

Effect of Exogenous Platelet-Activating Factor on Sperm Capacitation and Acrosome Reaction in Infertile Subjects

QAMAR SHAKOOR SANDHU, SAQIB SOHAIL*, JAWAD NAWAZ**, HAMID JAVAID QUERSHI***

ABSTRACT

Objective: To study the effect of exogenous platelet activating factor on capacitation and acrosome reaction in sperms of infertile subjects.

Subjects and methods: Twenty semen samples were collected as per WHO criterion for semen analysis from infertile males. The sperms were separated from the seminal fluid by using Sydney IVF Sperm gradient kit. Chlortetracycline (CTC) assay was employed to assess the specific immunofluorescent pattern in spermatozoa for incapacitation, capacitation and acrosome reaction. A group of sperms incubated in sperm washing medium was compared with the group of sperms incubated in exogenous PAF.

Results: The results were obtained as percentage of sperms stained with chlortetracycline for the specific immunofluorescent pattern. A significant difference was found within the individual group in relation with incubation time, though no statistically difference was found when the two groups are compared with each other.

Key words: Spermatozoa, Capacitation, Acrosome reaction, PAF, CTC.

INTRODUCTION

Infertility is said to be present when a couple is unable to achieve a spontaneous pregnancy even after a year of unprotected intercourse. It has been reported that infertility prevails among 15% of couples worldwide and both sexes have been found to be involved evenly^{1,2,3,4}.

Half of infertility cases are attributed to male factor^{4,5}. Recent evidences show that 50% of MFI is attributed to abnormal spermatogenesis resulting in oligospermia (less than normal numbers of sperm) and asthenospermia (low motility of sperm)^{5,6}.

The spermatozoa should have normal morphology and motility to be transported through female reproductive tract and for penetration into female egg. Mammalian sperms after ejaculation in vivo must reside in the female reproductive tract for sometime to acquire fertilizing ability. During this time, there are some biochemical and physiological changes in sperms that render them capable of fertilizing the eggs and are termed as capacitation. Sperms exposed to capacitation conditions show many altered properties like efflux of cholesterol, decrease in the net negative 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 make the sperm ready for acrosome reaction³.

Seminal plasma is diluted in the female reproductive tubes, resulting in efflux of cholesterol. This efflux of cholesterol with other factors brings about the changes in the membrane which initiate intracellular events leading to capacitation⁷.

Sperm-zona binding triggers acrosome reaction (AR). In AR, proteolytic enzymes are released from acrosome which prompts both zona pellucida penetration and oocyte fusion. This process known as acrosome reaction is irreversible, and includes both physiological and morphological changes. By releasing hydrolases, the sperm penetrates the egg. Acrosome reaction is confined to only those sperms that are previously capacitated⁸.

Fertility potential of spermatozoa is regulated by many exogenous and endogenous factors. Platelet-activating factor (PAF) is one of the endogenous factors responsible for the regulation of sperm fertilizing capacity⁹. It is unique, potent signaling phospholipids present in the human sperm in addition to other cells. Apart from the activation of platelets, PAF has numerous other effects related to reproduction, sperm motility¹⁰ ovulation, sperm capacitation¹¹, acrosome reaction¹² fertilization, pre-implantation, embryo development, implantation¹³ and parturition¹⁴. When PAF binds to the surface receptors, it provokes the formation of inositol triphosphate (IP₃) and diacylglycerol (DAG) and increases the intracellular calcium by stimulating the calcium release from intracellular stores as well as the entry of extracellular calcium through channels^{15,16}. The elevated intracellular calcium

* Senior Demonstrator Physiology, Lahore Medical and Dental College, Lahore

** Senior Demonstrator Physiology, Central Park Medical College, Lahore

***Professor of Physiology, Services Institute of Medical Sciences, Lahore

Correspondence to Dr. Qamar Shakoor Sandhu, Assistant Professor, Email: qamarshakoorsandhu@yahoo.com

causes depolymerization of the membrane actin membrane and activation of phospholipases, resulting in acrosome reaction¹⁷.

Several studies have shown that PAF may have a direct role in fertilizing capacity of human sperms. PAF treatment of sperms may enhance fertilizing capacity possibly by augmenting the acrosome reaction^{18,19}. Some recent trails could not demonstrate improved motility of sperms when they were exposed to PAF preparation marketed for treatment of human sperm cell prior to insemination²⁰⁻²¹. Controversial reports are available on the effect of PAF on motility and acrosome reaction of fresh and frozen human sperms. On the other hand, several other reports showed the beneficial effect of PAF treatment on motility characteristics of fresh or frozen sperms^{22,23}. There is significant enhancement of both percent penetration of zona free hamster oocyte and penetration index in PAF treated asthenospermic and normospermic specimens²⁴. It has recently been reported that exposure of sperm to exogenous PAF prior to intrauterine insemination resulted in a significant increase in human pregnancy outcomes. Patients whose sperms were treated with PAF had a significantly higher pregnancy rate (40%) than patients not receiving treatment (20%)^{25,26}. In another study, an increase in pregnancy rate was not found when male factor sperms were treated with PAF²⁷. No study on the effect of exogenous PAF on sperm capacitation and acrosome reaction has been documented in Pakistan.

MATERIALS AND METHODS

This analytical cross-sectional study was carried in the Department of Physiology and Cell Biology, University of Health Sciences, Lahore. Twenty semens samples of infertile subjects 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. 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)²¹. 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 (1×10^{-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 sperm 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 capacitating and acrosomal status of sperms. The different fluorescence patterns with CTC staining are:

- **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^[28, 29].

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. 20 µl of sperm suspension removed from each group was mixed rapidly with 20 µL of CTC staining solution by vortexing in a 0.5 ml light-protected centrifuge tube. 2 µl of fixing solution (12.5% glutaraldehyde in Tris buffer) was added to the tube and mixed thoroughly. 5 µl of the sperm sample was placed in a clean glass slide and covered with a cover slip²⁵. 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 F

pattern as CTC1, B pattern as CTC2, AR pattern as CTC3

RESULTS

The data was analyzed using SPSS 17.0. Two-Independent sample t-test, One-way ANOVA was applied to analyze the results.

1. Analysis of results within Sperm Washing Medium (SWM) group: Descriptive data for CTC1 (non-capacitation) is shown in Fig-1 the mean \pm SD percentage of non-capacitative sperms decreases from 84.80 ± 6.1 at 0 hr to 68.70 ± 10.5 at 4 hr. Descriptive data for CTC2 (capacitation) is shown in Fig-2. The mean \pm SD percentage of capacitated sperms increases from 13.95 ± 7.2 at 0 hr to 22.30 ± 5.6 at 4 hr. Descriptive data for CTC3 (acrosomal reaction) is shown in Fig-3. The mean \pm SD percentage of acrosome reacted sperms increases from 1.25 ± 1.0 at 0 hr to 9.00 ± 4.5 at 4 hr. ANOVA showed CTC1, CTC2 and CTC3 to be significantly different at different period times. The results of CTC1 indicate that non-capacitation in the sperms decreases significantly after being incubated in SWM for longer time. The results of CTC2 show that the numbers of capacitated sperms increase significantly though after longer incubation time. The results of CTC3 indicate a significant progression of acrosome reaction with the increase of incubation time.

2. Analysis of results within Platelet-Activating Factor (PAF) group: Descriptive data for CTC1 (non-capacitation) is given in Fig-4. The mean \pm SD percentage of non-capacitative sperms decreases from 84.80 ± 6.1 at 0 hr to 64.85 ± 10.1 at 4 hr. Descriptive data for CTC2 (capacitation) is depicted in Fig-5. The mean \pm SD percentage of capacitative sperms increases from 13.95 ± 5.6 at 0 hr to 24.40 ± 6.2 at 4 hr. Descriptive data for CTC3 (Acrosomal reaction) is shown in Fig-6. The mean \pm SD percentage of acrosome reacted sperms increases from 1.25 ± 4.5 at 0 hr to 10.80 ± 1.0 at 4 hr. ANOVA showed CTC1, CTC2 and CTC3 to be significantly different at different time intervals. There is a progressive decrease in number of non-capacitated sperms (CTC1) which is statistically significant. The capacitation (CTC2) and acrosomal reaction (CTC3) of sperms in PAF group show the same pattern as in CTC1.

Comparison of results between Sperm Washing Medium (SWM) and Platelet Activating Factor (PAF) groups: On analyzing the values in the two groups, it was revealed that values of non-capacitation, capacitation and acrosome reaction are not statistically different ($p > 0.05$) from each other as shown in table-1.

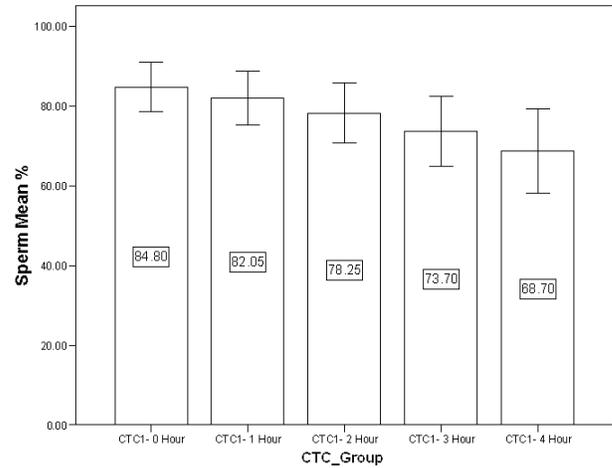


Figure-1: (Bar chart) CTC1 Sperm Washing Medium (SWM) patient group

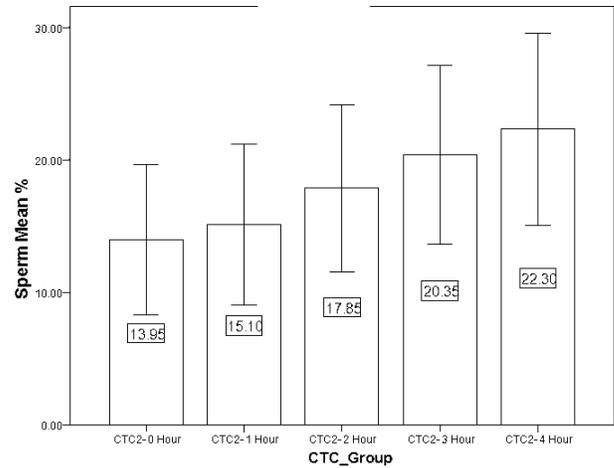


Figure-2: (Bar chart) CTC2 Sperm Washing Medium (SWM) patient group

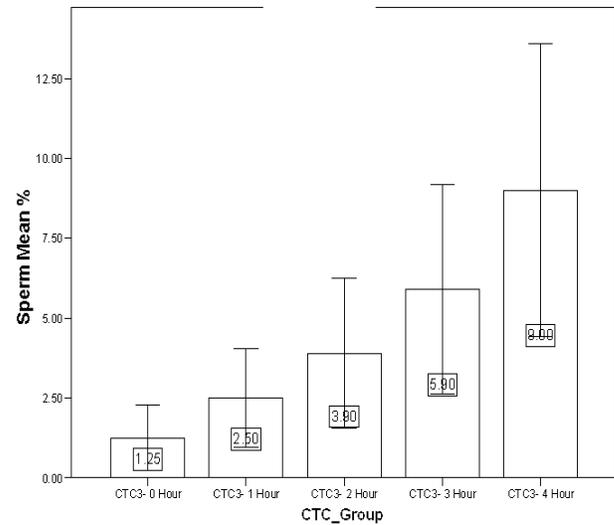


Figure-3: (Bar chart) CTC3 Sperm Washing Medium (SWM) patient group

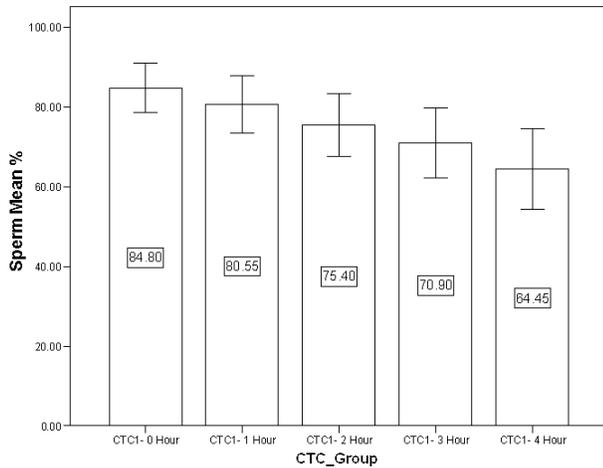


Figure-4: (Bar chart) CTC1 Platelet-Activating Factor (PAF) patient group

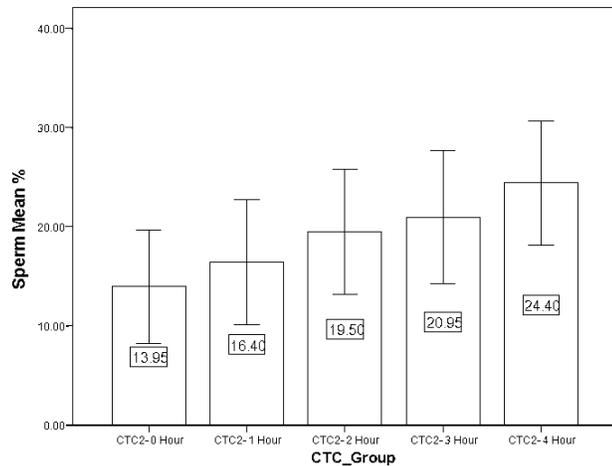


Figure-5: (Bar chart) CTC2 Platelet-Activating Factor (PAF) patient group

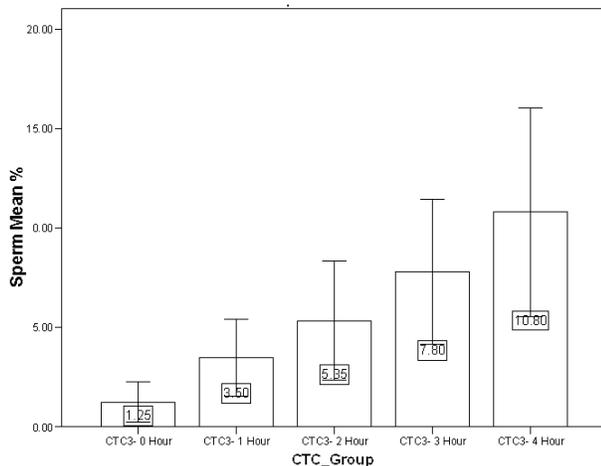


Figure-6: (Bar chart) CTC3 Platelet-Activating Factor (PAF) patient group
Independent samples t- test between Sperm Washing Medium (SWM) and Platelet-Activating Factor (PAF) groups within patient group

CTC Group-Hour	Mean Difference	p-Value
CTC1-0	.00000	1.000

CTC2-0	.00000	1.000
CTC3-0	.00000	1.000
CTC1-1	1.50000	.500
CTC2-1	-1.30000	.511
CTC3-1	-1.00000	.078
CTC1-2	2.85000	.250
CTC2-2	-1.65000	.413
CTC3-2	-1.45000	.096
CTC1-3	2.80000	.319
CTC2-3	-.60000	.780
CTC3-3	-1.90000	.091
CTC1-4	4.25000	.204
CTC2-4	-2.10000	.332
CTC3-4	-1.80000	.255

p-value <0.05 is significant

DISCUSSION

Capacitation is a physiological condition which matures the hyperactivated spermatozoa for the fertilization through acrosome reaction. It is believed that only those spermatozoa become acrosome reacted which are previously capacitated^[3]. There are mediators present in the spermatozoa themselves and female reproductive tubes which bring about capacitation and acrosome reaction. One of these mediators is Platelet-Activating Factor (PAF). This is an endogenous phospholipid which is positively associated with motility, capacitation and acrosome reaction of spermatozoa. PAF has also been associated with pre-implantation and early development of embryo³⁰. The action of PAF is found to be receptor-mediated. Studies show more concentration of PAF in the spermatozoa of infertile subjects than the spermatozoa of fertile subjects. This was attributed to inability of the spermatozoa to utilize PAF, so unable to get capacitated and acrosome reactive and resultantly failing in fertilizing the ovum.

Majority of the studies conducted were to evaluate the effects of exogenous PAF on normal spermatozoa. Few studies have been done to see the effects of exogenous PAF on spermatozoa of infertile subjects. In the present study, capacitation and the acrosome reaction were studied in spermatozoa in infertile subjects in respect of effects of exogenous PAF on them^{26,27, 31-33}.

In this study, CTC1 (non-capacitation) was noted decreased comparatively with longer period of incubation in both SWM and PAF groups. Values of CTC2 and CTC3 (capacitation and acrosomal reaction) increased with the same pattern i.e with longer period of incubation in both groups. At different time intervals the difference of immunofluorescent pattern was significant statistically within the individual groups.

When the two groups (SWM and PAF) were compared, no statistically significant difference was found. It is concluded that PAF promoted capacitation and acrosomal reaction in abnormal sperms when they were incubated over a longer period.

REFERENCES

1. Kolletis PN. Evaluation of the subfertile man. *Am Fam Physician*. 2003; 67:2165-72.
2. Nallela KP, Sharma RK, Aziz N, Agarwal A. Significance of sperm characteristics in the evaluation of male infertility. *Fertil Steril*. 2006; 85:629-34.
3. Nieshlag E, Leifke E. Empirical therapies for idiopathic male infertility. In Niechlag E and Behra MB (editors). *Andrology, Male Reproductive Health and Dysfunction*. Berlin Springer-Verlag, 2000; 313.
4. Nishimune Y, Tanaka H. Infertility caused by polymorphism or mutations in spermatogenesis-specific genes. *J Androl*. 2006; 27:326-34.
5. Dekrester DM, Baker HW. Infertility in men. Recent advances and continuing controversies. *J Clin Endocrinol Metab*. 1999; 84:3444-50.
6. Dickman AB, Goldberg E. Characterization of human antigen with sera from infertile patients. *Biol Reprod*. 1994; 50(5):1087-93.
7. Martinez P, Marros A. Membrane lipid dynamics during human sperm capacitation. *Frontiers in Biosciences*. 1996; 1:103-17.
8. Florman HM, Ducibella T. Fertilization in mammals. In: Neil JD, editor-in-chief. Minhas BS, Kumar, Ricker DD, The presence of platelet-activating factor-like activity in human spermatozoa. *Fertil Steril*. 1991; 55:372-6.
9. Minhas BS, Kumar, Ricker DD, Robertson JL. The presence of platelet-activating factor-like activity in human spermatozoa. *Fertil Steril*. 1991; 55:372-6.
10. Henkel RR, Schill WB. Sperm preparation for ART. *Reprod Biol Endocrinol*. 2003; 1:108.
11. Wu C, Stojanov T, Chamio, Ishii S. Evidence for the autocrine Induction of capacitation of mammalian spermatozoa. *J Biol Chem*. 2001; 276:26962-8.
12. Huo LJ, Yang ZM. Effects of platelet-activating factor in capacitation and acrosome reaction in mouse spermatozoa *Mol Reprod Dev*. 2000; 56:436-40.
13. Minhas BS, Ripps BA, Zhu P, Kim HN, Burwinkel TH, Gleicher N. Platelet-Activating factor and conception. *Am J Reprod Immunol*. 1996; 35:267-71.
14. Harper MJK. Platelet-activating factor: a paracrine factor in preimplantation stages of development \ *Biol Reprod*. 1989; 40:907-13.
15. Lapetina EG. PAF stimulate the phosphatidylinositol cycle. *J Biochem*. 1982; 257: 7314-7.
16. Putney JWJ. Formation and actions of calcium mobilizing messenger, inositol 1, 4, 5-triphosphate. *Am J Physiol*. 1987; 252:G149-57.
17. Benoffs. Modelling human sperm-egg interactions in vitro. Signal transduction pathways regulating the acrosome reaction. *Mol Hum Reprod*. 1998; 4:453-71.
18. Belker AM, Thomas AJJ, Fuchs EF. Result of 1,469 microsurgical vasectomy reversals by the vasovasotomy study group. *J Urol*. 1991;145(3):505.
19. Sigman M, Lipshultz LI, Howards SS. Evaluation of Subfertile male. In: Lipshultz LI, howardsss, editors. *Infertility in male 3d ed*. St Louis: Mosby; 1997: 173.
20. Angle MJ, Tom R, Javeri K, Mc Cluse RD. Effects of platelet-activating factor on human spermatozoa – oocyte interactions. *J Reprod Fertil*. 1993; 98:541-8.
21. Baeck K, Feliciane M, Neri QV, Rosenwaks Z, Palermo GD, platelet-activating factor: Not a panacea for sluggish sperm. *Fertil Steril*. 2005; 84:S450.
22. Minhas BS, Ripps BA. Methods for enhancement of sperm function. *Front bio-sci*. 1996; 1:65-71.
23. Briton-Jones C, Yeung Q SY, Tjer GCC, Chiu TTY, Cheung LP, Yim SF, Lok IH, Haines C. The effects of follicular fluid and platelet-activating factor on Motion characteristics of poor-quality cryopreserved Human sperm. *J Assist Reprod and Genet*. 2001; 18:165-70.
24. Minhas BS. Platelet-Activating Factor treatment of human spermatozoa enhances fertilization potential. *Am J Obstet Gynecol*. 1993; 168(4):1314-7.
25. Wild MD, Roundbush WE. Platelet-Activating Factor improves intra-uterine insemination success. *Am J Obstet Gynecol*. 2001; 184:1064-5.
26. Roundbush WE, Messey JB. Platelet-Activating Factors significantly enhances intrauterine insemination pregnancy rates. *Fertil Steril*. 2004; 82:52
27. Roundbush WE, Toledo AA, Kort HI, Mitchell-Leef D. Platelet-Activating Factor significantly enhances intrauterine insemination pregnancy rates in non-male factor infertility. *Fertil Steril*. 2004; 82:52-6.
28. Yao Y, HoP, Yeungs, Effect of Human Follicular Fluid on the capacitation motility of human spermatozoa *Fertil Steril*. 2000; 73:680-6.
29. Sanchez-Luengo S, Fernandez PJ, Romeu A. Insulin growth factor may be implicated in human sperm capacitation. *Fertil Steril*. 2005; 83:1064-6.
30. Bosch P, Roundbush WE, McGraw RA, DeJarnette JM. Bull spermatozoa express receptors for platelet-activating factor. *Revista Cientifica*. 2009; 21: 513-21.
31. Roundbush WE, Fukuda AI, Minhas BS. Enhanced embryo development of rabbit oocytes fertilized in vitro with platelet-activating factor – treated spermatozoa. *J Assist Reprod. Genet*. 1993; 10:91-4.
32. Aravindakshan TV, Sharma A. Induction of acrosome reaction in fresh and frozen–thawed bovine spermatozoa by Platelet–activating factor. *Indian J Exp Biol*. 1995; 33:87-90.
33. Chapman AM, Lefever RL, Kuehl TJ. The effects of platelet-activating factor as motility properties of human sperms cells in vitro. *Fertil Steril* 2005; 84:5460.