Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.4050

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (2):** 511-516 (2018)

International Journal of Pure & Applied Bioscience

Research Article

OPEN ACCESS

Cytolocalisation of PDC-109 Protein in Ejaculated Spermatozoa of Buffaloe (*Bubalus bubalis*)

Sunilkumar Patil[1*], K.V. Jamuna[1], Lakshminarayana Somashekar[2], Sellappan Selvaraju[2], R.V. Prasad[3] and Binsila B. Krishnan[2]

[1]Department of Veterinary Anatomy,Veterinary College, Bangalore, KVAFSU, Bidar

[2]Reproductive Physiology Laboratory, Animal Physiology Division, ICAR- NIANP, Adugodi, Bangalore.

3- Vice-chancellor, KVAFSU, Bidar

*Corresponding Author E-mail: drsunilpatil681@gmail.com

Received: 14.06.2017 | Revised: 26.07.2017 | Accepted: 1.08.2017

ABSTRACT

Semen quality evaluation is important for improving fertility of animals especially the dairy cows. The aim of the present study was to localize PDC-109, one of the fertility regulating proteins in buffalo spermatozoa. Indirect immunofluorescence was performed in spermatozoa to localize PDC-109. The semen samples were fixed in paraformaldehyde and the PDC-109 was localized using goat polyclonal primary antibody (1:50 dilution) and chicken anti-goat-FITC as secondary antibody (1:200 dilution). The PDC-109 was localized in the apical portion of the acrosomal membrane and post-acrosome region of the buffalo spermatozoa. Since, PDC-109 was localized in the acrosome membrane, it may bind to the choline phospholipids, and may play an important roles in capacitation and acrosome reaction, motility regulation as well as to form oviduct sperm reservoir before fertilization. PDC-109 also localized in the post acrosomal region indicating that PDC-109 might be involved in the sperm penetration deep into the oocyte cytoplasm. This is the first report localized PDC-109 in buffalo spermatozoa.

Key words: *Buffalo, Spermatozoa, Indirect immunofluorescence, PDC-109, Acrosome*

INTRODUCTION

Secretions from the testes, epididymis, seminal vesicles and other accessory glands contribute to the complex mixture of fluid and proteins that comprise seminal plasma. The seminal plasma contains many biological substances and regulates the fertilization and successful pregnancy. The proteins present in seminal plasma are being studied to assess the semen quality and fertility. These fertility-regulating proteins present in bull seminal plasma and spermatozoa have been studied in detail but not in buffaloes. The seminal plasma provides more than half of the proteins to the spermatozoa, the functions of these proteins depends on the localization on the spermatozoa. Hence studying the localization of these proteins might elucidate the possible functions during fertilization process and help to predict bull fertility.

Cite this article: Patil, S., Jamuna, K.V., Somashekar, L., Selvaraju, S., Prasad, R.V. and Krishnan, B.B., Cytolocalisation of PDC-109 Protein in Ejaculated Spermatozoa of Buffaloe (*Bubalus bubalis*), *Int. J. Pure App. Biosci.* **6(2):** 511-516 (2018). doi: http://dx.doi.org/10.18782/2320-7051.4050

Few of the most extensively studied proteins in bull spermatozoa are PDC-109 (protein with N-terminus aspartic acid, D and carboxy terminus cystine, having 109 amino acids), BSP-A3, and BSP-30-kDa belongs to family of acidic heparin-binding proteins called bovine seminal plasma proteins (BSPs) secreted by the seminal vesicles. These proteins represented approximately 70-86% of the total protein content of bovine seminal plasma[11]. PDC-109 is the abundant protein among all the three bovine seminal plasma proteins[12]. After ejaculation, PDC-109 protein coats the spermatozoal surface by specific interaction with choline containing phospholipids, resulting in cholesterol efflux from the sperm membrane and causes decapacitation and prevent premature acrosome reaction[8]. Thus, PDC-109 is known to increase sperm motility[13], capacitation and acrosome reaction[16], sperm–egg interaction and fertilization [17]and helps in the formation of oviductal sperm reservoir[6,7]. Also, prolonged exposure of spermatozoa to PDC-109 protein during processing for cryopreservation or at higher concentration in seminal plasma induces changes in sperm plasma membrane by stimulating lipid and cholesterol efflux resulting in membrane destabilization followed by cryoinjury manifested by premature capacitation and acrosome reaction[1,9].

Though, there is a wealth of information regarding the beneficial and or detrimental effects of the PDC-109 protein in cattle spermatozoa,[14,15] but no information is available on cytolocalization of PDC-109 in buffalo spermatozoa. Based on the locations of proteins binding, for example, either acrosome, equatorial segment, post-acrosome, mid piece or tail, it may be possible to predict the functions of these proteins and may provide insights into their role in male fertility.

Hence, the objective of the present study was to evaluate the topographical distribution of PDC-109 protein on ejaculated buffalo spermatozoa.

MATERIAL AND METHODS

The ejaculated buffalo semen samples were collected from Nandini Sperm Station, Hessaraghatta, Bengaluru, Karnataka. All these chemicals were of analytical grade or molecular biology grade. All the experimental steps were carried out at room temperature (25-27°C) except incubation of cells with primary antibody, which was carried out at 4°C.

The neat semen samples were washed in 50% Percoll (Sigma-Aldrich, USA) in Phosphate Buffered Saline (PBS, pH 7.2) by centrifuging at 1000g (Remi, CM-12, India) for 5 min at room temperature (25-27°C). The sperm pellet was resuspended in 1 mL PBS and centrifuged at 300g for 5 min. The resulting sperm pellets were resuspended in 1mL of 4% paraformaldehyde in PBS and incubated for 20 min at room temperature and further centrifuged at 300g for 5 min. The fixed spermatozoa were resuspended and smeared onto the slides coated with 0.01% poly-L-lysine (Sigma-Aldrich, USA) and incubated at room temperature for 5 min. Permeabilization of sperm membrane was carried out in 0.1% Triton X-100 (SRL, India) in PBS for 30 min. The blocking was carried out by incubating the smeared slides in 5% chicken serum diluted in PBS for 30 min. After incubation, the slides were washed thrice in PBS for 5 min each and were incubated with primary antibody, BSPH-1 goat polyclonal IgG (Santa Cruz, USA) at 1:50 dilution in PBS for overnight at 4°C. Negative controls were also incubated for overnight in blocking buffer without primary antibody. After overnight incubation, the slides were washed twice in PBS for 5 min each. All the slides were incubated with secondary antibody, chicken anti-goat conjugated with fluorescein isothiocyanate (Santa Cruz, USA) at 1:200 dilution in PBS for 1 hour at room temperature. After incubation, the slides were washed thrice in PBS for 5 min each. The cells were counterstained with 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI, Himedia, India),10µg in 1000µL of PBS by incubating for 1 hour with gentle rocking. The excess of DAPI was removed by washing with PBS for 5 min and allowed to dry in dark. Further the slides were mounted with antifade

agent (Prolong diamond antifade, Invitrogen, Thermofisher, USA) and incubated for overnight in dark. The fluorescent signals were captured at a magnification of x1000 for FITC (excitation at 450-490, dichroic mirror (DM) at 505 and barrier filter (BA) at 520) and DAPI (excitation at 330-380, DM at 400 and BA at 4200 using fluorescent microscope (Eclipse E50i, Nikon, Japan). The RGB images of FITC and DAPI labeling in sperm was compared with negative controls, the merged image of FITC and DAPI was considered to measure the intensities respectively. The intensity of PDC-109 localization in sperm was measured in pixels and represented as intensity profile graph on validating with negative control.

RESULTS AND DISCUSSION

Our results demonstrate for the first time in buffalo spermatozoa that the antibody against PDC-109 (BSP A1/A2) protein was bound to apical portion of the acrosome membrane and post-acrosome region of ejaculated buffalo spermatozoa. Negative control showed absence of PDC-109 localisation in acrosome membrane and post-acrosome region of buffalo spermatozoa (Fig.1), (Fig.2, Intensity profile of merged image of Buffalo spermatozoa expressing PDC-109),(Fig.3 Intensity profile of merged image of negative control of Buffalo spermatozoa showing absence of expression and showing peak of counter stain, DAPI). The expression of PDC-109 was also not consistent in all the spermatozoa in an ejaculate. Similar to the present study, in bull spermatozoa, PDC-109 protein binds to the sperm surface at the acrosome, post-acrosome and mid piece region[10]. However, in another study reported that the PDC-109 was localized to only on the acrosomal region of the sperm head which is the region known to bind to oviductal epithelium[4,5].

Association of PDC-109 protein with membranes of the ejaculated buffalo sperm head suggests that it may be involved in cholesterol and phospholipid removal from the membrane, as reported in bovine spermatozoa[8]. Not all buffalo spermatozoa expressed in the acrosome region, may be due to PDC-109 present in seminal plasma might have caused membrane changes by rearrangement of cholesterol and phospholipids.

The spermatozoa acquire PDC-109 protein from the seminal plasma during the time of ejaculation. During this brief period, PDC-109 binds to acrosomal membrane or post acrosomal (plasma) region of the sperm head. PDC-109 stabilizes the sperm membrane by preventing free movement of phospholipids (arrested state). This arrested (decapacitated), state may prevent premature acrosome reaction of spermatozoa[3]. Binding of PDC-109 in the acrosomal membrane also causes cholesterol efflux (first cholesterol efflux) and release of some phospholipids resulting in decreased in cholesterol/phospholipid ratio. This leads to destabilization of sperm membrane during cryopreservation or in response to the chemokine signalling in the female reproductive tract. These actions of PDC-109 may depend on the abundance of PDC-109 in spermatozoa[14].

These PDC-109 in spermatozoa act as an anchoring protein to bind to oviductal epithelium and thus helping spermatozoa to preserve viability and motility in the oviductal reservoir[6]. When oviductal/follicular fluid enters the oviduct, high density lipoproteins present in them docks to PDC-109 present in sperm membrane and PDC-109 leave the sperm membrane complexing with HDL. Because cholesterol is recognized to have an important stabilizing effect on membranes, its outflow (second cholesterol efflux) results in further destabilization of the membrane. As calcium enters through this altered sperm membrane, Phospholipase A2 is activated, converting phospholipids to lysophospholipids that are known to destabilize membranes leads to activated sperm motility due to calcium influx. As a result, it provides hypermotility and thrust for the release of sperm from oviductal reservoir and to penetrate zona pellucida.

The adhesion to zona pellucida would trigger acrosome reaction. Thus, PDC- 109 protein not only decapacitates the spermatozoa at early stages but may eventually promote capacitation and helps in process of fertilization[15].Thus, major functions of interacting proteins of PDC-109 in relation to the *Bos taurus* genome were identified as cell recognition, cholesterol efflux, capacitation, acrosome reaction, and fertilization[14].

Excess of PDC-109 expression in spermatozoa was correlated with bulls with low fertility[14]. Higher concentration of PDC-109 in seminal plasma or prolonged exposure of spermatozoa to PDC-109 protein during processing for cryopreservation causes changes in sperm plasma membrane by stimulating lipid and cholesterol efflux resulting in membrane destabilization followed by cryoinjury manifested by premature capacitation and acrosome reaction[9].

The protein is also localized in the post acrosmal region suggesting that this protein might be involved in deep penetration of sperm into the cytoplasm[2] to facilitate efficient transfer of nuclear material to the oocyte.

In cattle, though localization of PDC-109 in mid-piece region has been reported, in buffalo spermatozoa, PDC-109 was not observed to be localized in the mid piece. Since, the proteins present close to mitochondria has been involved in sperm motility[13], whether absence of this protein in mid piece could be the reason for sluggish motility of buffalo spermatozoa need to be studied.

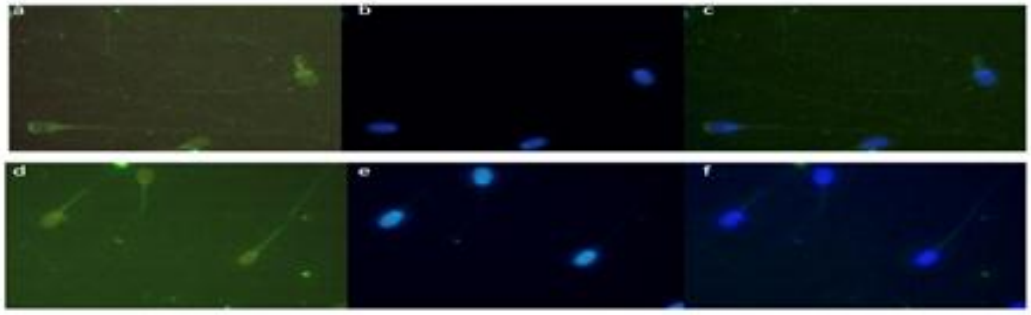

Fig. 1: Immunocytolocalisation of PDC-109 protein in apical portion of acrosome membrane and post-arcosome region of buffalo spermatozoa(a) counter stained with DAPI (b), (c) merged image of a& b. Negative control showing absence of fluorescent signal over acrosome and post-acrosome region of buffalo spermatozoa(d), counterstained with DAPI (e), (f) Merged image of (d) &(e)

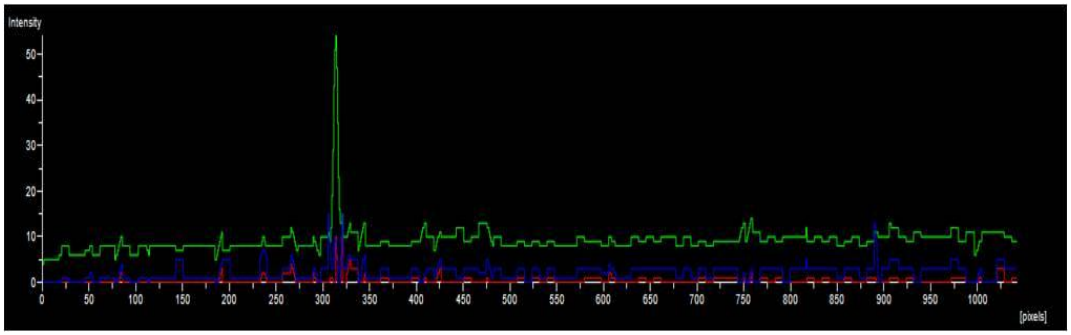

Fig. 2: Intensity profile of merged image of PDC-109 localization in Buffalo spermatozoa showing peak in the florescent area

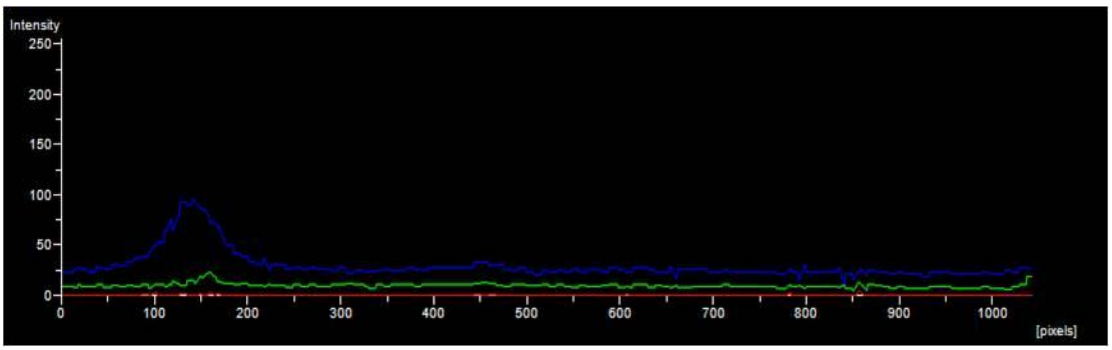

Fig. 3: Intensity profile of merged image of negative control of PDC-109 in Buffalo spermatozoa showing absence of peak for flouroscent area and intensity of counterstain DAPI (nuclear stain) is showing peak

CONCLUSION

In buffalo ejaculated spermatozoa, PDC-109 is localized in the apical region of acrosome and post acrosomal region suggesting the importance of this protein in capacitation process and sperm penetration to oocyte cytoplasm for successful fertilization Acknowledgement: The authors like express their gratitude to Director, NIANP, Adugodi, Bengaluru for providing necessary facilities for carrying out experiment.

REFERENCES

1. Bergeron, A., Crete, M.H., Brindle, Y. and Manjunath, P., Low density lipoprotein fraction from hen's egg yolk decreases the binding of the major proteins of bovine seminal plasma to sperm and prevents lipid efflux from the sperm membrane. *Biol. Reprod*. **70:** 708–717 (2004).
2. Delgado, B.N.L., Hernandez, G.E.O., Segura, N.M. and Mujica, A., Actin polymerization in the equatorial and post acrosomal regions of guinea pig spermatozoa during the acrosome reaction is regulated by G proteins. *Mol. Reprod. Develop.* **70(2):** 198-210 (2005).
3. Desnoyers, L. and Manjunath, P., Major proteins of bovine seminal plasma exhibit novel interactions with phospholipids. *J. Biol. Chem*. **267:** 10149-10155 (1992).
4. Dobrinski, I., Suarez, S.S. and Ball, B.A., Intracellular calcium concentration in equine spermatozoa attached to oviductal epithelial cells in vitro. *Biol. Reprod.* **54:** 783–788 (1996).
5. Gwathmey, T.M., Ignotz, G.G. and Susan, S.S., PDC-109 (BSP-A1/A2) Promotes bull sperm binding to oviductal epithelium in vitro and may be involved in forming the oviductal Sperm Reservoir. *Biol. Reprod*. **69:** 809–815 (2003).
6. Gwathmey, T.M., Ignotz, G.G., Mueller, J.L., Manjunath, P. and Suarez, S., Bovine seminal plasma proteins PDC-109, BSP-A3, and BSP-30 kDa share functional roles in storing sperm in the oviduct. *Biol. Reprod***. 75:** 501–507 (2006).
7. Hunter, R.H.F. and Leglise, P.C., Polyspermic fertilization following tubal surgery in pigs, with particular reference to the role of the isthmus. *J. of Reprod. Fert*. **24:** 233–246 (1971).
8. Manjunath, P. and Therien, I., Role of seminal plasma phospholipid-binding proteins in sperm membrane lipid modification that occurs during capacitation. *J. Reprod. Immun*. **53:** 109–119 (2002).
9. Manjunath, P., Bergeron, A., Lefebvre, J. and Ton, J., Seminal plasma proteins: function and interaction with protective agents during semen preservation. *Biochem. Et Biophys. Act*. **1784:** 891–899 (2008).
10. Manjunath, P., Chandonnet, L., Leblond, E. and Desnoyers, L., Major proteins of bovine seminal vesicles bind to spermatozoa. *Biol. Reprod*. **50:** 27–37(1994).
11. Moura, A.A., Koc, H., Chapman, D.A. and Killian, G.J., A comprehensive proteomic

analysis of the accessory sex gland fluid of mature Holstein bulls. *Ani. Reprod. Sci*. **98:** 169–188 (2007).

12. Nauc, V. and Manjunath, P., Radioimmunoassays for bull seminal plasma proteins (BSP-A1/-A2, BSP-A3, and BSP-30-kilodaltons) and their quantification in seminal plasma and sperm. *Biol*. *Reprod*. **63:** 1058–1066 (2000).
13. Sanchez-Luengo, S., Aumuller, G., Albrecht, M., Sen, P.C., Rohm, K. and Wilhelm, B., Interaction of PDC-109, the major secretory protein from bull seminal vesicles, with bovine sperm membrane Ca2 + -ATPase. *J. Andr*., **25:** 234–244 (2004).
14. Somashekar, L., Selvaraju, S., Parthipan, S. and Ravindra, J.P., Profiling of sperm proteins and association of sperm PDC-109 with bull fertility. *Sys. Biol. in Reprod. Med.*, **61(6):** 376-87 (2015).
15. Srivastava, N., Jerome, A., Srivastava, S.K., Ghosh, S.K. and Amit, K., Bovine seminal PDC-109 protein: An overview of biochemical and functional properties. *Ani. Reprod. Sci*. **138:** 1–13(2013).
16. Therien, I., Moreau, R. and Manjunath, P., Major proteins of bovine seminal plasma and high density lipoprotein induce cholesterol efflux from epididymal sperm. Biol. Reprod. **59:** 768–776 (1998).
17. Yanagimachi, R., Mammalian fertilization. Physiology of Reproduction, 2nd Ed, In: Knobil, E., Neill, J.D. (Eds.), Raven Press, New York, 1994, 189-317.