031*
CTC3_4	SWM PAF	22.15 26.50	-4.350000	.000*

P-value <0.05 is significant.

Table 2: Comparison between SWM and PAF groups

CTC Incubation period	Group	Mean	Mean Difference	P-Value
CTC1_0	SWM PAF	84.8000 84.8000	.0000	>0.999
CTC2_0	SWM PAF	13.9500 13.9500	.0000	>0.999
CTC3_0	SWM PAF	1.2500 1.2500	.0000	>0.999
CTC1_1	SWM PAF	82.0500 80.5500	1.5000	0.499
CTC2_1	SWM PAF	15.1000 16.4000	-1.3000	0.5109
CTC3_1	SWM PAF	2.5000 3.5000	-1.0000	0.07
CTC1_2	SWM PAF	78.2500 75.4000	2.8500	0.25
CTC2_2	SWM PAF	17.8500 19.5000	-1.6500	0.3998
CTC3_2	SWM PAF	3.9000 5.3500	-1.4500	0.107
CTC1_3	SWM PAF	73.7000 70.9000	2.8000	0.3152
CTC2_3	SWM PAF	20.3500 20.9500	-0.6000	0.778
CTC3_3	SWM PAF	5.9000 7.8000	-1.9000	0.08
CTC1_4	SWM PAF	68.7000 64.4500	4.2500	0.229
CTC2_4	SWM PAF	22.3000 24.4000	2.1000	0.3293
CTC3_4	SWM PAF	9.0000 10.8000	-1.8000	0.2542

P value <0.05 is significant.

Table 3: Comparison of SWM groups of controls & patients

Group	Controls (Mean±S.D)	Patients (Mean±S.D)	P Value
CTC1_0	68.15±4.86	84.80±6.15	0.00*
CTC2_0	27.35±4.89	13.95±5.69	0.00*
CTC3_0	4.85±1.81	1.25±1.01	0.00*
CTC1_1	53.75±8.40	82.05±6.74	0.00*
CTC2_1	37.35±6.06	15.10±6.07	0.00*
CTC3_1	8.40±2.21	2.50±1.53	0.00*
CTC1_2	45.75±4.77	78.25±7.52	0.00*
CTC2_2	42.45±5.90	17.85±6.31	0.00*
CTC3_2	13.90±6.54	3.90±2.35	0.00*
CTC1_3	38.50±4.91	73.70±8.72	0.00*
CTC2_3	43.30±8.16	20.35±6.76	0.00*
CTC3_3	17.75±2.55	5.90±3.27	0.00*
CTC1_4	33.00±7.41	68.70±10.58	0.00*
CTC2_4	46.85±5.32	22.30±7.25	0.00*
CTC3_4	22.15±2.32	9.00±4.58	0.00*

P value <0.05 is significant.

Table 4: Comparison of PAF groups of controls and patients

Group	Controls (Mean ±S.D)	Patients (Mean ±S.D)	P Value
CTC1_0	68.15±4.86	84.80±6.15	0.00*
CTC2_0	27.35±4.89	13.95±5.69	0.00*
CTC3_0	4.85±1.81	1.25±1.01	0.00*
CTC1_1	44.10±7.30	80.55±6.718	0.00*
CTC2_1	45.75±7.83	16.40±6.31	0.00*
CTC3_1	11.50±3.48	3.50±1.93	0.00*
CTC1_2	35.45±7.74	75.40±7.91	0.00*
CTC2_2	48.45±6.99	19.50±6.30	0.00*
CTC3_2	16.15±3.69	5.35±2.97	0.00*
CTC1_3	27.80±6.92	70.90±8.79	0.00*
CTC2_3	48.75±11.38	20.95±6.73	0.00*
CTC3_3	21.00±2.36	7.80±3.65	0.00*
CTC1_4	22.25±6.18	64.45±10.19	0.00*
CTC2_4	51.35±7.22	24.40±6.23	0.00*
CTC3_4	26.50±2.01	10.80±5.23	0.00*

P-value <0.05 is significant.

DISCUSSION

There are many factors which influence the fertilizing capability of sperms. One of these factors is PAF. PAF is believed to be involved in capacitation and AR of sperms[39]. Capacitation is a hyperactivated form of sperm with some biological and physiological changes[40]. In AR there is release of proteolytic enzymes and hydrolases which help the sperm to penetrate the ovum[10]. PAF is also said to be helpful in pre-implantation, implantation, embryo development and parturation[21,24,41]. Reseacher gleaned that there are more PAF recepters in motile sperms than in non-motile sperms. They also found an inverse coorelation between actual concentration and motility. This indicated that non-motile sperms contained more PAF than motile sperms because non-motile sperms were unable to utilize PAF[42,43]. PAF content in human sperm has a significantly positive relation not only to sperm motility but to the pregnancy outcome as well. Medical literature endorses the beneficial effects of exogenous PAF on sperms of fertile subjects by finding more pregnancy outcomes[14,19,29,34]. Little information available about effects of PAF on sperms of infertile subjects. Capacitation and AR are two indicators to judge the fertilizing functionality of the sperm. In the present study both capacitation and AR were evaluated in sperms of both groups, controls and patients after exposing them to SWM and exogenous PAF.

In both groups, SWM and PAF, incapacitation decreased and capacitation and acrosome reaction increased as the incubation progressed. Significant differences were noted at different times. These time related findings are in agreement with similar studies worldwide. Some researchers showed the same effect by obtaining increase in pregnancy rate where sperms were exposed to exogenous PAF before insemination. In patients when CTC1, CTC2, and CTC3 were compared between SWM and PAF groups, no significant differences were found. This shows PAF does not affect the sperms of infertile subjects to get capacitation and acrosome reaction. This is believed to be because of lack of ability of sperms to make use of exogenous PAF.

When values of SWM groups in controls and patients were compared, significant differences were found, showing facilitation in obtaining capacitation and AR when conducive environments were provided to sperms. Similar results were obtained on comparing PAF groups of controls and patients, showing the probable effect of PAF on capacitation and acrosome reaction. It is interesting to note that within PAF control and PAF patient groups the difference in mean values of CTC1, CTC2, and CTC3 were profoundly different. For CTC1 control the difference between values at 0 hour and 4 hour was calculated to be 46 (Table 1), while the corresponding values for CTC1patient was 20 (Table 2), indicating a difference of more than double. This again symbolizes the inability of infertile sperm to utilize PAF. Similarly, the differences between CTC2, CTC3 controls and CTC2, CTC3 patients were similar, indicating the effect of PAF on capacitation and acrosome reaction in normal versus infertile sperms.

CONCLUSION

This study concludes that PAF promotes capacitation and acrosome reaction in sperms of fertile subjects and has little effect on sperms of infertile subjects for the same indicators. This endorses the fact that infertile sperms are unable to make use of PAF.

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