Research Article

Comparative Study of Agonists Induced Acrosome Reaction in Murrah Buffalo Spermatozoa

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Abstract: Capaciatation and acrosome reaction are two very important processes for achieving the fertilizing competence in spermatozoa. In our study acrosome reaction in capacitated murrah buffalo spermatozoa was induced in the absence (control) or presence of LPC (positive control, 100g/mL) or inducers such as Spermine-NONOate (100 µM), progesterone (P4, 20 µM), and estradiol (E2, 50 µM). Progesterone induced significantly (*P<0.05*) highest percentage of AR (53.86±0.30%) followed by LPC (52.07±0.46%), 17-β Estradiol (45.13±0.15%) and spermine-NONOate (43.85±0.42%). Subset of protein p32, p38, p45, p49, p80 and p105 were significantly (*P<0.05*) more phosphorylated in progesterone also as compared to the spermine-NONOate followed by LPC. In comparison to progesterone and LPC-induced phosphorylation, p69 was higher phosphorylated in the spermine-NONOate treated spermatozoa. This study came to the conclusion that the agonist-induced acrosome response in buffalo spermatozoa increased protein tyrosine phosphorylation of the group of proteins described above.

Keywords: *Acrosome Reaction, Agonists, Progesterone, Estradiol, Spermatozoa, Murrah buffalo*

1. Introduction

Mammalian spermatozoa are unable to fertilize the egg after ejaculation, but they eventually achieve fertilization competence through a sophisticated series of modifications over a set period of time in the female reproductive tract or incubation in a particular medium. Capacitation is a biochemical and biophysical remodeling process in which spermatozoa obtain the ability to fertilize as a result of a series of profound biochemical and biophysical alterations (Yanagimachi, 1994) leading to hyperactivation in sperm motility patterns (Suarez, 1996) and acrosome response (Visconti et al., 2002; Bernecic et al., 2019; Bhakta et al., 2019). Progesterone is secreted by cumulus oophorous cells in the female reproductive system and has role in sperm capacitation and acrosome reaction (Wang et al., 2013).

The acrosome response is a unique type of exocytosis that involves the opening and fusing of the outer acrosomal membrane with the plasma membrane at numerous locations (Yanagimachi, 1994). In acrosome reaction calcium influx, actin polymerization, and intracellular pH, high levels, protein activation (phospholipases, kinases, protein G, etc.) all contribute to the release of hydrolases and the exposure of new membrane domains which involves the opening and fusing of the outer acrosomal membrane with the plasma membrane at numerous locations (Yanagimachi, 1994) and necessary for fertilization (Florman et al., 2008 and Breitbart, 2003; Barbaux et al., 2020; Yamatoya et al., 2020).

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Phosphorylation of linked proteins at tyrosine (Tyr), serine (Ser), and threonine (Thr) residues via intracellular signals controls both capacitation and acrosome reaction (Naz & Rajesh, 2004, Visconti et al. 2002, Liguori et al. 2005). During capacitation and fertilization, sperm tyrosine phosphorylated proteins are primarily found in the flagella (Lewis and Aitken, 2001, Urner et al., 2001, Pommer et al., 2003), and the presence of tyrosine phosphorylation in the main fragment is responsible for hyper-activated motility (Nassar et al., 2003).

Cumulus cells, zona pellucid and follicular fluid, (Tesarik et al., 1993) can all induce the acrosome response (Cross et al., 1988). Progesterone is the major inducer of the acrosomal reaction in follicular fluid (Meizel and Turner, 1991). Ca^{2+} is a major regulator of sperm motility and hyperactivation (Tateno et al., 2013) at the molecular due to its unique calcium channel of CatSper sperm (Lishko et al., 2011), and its participation is regulated by nitric oxide. NO controls the mobilization of accumulated Ca^{2+} in human sperm via protein S-nitrosylation, which is synergistic with progesterone, implying that this synergy is important in gamete interactions leading to fertilization (Machado-Oliviera et al., 2008). Furthermore, studies show that estrogens in the environment can significantly enhance sperm capacitation and acrosome response in animals (Adeoya-Osiguwa et al., 2003). Several studies have demonstrated the nongenomic effects of steroid hormones on the regulation of sperm capacitation, hyperactive motility, and acrosome response (AR) during their transit through the vaginal canal (Fujinoki et al., 2016).

Steroid hormones can control all of these processes by interacting with nuclear receptors or other possible novel membrane receptors (Luconi et al., 2004).17-estradiol (E2) is the most powerful oestrogen and it was believed that responses to E2 were mediated by the nuclear oestrogen receptors ER^{α} and ER^{β} (ER, ER; Levin, 2001). However, non-genomic effects of estrogens have also been identified in a number of cell types, including those from the reproductive system (Revelli et al., 1998) and may be sperm (Kelly and Levin, 2001; Luconi et al., 2001). Both membrane-bound receptors and/or interactions with other proteins and/or membrane lipids are used to mediate these effects (Levin, 2001).

A previous study found novel membrane progesterone receptors (PR) in ovine spermatozoa, as well as the two conventional nuclear estrogen receptors (ER^{α} and ER^{β}) (Gimeno-Martos et al., 2017). Estrogen may also stimulate the AR on its own in ram spermatooza. Nitric oxide (NO) is a cellular messenger that activates soluble guanylate cyclase and is generated by nitric oxide synthase (endothelium, neurons, and inducible; eNOS, nNOS, and iNOS) (Siddique et al., 2021; Ahern et al., 2002). Inhibitors of Tyr kinases and Tyr phosphatases inhibited or reduced both spermatozoa responses to Progesterone (Baron et al., 2016). Tomes et al. (2004) also reported that several Tyr kinase inhibitors and tyrosine phosphatase inhibitors blocked the calcium-triggered exocytosis.

Nitric oxide synthases (NOSs) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. The family of nitric oxide synthases (NOS) has a vital role in many pathological disorders as well as several physiological systems. There are three different types of NOS: neuronal NOS (nNOS, also known as NOS 1), endothelial NOS (eNOS, also known as NOS 3), and an inducible NOS (iNOS or NOS 2). The expression of eNOS and nNOS is constitutively present. eNOS is traditionally regarded as the primary isoform involved in the regulation of vascular function. Nitric oxide synthase is found in the reproductive tract cells of both male and female mammals, as well as in the gametes of vertebrates and invertebrates, leading to the notion that NO may have a role in fertilization (Siddique et al., 2013; Kim et al., 2004; Kadlec et al.,

2020) and induces the acrosome reaction (Siddique et al., 2012; Yang et al., 2005; Herrero et al., 2003; Siddique et al., 2021; Siddique et. al., 2019). Spermicine-NONOate (SPER/NO, (Z)-1-N-[3-Aminopropyl]-N-[4-(3-aminopropylammonio) butyl]-amino Diazenium-1, 2-diolate) is a mixture of spermine and nitric oxide (NO). It is an easy-to-use reagent for creating aqueous solutions of NO, with a half-life of 230 minutes at 22 °C. Spermine NONOate may be used for dependable and predictable NO production in solution by dissociating to release two moles of NO for every mole of the parent molecule in a pH-dependent manner. This process is known as first order kinetics. Nitric oxide is beneficial at physiological concentration while cytotoxic at higher concentration. Hence, maintenance of a balanced redox state is crucial for normal male reproductive functions (Baskaran et al., 2020).

2. Material and Methods

2.1 Semen collection and evaluation

The semen was collected twice a week from three Murrah buffalo (*Bubalus bubalis*) bulls (six ejaculates from each bull) under uniform dietary and managerial conditions maintained at National Dairy Research Institute, Karnal. These buffalo bulls' semen with a score of +3 or higher are considered for our experiments. Ejaculates were submerged in a warm water bath at 38.5°C until sperm were stretched, and mass motility was assessed immediately after collection using light microscopy (Eclipse-200, Nikon, Japan). In this study, ejaculates containing spermatozoa with > 80 percent progressive forward motility and $1x10^9$ cells per mL were used (WHO, 2010).

2.2 Sperm cell processing and culture

Sperm culture was done by standard procedure developed by Parrish et al. (1988) and modified by Galantino-Homer et al. (1997) subsequently. The medium was devoid of sodium bicarbonate and BSA wherever spermatozoa were to be maintained under noncapacitating conditions. After being centrifuged at 275 g for 6 minutes with sp-TALP and cleaned, 500 microliters of freshly ejaculated semen were collected in 15 mL tubes. The loose sperm pellet was once again washed with sp-TALP (6 mg BSA/mL). A haemocytometer was used to measure the sperm concentration, which was then adjusted to $100x10^6$ cells/ml after the pellet was resuspended in sp-TALP (6mg BSA/mL). The Roy and Atreja, 2008 procedure was used for capacitation of buffalo spermatozoa. Heparin, at a concentration of 10 g/mL, was used to induce sperm capacitation. Tubes were incubated at 38.5 °C with 5% CO_2 and 85% relative humidity in the air for 6 hours and observe it under an inverted bright field microscope at 200 x magnifications every hour to verify the fluidity of all suspensions. The sperm sample was processed after 6 hours of incubation to determine the acrosome response.

2.3 Assessment of acrosome reaction in presence of its agonists

Spermine-NONOate, progesterone (P4), and estradiol (E2) were used to induce the AR in heparin-capable spermatozoa in the absence (control) or presence (positive control, 100g/mL) of LPC or Spermine-NONOate, progesterone (P4), and estradiol (E2). Agonists and inhibitors of nitric oxide generation are examples of nitric oxide modulators. LPC is known to trigger the acrosome response exclusively in capacitated cells. These treatment groups were then incubated for 15 minutes at 38.5 °C with 5% CO_2. Suraj and Atreja (2002) method dual staining techniques were used to distinguish between healthy and degenerative acrosomal depletion. Heparin capacitated spermatozoa were collected and treated with various concentrations of 17-β-Estradiol (10,

50, and 100 µM) in order to standardize the optimal 17-β-Estradiol concentration. The concentration of hemoglobin (a nitric oxide scavenger) was also standardized by using different (10, 20, 40, 60, and 100 µg/ mL) concentrations of it. After standardization, heparin-capable spermatozoa were collected again and treated for 15 minutes with LPC, Spermine-NONOate, Spermine-NONOate+Hb (40 g/ mL), 17--Estradiol (50 µM), and progesterone (20 µM), and percent AR determined by counting 200 cells after dual staining.

2.4 Protein Tyrosine Phosphorylation in presence of Inducer of AR

Fresh spermatozoa were processed and diluted before being cultivated in Sp-TALP media with heparin (10g/mL) for 6 hours. The degree of sperm AR was evaluated by treating the heparin capacitated samples in the absence (control) or presence (100M), LPC (100g/mL), and progesterone after 15 minutes in a CO2 incubator (20M).

Fresh spermatozoa were processed and diluted before being cultured for 6 hours in Sp-TALP medium with heparin (10g/mL). After 15 minutes in a CO_2 incubator, the degree of sperm AR was determined by treating the heparin capacitated samples in the absence (control) or presence of LPC (100g/mL), Spermine-NONOate (100 µM), and progesterone (20µM).

2.5 SDS-PAGE and Immunoblotting

Galantino-Homer et al. (1997) technique with slight modifications was used for the isolation of proteins from buffalo spermatozoa. After varying periods of incubation and at time zero, sperm preparations from three tubes of the same treatment were merged into one tube, representing $75x10^6$ cells. The Lowry method (Lowry et al., 1951) was used to determine protein concentrations, and the same quantity of protein was injected into each SDS-PAGE well. The electrophoresis procedure followed Laemmli's 1970 description. Coomassie Brilliant Blue R-250 was used to stain one gel while immunoblotting procedures were carried out on the other. Using the Otter et al. (1987) procedure, the separated proteins on gel were transferred to Immobilon-P membrane (0.45 µ). The membrane was removed from the sandwich, placed in 0.5% ponceau S reversible dye in 1% glacial acetic acid, and incubated at room temperature for 5 min with mild shaking or until the protein bands appeared up to desired level as per the method of Salinovich and Montelaro (1986). The membrane was removed from dye solution and placed in distilled water for 2-3 min or until the background became clear. The membrane was destained completely in distilled water for 5-10 min. This method is used to know the equal protein loading and transfer efficiency of protein onto the membrane.

The tyrosine phosphorylated proteins generated by agonists in capacitated buffalo spermatozoa were identified using an indirect immunoblotting technique. This process involves immobilizing the antigen on a solid protein binding matrix such as PVDF or nitrocellulose membrane. The membrane with the blocking solution was removed from the refrigerator the next day and allowed to equilibrate at ambient temperature for 2 hours. Simply clean the membrane with 20 mM TBST solution before incubating for 2 hours at room temperature with anti-phosphotyrosine monoclonal antibody (Sigma Clone PT-154) while gently shaking occasionally. Remove the membrane from the primary antibody solution and wash it briefly (30s x2) with TBST by gentle agitation, and then thoroughly (15 min x 4). The membrane was removed from the final wash solution and treated with goat anti-mouse IgG peroxidase conjugate Sigma; A2554 diluted (1:70,000) in TBS-TV for 1 hour at room temperature with brief moderate agitation. The membrane was taken from the second antibody solution and carefully washed with TBS-T for 30 seconds and 15 minutes respectively.

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2.6 Visualization of immunoblot and analysis of the tyrosine phosphorylated proteins

Enhanced chemiluminescent reagent (ImmobilonTM western chemiluminescent HRP substrate) was produced by combining the ECL Solution-A and -B in a 1:1 ratio while the membrane was in the final TBS-T wash solution. For a 8 x 7 cm membrane, 1.25 mL of Solution-A and 1.25 mL of Solution-B were adequate. The mixture was used straight soon after being immediately covered in aluminium foil to block off light and processed X-ray film. The photograph was taken using an Alpha-Imager (Alpha-Innotech, USA), which was used to place the X-ray film and CBB R-250 Stained Membrane on a white sheet. The band intensities were then calculated using Alpha Ease software, version FC 6.0.1, after the image had been processed.

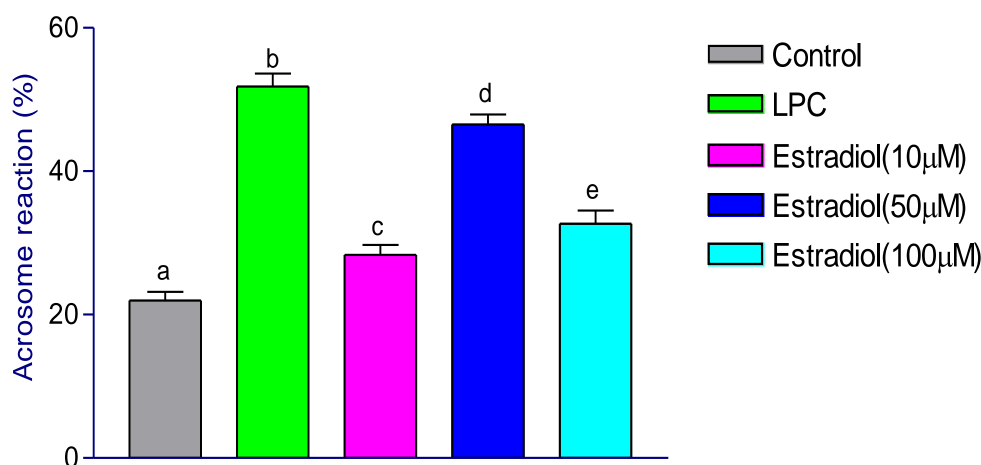
2.7 Statistical Analysis

Each experiment was run at least three times, and one-way ANOVA was used to evaluate the normally distributed data (variance analysis). The Statistical Product and Service Solutions, version 17.0.1 software is used to display the Duncan's Multiple Range Test (DMRT) findings as mean S.E.M. (SPSS Inc., Chicago, IL, USA). It has demonstrated statistical differences among the different treatments, and a difference was deemed statistically significant with $P < 0.05$.

3. Results

3.1 Effect of different concentration of 17-β-Estradiol on acrosome reaction

17-β-Estradiol at all concentrations (10 µM, 50 µM and 100 µM) demonstrated significantly (*P<0.05*) increased acrosome reaction as compared to the control value (21.97±0.21%). 17-β-Estradiol at 50 µM concentration showed significantly (*P<0.05*) highest increase in the percent AR (46.51±0.41 %) as compared to the 10 µM (28.31±0.41%) and 100 µM (32.69±0.85%). On increasing the concentration from 10 µM to 50 µM, there was significant (*P<0.05*) increase in the percent AR which decreased on further increasing the concentration upto 100 µM (Fig-1). LPC (positive control) induced significantly (*P<0.05*) higher percent of AR than the control value (51.83±0.78% vs. 21.97±0.21%). According to Kelly and Levin (2001), E_2 has been shown to alter Ca^{2+} fluxes, produce cyclic nucleotides, activate a number of kinases, alter ion channels, and enhance protein tyrosine phosphorylation, which in turn causes an increase in acrosome response.



3.2 Comparison of spermine-NONOate with other physiological agonists of AR

Results of AR induced by different inducers are presented in the Fig-2. Inducers of AR induced the AR significantly ($P<0.05$) as compared to control. Progesterone induced significantly ($P<0.05$) highest percentage of AR (53.86±0.30%) as followed by LPC (52.07±0.46%), 17-β Estradiol (45.13±0.15%) and spermine-NONOate (43.85±0.42%). Spermine-NONOate also induced significantly ($P<0.05$) higher percentage of AR as compared to control (43.85±0.42% vs. 22.81±0.12%). Further on addition of haemoglobin (40 µg/mL) to this system (spermine-NONOate+Hemoglobin) decreased the percentage of AR significantly ($P<0.05$) than the corresponding spermine-NONOate alone (20.66±0.25 % vs. 43.86±0.42%). And the significant decrease of percent AR in the presence of 40 µg/mL haemoglobin was due to the scavenging activity of haemoglobin.

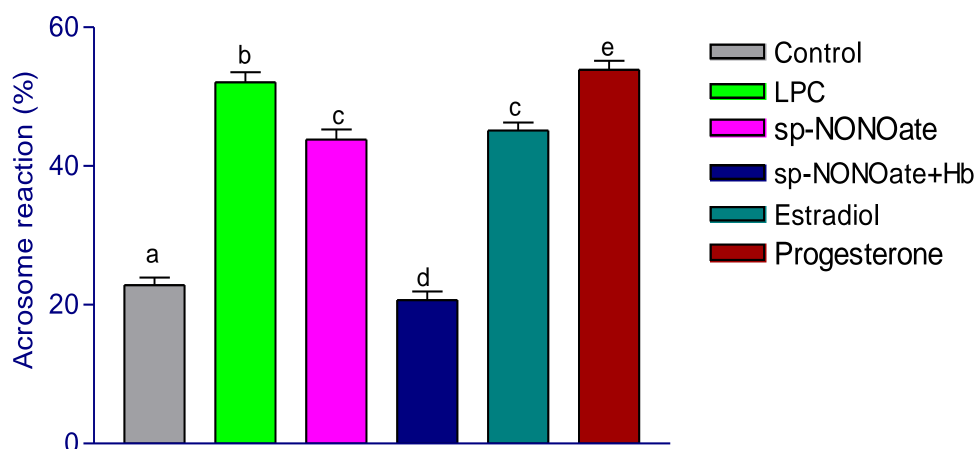


Fig-2. Comparison of spermine-NONOate with other physiological agonists of acrosome reaction.

3 Agonist Induced protein tyrosine phosphorylation during acrosome reaction

Protein with Laemmli non-reducing sample buffer containing 5 mM DTT (dithiothreitol) was extracted from different treatments from Murrah buffalo spermatozoa. In the micro Lowry protein assay, DTT is known for its interference; therefore, its concentration was kept low. By including the same quantity in the standards, the interference caused by DTT was nullified. As calculated by the updated micro-Lowry process, the protein yield was 290.41±6.231 µg/10^8 cells, ranging from 180.96 to 365.45 µg/10^8 cells (n=40). Twenty protein bands were found in buffalo spermatozoa after staining the gel with bright blue coomassie (R-250). It was found that their molecular weights were in the 17-120 kDa range. Ponceau S staining of the PVDF membrane confirmed equal loading and successful blot transfer of proteins on the PVDF membrane after electro transfer of proteins. After immunoblotting the PVDF membrane, the transfer efficiency was further confirmed by staining the PVDF membrane with coomassie brilliant blue (R-250).

3.4 Effect of different agonists of AR on protein tyrosine phosphorylation during AR

Densitometric analysis revealed that a total of eight proteins with molecular weights of 20, 32, 38, 45, 49, 69, 80, and 105 kDa (designated as p20, p32, p38, p45, p49, p69, 80, and p105, respectively) were tyrosine phosphorylated and had various densities (Fig-3; Table-1). It was discovered that distinct inducers of AR are in charge of phosphorylating a certain group of proteins

with a molecular mass between 20 and 105 kDa. Spermine-NONOate caused the p20, p32, p38, p45, p49, p69, p80, and p105 proteins to become phosphorylated. In comparison to spermine-NONOate and LPC, progesterone substantially (P 0.05) increased the phosphorylation of several proteins, including p32, p38, p45, p49, p80, and p105. In comparison to progesterone and LPC-induced phosphorylation, p69 was higher phosphorylated in the spermine-NONOate treated spermatozoa.

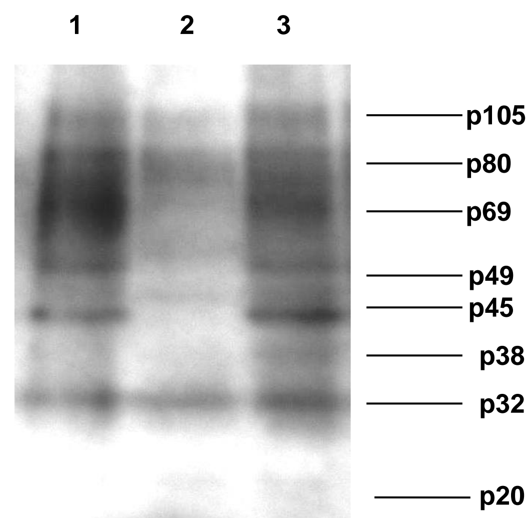


Fig-3. Effect of inducers of acrosome reaction on protein tyrosine phosphorylation.

Discussion

4.1 Comparison of spermine-NONOate with other physiological agonists of acrosome reaction

Acrosome reaction is normally triggered by ZP3 but can be reproduced *in vitro* using substances such as LPC (Fleming And Yangimachi, 1981), nitric oxide (Hererro *et al.*, 2003; Siddique et al., 2021; Yang *et al.*, 2005), 17-β Estradiol (Adeoya-Osiguwa *et al.*, 2003) or progesterone (Moseley *et al.*, 2005; Hererro *et al.*, 1998). In our study spermine-NONoate induced the AR in heparin capacitated spermatozoa. On addition of hemoglobin (40µg/mL) there was decrease in the percent AR due to nitric oxide scavenging activity of hemoglobin, which suggests the role of nitric oxide during AR in buffalo spermatozoa because on addition of spermine-NONOate acrosome reaction increased while addition of hemoglobin decreased the acrosome reaction in buffalo spermatozoa.

The results of this study reveal that progesterone induced significantly higher AR which is comparable to the AR induced by LPC. There are some controversies about the specific but non-gnomic action of steroid hormones (Maller, 2001). There is evidence that Progesteron P4 induces an AR in sperm that lack the nuclear receptors acquired by a non-genomic pathway (Sabeur *et al.* 1996). In human and stallion sperm, there is evidence for a P4 receptor (P4r) at the sperm surface (Blackmore *et al.*, 1991; Cheng *et al.*, 1998b). In human sperm P4 induces a rapid influx of calcium, calcium dependent phosphoinositide hydrolysis, an increase in tyrosine phos-

phorylation of sperm proteins and chloride efflux, which ultimately leads to the stimulation of AR. Progesterone promotes the influx of intracellular calcium rendering activation of a calcium-dependent isoform of sperm NOS. This would lead to an increase in NO synthesis, which in turn would activate COX enzyme that finally results in the acrosome reaction (Hererro *et al.*, 1998).

Non-genomic effects of estrogens have also been characterized in spermatozoa (Luconi *et al.*, 2001). 17-β Estradiol can significantly stimulate mammalian sperm capacitation, AR and fertilizing ability in spermatozoa (Adeoya-Osiguwa *et al.*, 2003). In our study 17-β Estradiol induced AR which is comparable to the AR induced by spermine-NONOate. In human sperm, it has been reported that 17-β Estradiol initiates a rapid increase in intracellular Ca^{2+} which renders the stimulation of the AC/cAMP signal transduction pathway, resulting in increased protein tyrosine phosphorylation (Luconi *et al.*, 1999, 2001) and subsequently inducing the AR.

4.2 Agonist Induced Protein Tyrosine Phosphorylation during Acrosome Reaction

Buffalo spermatozoa capacitated with heparin were acrosome reacted in the presence or absence of spermine-NONOate and compared with LPC and progesterone. Spermine-NONOate caused tyrosine phosphorylation of p20, p32, p38, p45, p49, p69, p80 and p105 proteins. Different inducers of AR like LPC and progesterone were found to be responsible for the tyrosine phosphorylation of a specific set of proteins in the molecular range of 20-105 kDa. And they had different levels of phosphorylation as evidenced by densitometric analysis.

p20, p32, p38, p45, p49, p69, p80 and p105 were phosphorylated in the LPC induced AR and this is in agreement with the results of Leclerc *et al.*, 1997, 1998). NO generated by spermine-NONOate also involved in the tyrosine phosphorylation of p80 and p105 in AR and it was also associated with sperm capacitation as reported by Thundathil *et al.*, (2003). A progressive increase in P-Thr-Glu-Tyr-P level occurred only in proteins of p80 and p105 kDa during the course of human sperm capacitation (Thundathil *et al.*, 2003) and L-NAME reverses the inhibition of the phosphorylation.

In our study LPC caused the protein tyrosine phosphorylation of p20, p32, p38, p45, p49, p80 and p105 in addition to the partial phosphorylation of p69 and p105. The above observations are in close agreement with previous reports in which, LPC resulted in phosphorylation of two proteins p80 and p105 during AR (De Lamirande and Gagnon, 2002) similar to those observed during capacitation (Thundathil *et al.*, 2002, 2003). The level of P-Tyr in p80 and p105 increased after addition of LPC and was higher in acrosome reaction. Liguori *et al.* (2005) demonstrated that PKC, PKA, PTK, PI3K, Akt and the ERK pathway are involved in human sperm AR induced by LPC.

Progesterone induced AR resulted in P-Tyr of p20, p32, p38, p45, p49, p80 and p105 proteins which are in conformity with the results of Bajpai And Doncel, 2003; LUCONI *et al.* 2005) which shows significant increase in protein tyrosine phosphorylation of p80 and p105 during AR. Progesterone has been involved in AR in human (Osman *et al.*, 1989; boar (Jang And Yi, 2002); mouse (Melendrez *et al.*, 1994), staliion (Meyers *et al.*, 1995), dog (Sirivaidyapong *et al.*, 1999); Patrat *et al.*, 2000), golden hamster (Meizel *et al.*, 1990) and goat (Somanath *et al.*, 2000). Progesterone by binding to a non-genomic mPR, has been reported to induce AR via intracellular signal transduction cascades (Kopf, 2002). Parinaud and Milhet (1996) demonstrated that progesterone-induced AR may involve a cAMP mediated pathway by showing that concentrations of cAMP increase in a Ca^{2+} dependent manner from human sperm. But conversely, Moseley *et*

al. (2005) reported that progesterone induced acrosome reaction is not dependent on the PKA activation. In addition, Blackmore *et al.* (1990) and Baldi *et al.* (1991) reported that progesterone induced a significant increase in intracellular Ca^{2+} concentration in sperm.

Table-1: Relative Band Intensities (Mean ± SE) of Tyrosine Phosphorylated Proteins in Buffalo Spermatozoa in presence of different Inducers of AR

Group	Control (LPC)	Spermine-NONOate	Progesterone
p105	100	105.7313±1.962[a]	116.4626±3.753[b]
p80	100	119.4666±2.9627[a]	125.6±2.0816[b]
p69	100	113.9909±2.9627[a]	103.6698±2.9627[b]
p49	100	138.1356±2.6457[a]	151.6949±3.2145[b]
p45	100	138.764±2.0275[a]	148.3146±2.3094[b]
p38	100	108.0264±0.881[a]	117.5529±1.452[b]
p32	100	103.6947±3.179[a]	114.3187±2.886[b]
p20	100	87.56906±2.905[a]	85.55302±2.02758[a]

Values are the mean ± SEM of three different samples. Different letters ([a, b]) indicate significant differences ($p<0.05$).

Conclusion

Therefore, this study came to the conclusion that the agonists caused the buffalo spermatozoa to respond in an acrosome reaction and were linked to an increase in the protein subsets p20, p32, p38, p45, p49, p80, and p105 being phosphorylated on their tyrosine kinases.

Author Contributions

Conceptualization, R.S. and S.A.; methodology, R.S.; software, A.K.; validation, R.S., S.A. and K.S.; formal analysis, F.A.; investigation, R.S.; resources, A.K.; data curation, R.S.; writing—original draft preparation, R.S.; writing—review and editing, S.A.; visualization, A.M.; supervision, S.A.; project administration, S.A.; funding acquisition, S.A. All authors have read and agreed to the published version of the manuscript."

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Conflicts of interest

The authors affirm that they do not have any competing interests.

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