ISSN: 2576-2842

Research Article

Journal of Gynecology & Reproductive Medicine

Enhanced Fc Receptor Expression In Sea Urchin Spermatozoa By Platelet-Activating Factor

Nicole Russell and William E. Roudebush*

Department of Biomedical Sciences, University of South Carolina School of Medicine Greenville Greenville, South Carolina

***Corresponding Author**
William E. Roudebush, Department of Biomedical Sciences, University of South Carolina School of Medicine Greenville Greenville, South Carolina

Submitted: 2023, Dec 12; **Accepted**:2024, Jan 11; **Published**:2024, Jan 17

Citation: Roudebush, E. W., Russell, N. (2024). Enhanced Fc Receptor Expression In Sea Urchin Spermatozoa By Platelet-Activating Factor. *J Gynecol Reprod Med, 8*(1), 01-04.

Abstract

Platelet-activating factor (PAF) is a signaling phospholipid that has a variety of reproductive roles, e.g. sperm capacitation and the acrosome reaction (an exocytotic process). PAF's mechanism action involves G-protein receptor activation induction of intracellular calcium, which enhances sperm motility. In nonreproductive cells, the Fc receptor (FcR) functions in ligand-triggered transmission signals across the membrane which alters secretion, exocytosis, and increased intracellular calcium. This study looked at the impact of PAF on FcR expression in sea urchin spermatozoa. Sea urchin sperm were exposed to exogenous PAF (10-7M; 15 minutes) and evaluated for FcR activity via flow cytometry. A significant (P=0.01) difference (64.30%) in FcR wave activity between the PAF (mean 3.286) and control (2.0) groups was observed. Within waves, there were more (83.31%) high points (crests) in the PAF (mean 1.571) group than the control (mean 0.857) group. Similarly, there were more (49.96%) low (troughs) points in the PAF (mean 1.714) group than the control (mean 1.143) group. This is the first report to demonstrate a relative positive change in spermatozoa FcR expression when exposed to PAF. The FcR functions in ligand-triggered transmission of PAF via a G-protein coupled receptor mediated pathway leading to physiological alterations. Provided the known mechanism of action of PAF on spermatozoa physiology, the data suggests that the interaction of PAF and FcR play a pivotal role in sperm activity and fertilization potential.

Keywords: Platelet-activating factor, Fc Receptor, Spermatozoa, Fertility

1. Research Highlights

Our results indicate that PAF has a positive effect on sea urchin spermatozoa FcR expression cycling patterns (associated with fertility potentials) and quantity which may correlate with increased physiological processes. Not only does PAF increase the number of waves (cycling pattern of highs and lows) of the FcR, but it also effects and maintains the persistence of function overtime preserving the fertility period. These results would suggest that the treatment of spermatozoa with PAF prior to IUI procedures could increase the rates of success.

2. Introduction

Uncovering the molecular mechanisms that regulate early gamete activity and fertilization potential is crucial to understanding why fertilization does not always result in a live birth. Many endogenous and exogenous elements impact human gamete viability. One such element is platelet-activating factor (PAF), a signaling phospholipid that has been found to have a variety of reproductive roles. The PAF receptor is found in the neck region of spermatozoa and positively correlates to fertility status of males [1]. Upon exposure to progesterone in the female reproductive tract, spermatozoa are stimulated to synthesize and secrete PAF, which then works in an autocrine signaling cascade to enhance spermatozoa functionality [2]. This autocrine signaling cascade increases intracellular calcium which triggers an acceleration of sperm cell metabolism, activation of secondary transmitters, changes on the plasmalemma surface, capacitation and the acrosome reaction [3]. PAF receptor concentration has been associated with increased spermatozoa motility [1].

Witkin et al. has demonstrated the presence of the Fc Receptor (FcR) on spermatozoa [4]. In nonreproductive cells, the FcR is a membrane bound receptor that functions in ligand-triggered transmission signals across the plasma membrane which results

in alteration in secretion, exocytosis, and increased intracellular calcium [5]. The FcR functions in ligand-triggered transmission of PAF via a G-protein coupled receptor mediated pathway leading to alterations in secretion, exocytosis, and cellular metabolism [5].

The sea urchin embryo is a time-honored model for investigational studies in developmental biology. By experimenting with sea urchin spermatozoa, we better understand the events concerning spermatozoa physiology that are beneficial for understanding similar events in human spermatozoa. The relationship between PAF and FcR expression in spermatozoa is not known. Therefore, the study objective was to determine the effect of PAF on FcR expression in sea urchin spermatozoa.

3. Materials & Methods

This study was conducted at Gulf Specimen Marine Laboratory (Panacea, FL).

3.1. Sea Urchins

Animal Collection: Short spined sea urchins (Lytechinus variegatus) were harvested in situ from the seafloor of coastal waters (Gulf of Mexico) and held in filtered, aerated and ozone treated sea water that is sourced from Dickerson Bay (Panacea, FL).

Gamete Collection: Gravid sea urchins were induced to shed their gametes by injection of 1mL of KCl (0.5M) into the test cavity via the membranous peristome. Following the KCl injection, gametes are released typically within 2 minutes from each of the five gonopores. Once gamete shedding has been observed, the spermatozoa producing animals were isolated and utilized for the study.

Spermatozoa exposure to PAF: Within five minutes of collection, spermatozoa were exposed to PAF (10-7M) in sea water for 15 minutes (step 1). After exposure the tubes were centrifuged at 350g for 5 minutes (step 2). The supernatant was decanted and 2mL of PBS was added to the remaining pellet of each tube (step 3). The pellet was resuspended in the PBS (step 4). Steps 2 through step 4 were repeated then a final centrifuge was performed, and 1 mL of PBS was added to each tube.

FcR Expression: PAF-exposed spermatozoa were measured for FcR activity utilizing a specific FcR assay (Arex Life Sciences, Watertown, MA). An aliquot (5ml) of the sperm suspension were added to an Arex reagent tube which is a 1.5mL microfuge tube containing 120mL of the pre-aliquoted assay mixture containing Assay Buffer, the FcR-recognizing ligand, and DNA stain (step 5). 20mL of the Arex Developer was added and samples were incubated for 20 minutes (Step 6). Assay buffer (1mL) was added, mixed and centrifuged for 30 seconds at 350g (step 7). The supernatant was aspirated with special precaution not to disturb sperm pellets (step 8). Assay buffer (300mL) was added to cell pellet for resuspension and mixed (step 9). A control group (no PAF treatment) followed the same FcR assay procedure. Samples were measured for Fc receptor expression quantity via spectrometry (NanoDrop 3300, ThermoFisher Scientific, Waltham, MA) at various time points, as follows: 30 minutes, 1 hour, 2 hours, 3 hours, 6 hours, 9 hours, 12 hours, and 24 hours.

3.2. Fc Receptor Nanodrop

Samples were measured for Fc receptor expression via spectrometry (NanoDrop, ThermoFisher Scientific, Waltham, MA) at various time points (minutes). The Arex Buffer (1mL) was placed directly on the measurement pedestal and used as blanking buffer. The Arex buffer was removed thoroughly via lab wipes. A fresh aliquot of the sample was pipetted onto the pedestal and measured. This was repeated for all samples.

4. Results

The PAF group showed increased cycling patterns and overall FcR expression quantity as compared to control samples (Figure 1 and Figure 2). There was a significant ($P<0.01$) difference in FcR expression levels between the PAF (6,452.14 RFU) group and the control (3,113.77 RFU) group (Figure 1). There was a positive upward trend of FcR expression in the PAF exposed group ($Y=303.64X+4782.1$; $R2=0.0777$) while the control group had a negative trend ($Y=247.15X+4473.1$; $R2=0.1672$). Additionally, the PAF exposed group demonstrated a much greater variance in FcR cycling patterns over time (Figure 2)

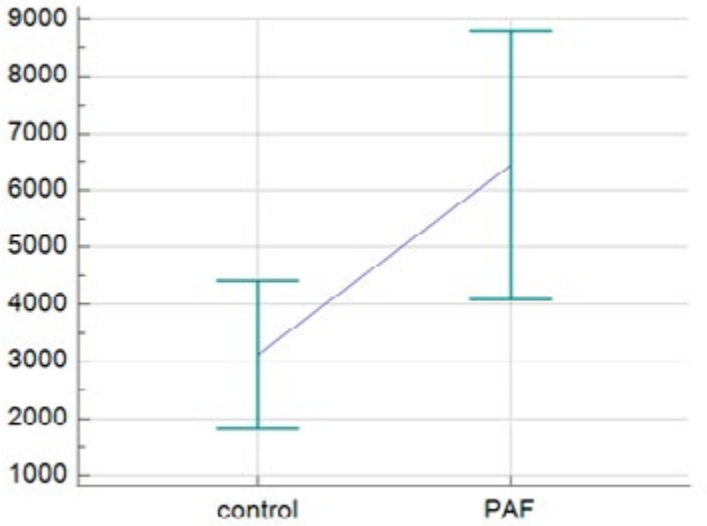

Figure 1: Overall expression of FcR in PAF treated spermatozoa vs. control spermatozoa

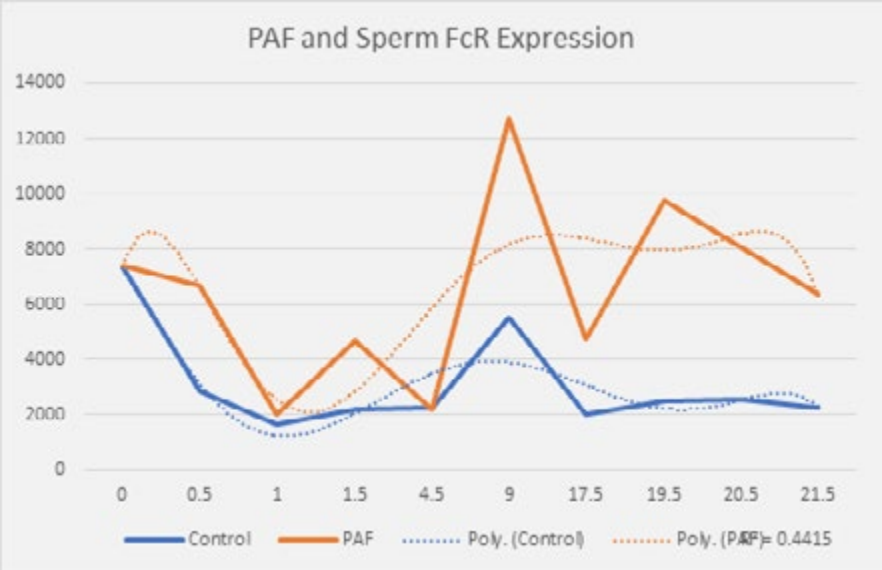

Figure 2: The difference in cyclical expression of FcR in PAF treated spermatozoa vs. control spermatozoa.

5. Conclusion

While the sea urchin is a time-honored model for investigational studies in developmental biology, little is known regarding the role of PAF in spermatozoa activity, fertilization potential, and the relationship between PAF and the FcR. Both PAF and FcR have an exocytotic function and alter cell metabolism. Through the transmission of second messengers and signal transductions, PAF and the FcR play a role in capacitation and acrosome reaction of spermatozoa, which is necessary for spermatozoa penetration and fertilization of an ovum. FcR expression is cyclical with increased cycling associated with increased fertilization potential. Our results indicate that PAF has a positive effect on sea urchin spermatozoa FcR expression cycling patterns (associated with fertility potentials) and quantity which may correlate with increased physiological processes, cellular metabolism, signal transmissions, and fertilization outcomes. Not only does PAF increase the number of waves (cycling pattern of highs and lows) of the FcR, but it also effects and maintains the persistence of function overtime preserving the fertility period. These results would suggest that the treatment of spermatozoa with PAF prior to IUI procedures could increase the rates of success.

References

1. Purnell, E. T., & Roudebush, W. E. (2001). Platelet-activating factor activity (ligand and receptor transcript) content in sperm: motile versus nonmotile. In Andrology in the 21st century, Proceedings of the VIIth International Congress of Andrology. Englewood, NJ: Medimond Publishing Company,

Inc (pp. 71-6).
2. Minhas, B. S. (1994, August). Efficiency enhancement of manipulated male and female gametes. In Seminars in reproductive endocrinology (Vol. 12, No. 03, pp. 177-183). Copyright© 1994 by Thieme Medical Publishers, Inc..
3. Kordan, W., Lecewicz, M., & Tobolski, J. (2009). Effect of platelet-activating factor on motility. Pol J Vet Sci, 12(2), 175-81.
4. Witkin, S. S., Shahani, S. K., Gupta, S., Good, R. A., & Day, N. K. (1980). Demonstration of IgG Fc receptors on spermatozoa and their utilization for the detection of circulating immune complexes in human serum. Clinical and Experimental Immunology, 41(3), 441.
5. Hajela, K. (1991). Structure and function of Fc receptors. Biochemical education, 19(2), 50-57.