Osteopontin ameliorates sodium nitroprusside induced free radical damage on sperm motility of frozen thawed buffalo semen

Visakh Viswam, K Loganathasamy, VS Gomathy and D Reena

DOI: https://doi.org/10.22271/j.ento.2020.v8.i6y.8093

Abstract

The experiment was undertaken to study sodium nitroprusside (SNP) induced free radical damage on sperm motility of frozen thawed buffalo semen and the ameliorative effects of osteopontin (OPN) supplementation on sperm motility. Buffalo frozen semen straws from 8 ejaculates of 6 bulls were procured from Central Frozen Semen Production and Training Institute, Hessarghatta, Bangalore and stored at Semen Bank, Madras Veterinary College, Chennai. The semen straws were thawed and seminal plasma and semen extender were removed from spermatozoa by centrifugation. Spermatozoa were suspended in 1mL capacitation medium (control), with the addition of 100µg/mL of OPN (treatment I) or 100µg/mL of SNP (treatment II) or 100µg/mL of OPN + 100µM/mL of SNP (treatment III). Sperm with capacitation medium alone without any supplementation served as control. The contents were incubated at 37°C for 4 h and the post capacitation sperm motility was observed under bright field microscopy. The post capacitation sperm motility was significantly (P<0.05) higher in treatment I (71.80% ± 0.04) as compared to control (61.71% ± 0.03), treatment II (24.82% ± 0.07) and treatment III (46.64% ± 0.05). But, the post capacitation motility in treatment II and III were significantly (P<0.05) lower than control. The post capacitation motility in treatment III was significantly (P<0.05) higher than treatment II. The study indicated that addition of SNP alone in the capacitation medium has detrimental effects on the sperm motility and addition of OPN alone in the capacitation medium exerts beneficial effects on sperm motility. When both OPN and SNP were added in the capacitation medium, OPN partially ameliorated the toxic effects of SNP on the sperm motility of frozen thawed buffalo semen.

Keywords: Osteopontin, sodium nitroprusside, sperm motility, buffalo semen

Introduction

The influence of seminal proteins on male reproduction has drawn attention because many studies proved that their expression is associated with fertility scores in dairy cattle [1], beef cattle [2] and horses [3]. Proteins such as osteopontin (OPN), prostaglandin D synthase, bovine seminal plasma proteins (BSP A1, A2 and A3), heparin binding proteins (HBPs), fertility associated antigen (FAA), phospholipase A2, sperm adhesion Z13, clusterin (CLU) and heat shock proteins (HSPs) have been identified in seminal plasma and documented as indicators of fertility [4,5,6,7,8]. OPN is an acidic protein belongs to a family of proteins named SIBLING (small integrin-binding ligand N-linked glycoprotein) [9]. In bulls, OPN in seminal plasma is secreted by ampulla and vesicular gland [10]. High fertility Holstein bulls have greater concentrations of OPN in accessory sex gland fluids than low-fertility bulls [7]. In cattle, studies with the use of specific antibodies have shown that OPN is one among the seminal plasma proteins that are associated with fertility. OPN has also been detected at greater concentrations in the seminal plasma than in the sperm cells in buffalo as OPN is produced by ampulla and seminal vesicles, as in case of cattle [11]. Treatment with OPN in the presence of heparin improved sperm in vitro sperm capacitation, synchronous pronuclear (PN) formation, blastocyst yield, and embryo quality in buffalo [12]. The importance of OPN in reproduction was also demonstrated in experiments using in vitro fertilization [11].

Nitric oxide (NO) has been proved as an inter and intracellular messenger molecule controlling many physiological processes. NO is synthesized from L-arginine by the action of nitric oxide synthase (NOS), an enzyme existing in three isoforms. Two of them, endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) collectively called as constitutive nitric oxide synthase, are responsible for continuous basal release of NO and both require calcium/calmodulin for activation.
The other one is called as inducible nitric oxide synthase (iNOS), is responsible for prolonged release of NO and does not require calcium/calmodulin for activation. It is expressed in response to inflammatory cytokines and lipopolysaccharides [14, 15, 16]. NO is involved in regulation of mammalian sperm functions such as motility, capacitation and acrosomal reaction [17]. Narrow range of NO concentration enhanced the early events in reproduction. But either the lack of NO or excess of NO had negative consequences [18]. NO produced by sperm NOS participates in capacitation and acrosome reaction of cryopreserved bovine semen [19]. Higher concentration of NO exerts many adverse effects on sperm characteristics like motility, morphology and viability [20].

Motility is one of the most important features of fertile spermatozoa. It was the first and continues to be the most widely used indicator of sperm function. Sperm motility is an important attribute, because it is readily identifiable and reflects several structural and functional competence, as well as essential aspects of spermatozoa metabolism [21]. Hence, the present experiment was undertaken to study influence of both sodium nitroprusside (SNP), a NO donor and OPN, a seminal plasma protein on sperm motility of frozen thawed buffalo semen.

Materials and Methods

Materials
All the plasticware used for this study viz., centrifuge tubes, microcentrifuge tubes, microtips (different graduations) were purchased from Tarsion India. All the glassware used in this study viz., laboratory bottles, microscope cover slips 18mm × 18mm, microscopic slides, conical flasks and beakers were purchased from Borosil, India. All the laboratory chemicals used in this study were procured from Sigma-Aldrich chemicals Co., USA.

Methods

Sperm treatment
Buffalo semen straws from 8 ejaculates of 6 bulls were procured from Central frozen Semen Production and Training Institute, Hessarghatta, Bangalore-51. The straws were collected in liquid nitrogen (LN₂;−196 °C) container, transported and stored in the Semen Bank, Madras Veterinary College, Chennai- 600 007. The semen straws were thawed at 37°C for 30 sec. and transferred to a test tube containing 5 ml of sperm capacitation medium (Table 1) and centrifuged at 50 g for 10 min. Supernatant was discarded and sperm pellet was reconstituted with 5 ml of fresh sperm capacitation medium and centrifuged as the same rate. Again, the supernatant was discarded and the sperm pellet was finally reconstituted in 1 ml of sperm capacitation medium (Control); supplemented with 100µg/ml OPN alone (Treatment I); 100µM/ml SNP alone (Treatment II); 100µg/ml OPN and 100µM/ml SNP (Treatment III). Sperm sample was incubated at 38±1°C and 5% CO₂ in humidified air for 4 h. After incubation, the post capacitation sperm motility was examined from the above groups as described below.

Table 1: Effects of OPN and SNP supplementation on post capacitation sperm motility of frozen thawed buffalo semen

| Groups | Number of experimental animals used for collection of semen straws | Post capacitation Motility (%±SE) |
|--------|------------------------------------------------------------------|----------------------------------|
| Control | 6                                                                | 61.71 ± 0.03                     |
| Treatment I (OPN-100µg/mL) | 6                                                                 | 71.80 ± 0.04                    |
| Treatment II (SNP-100µM/mL) | 6                                                                 | 24.82 ± 0.07                    |
| Treatment III (OPN-100µg/mL + SNP 100µM/mL) | 6                                                              | 46.64 ± 0.05                    |

Mean with different superscripts (a, b and c) are significantly different (P<0.05)

Data are presented as mean% ± SE.

Evaluation of sperm motility
The sperm motility was assessed by placing a drop of semen from each group on separate clean grease free glass slide and covered with cover slip. Minimum of three fields were scanned under bright field microscopy to assess the per cent progressive motile spermatozoa and graded in terms of per cent ranging from 0-100 in multiples of 10 [22].

Statistical analysis
Data were fed in Microsoft Excel and statistical analyses were conducted using SPSS for Windows 23.0 (IBM Corp.). Statistical design was carried out by completely randomized design (CRD). All the data were analyzed by one way analysis of variance followed by Duncan’s multiple comparison test.

Results
Table 1 (Figure-1) shows the effects of OPN and SNP supplementation on post capacitation sperm motility. The post capacitation sperm motility was significantly (P<0.05) higher in treatment I (71.80% ± 0.04) as compared to control (61.71% ± 0.03), treatment II (24.82% ± 0.07) and treatment III (46.64% ± 0.05). The post capacitation motility in treatment II and III was significantly (P<0.05) lower than control. Among the treatment groups, post capacitation sperm motility was significantly (P<0.05) low in treatment II. But, post capacitation motility in treatment III was significantly (P<0.05) higher than treatment II.
Discussion
The results of the present study revealed that spermatozoa treated with SNP significantly decreased sperm motility. This study corroborated with several studies which demonstrated that sperm motility was decreased in the presence of different concentrations of SNP [23, 24, 25, 26]. In other studies, NO was shown beneficial for motility of spermatozoa at low concentration but, high concentration was harmful to spermatozoa [27, 28]. Variation in sperm motility among the bulls may be due to regulation of nitric oxide (NO) synthesis by OPN because OPN is a general modulator of NO synthesis [29]. Nitric Oxide (NO) is synthesized in epithelial cells of male reproductive tract [30]. NO induces OPN production in macrophage, thereby providing a negative feedback loop to limit NO mediated tissue injury [30]. NO at higher concentration inhibits cellular respiration of spermatozoa by nitrosoylation of heme in mitochondrial enzyme, aconitase and glyceraldehydes 3- phosphate dehydrogenase leading to depletion of ATP and consequent loss of motility in the spermatozoa [31]. Spermatozoa obtained from bull semen displaying increased OPN gene expression had high motility than spermatozoa obtained from bull semen with lower gene expression [32]. OPN increases intracellular calcium [33] and thereby increase sperm motility [34]. OPN concentration was significantly high in Arabian horse with higher percentage of motile spermatozoa and they showed a higher fertilization capacity [35]. Spermatozoa treated with SNP and OPN showed significantly higher motility than spermatozoa treated with SNP alone. This could be due to down regulation of NO synthesis by OPN [36].

Conclusions
The study indicated that addition of SNP alone in the capacitation medium has detrimental effects on the sperm motility and addition of OPN alone in the capacitation medium exerts beneficial effects on sperm motility. When both OPN and SNP were added in the capacitation medium, OPN partially ameliorated the toxic effects of SNP on the sperm motility of frozen thawed buffalo semen.

Acknowledgements
The authors are grateful to Authorities of Tamil Nadu Veterinary and Animal Sciences University for providing necessary funds and facilities to carry out this experiment.

References
1. Cancel AM, Chapman DA, Killian GJ. Osteopontin localization in the Holstein bull reproductive tract. Biol. Reprod 1999;60:454-460.
2. Parent S, Lefievre L, Brindle Y, Sullivan R. Bull subfertility is associated with low levels of a sperm membrane antigen. Mol. Reprod. Dev 1999;52:57-65.
3. Brandon CI, Heusner GL, Caudle AB, Fayrer RA. Two dimensional polyacrylamide gel electrophoresis of equine seminal plasma proteins and their correlation with fertility. Theriogenology 1999;52:863-873.
4. Fouchecourt S, Metayer S, Locatelli A, Dacheux F, Dacheux J, Stallion epididymal fluid proteome: qualitative and quantitative characterization; secretion and dynamic changes of major proteins. Biol. Reprod 2000;62:1790-1803.
5. Sprott LR, Harris MD, Forrest DW, Young J, Zhang HM, Oyarzo JN et al., Artificial insemination outcomes in beef females using bovine sperm with a detectable fertility-associated antigen. J Anim. Sci 2000;78:795-798.
6. McCauley TC, Zhang HM, Bellin ME, Ax RX. Identification of a heparin-binding protein in bovine seminal fluid as tissue inhibitor of metalloproteinases-2. Mol. Reprod. Dev 2001;58:336-341.
7. Moura AA, Chapman DA, Killian GJ. Proteins of accessory sex glands associated with the oocyte-penetrating capacity of cauda epididymal sperm from Holstein bulls of documented fertility. Mol. Reprod. Dev 2006a;74:214-22.
8. Moura AA, Chapman DA, Koc H, Killian GJ. A comprehensive proteomic analysis of cauda epididymal fluid and identification of proteins associated with fertility scores of mature dairy bulls. J Andro 2006b;98(5):71-77.
9. Franzen A, Heinegard D. Isolation and characterization of two sialoproteins present only in bovine calcified matrix. Biochem. J 1985;232:715-724.

10. Siertert JE, Ensrud KM, Moore A, Hamilton DW. Identification of osteopontin (OPN) mRNA and protein in the rat testis and epididymis and on sperm. Mol. Reprod. Dev 1995;40:16-28.

11. Pero ME, Killian GJ, Lombardt P, Zicarelli L, Vallone L, Gasparini B. Identification of osteopontin in water buffalo semen. Reprod. Fertil. Dev 2007;19:279.

12. Souza CE, Moura AA, Monaco E, Killian GJ. Binding patterns of bovine seminal plasma proteins A1/A2, 30 kDa and osteopontin on ejaculated sperm before and after incubation with asthmic and ampullary oviductal fluid. Anim. Reprod. Sci 2008;105:72-89.

13. Gioncalves R, Chapman DA, Killian GJ. Effect of osteopontin on in vitro bovine embry development. Biol. Reprod 2013;88(1):545.

14. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacological Reviews 1991;43:109-141.

15. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases. Structure, function and inhibition. Biochemistry Journal 2001;357:593-615. http://dx.doi.org/10.1042/0264-6021:3570593

16. Forstermann U, Sessa WC. Nitric oxide synthases: regulation and function. European Heart Journal 2012;33:829-837.

17. Herrero MB, E de Lamirande, Gagnon C. Nitric oxide is a signalling molecule in spermatozoa. Current Pharmaceutical Design 2003;9:419-425.

18. Thaler CD, Epel D. Nitric oxide in oocyte maturation, ovulation, fertilization, cleavage and implantation: a little dab’ll do ya. Current Pharmaceutical Design 2003;90:399-409.

19. Cristian O, Pablo R, Sudha S. L-arginine promotes capacitation and acrosomal reaction in cryopreserved spermatozoa. Biochem. Biophys. Acta 2004;1674:215-221.

20. Vidya G, Garg SP, Rawekar AT, Deshpande VK, Biswas DA, Sawane MV, Akarte AN. Effect of oxidative stress on sperm quality in leukocytespermic infertile men. Biomedical Research 2011;22:329-332.

21. Mortimer ST. A critical review of the physiological importance and analysis of sperm movement in mammals. Hum. Reprod 1997;3:403-439.

22. Bansal AK, Bilasupi GS. Impacts of oxidative stress and antioxidants on semen functions. Veterinary Medicine International 2011;4060:1-7.

23. Herrero MB, Cebral E, Boquet M, Viggiano JM, Vitullo A, Gimeno MA. Effect of nitric oxide on mouse hyperactivation. Acta. Physiol. Pharmacol. Ther. Latinoam 1994;44:65-69.

24. Rosselli M, Dubey RK, Imthurn B, Macas E, Keller PJ. Effects of nitric oxide on human spermatozoa: evidence that nitric oxide decreases sperm motility and induces sperm toxicity. Hum. Reprod 1995;10:1786-1790.

25. Tomlinson MJ, East SJ, Barrat CL, Boltan AE, Cooke ID. Possible role of reactive nitrogen intermediates in leukocyte mediated sperm dysfunction. Am. J Reprod. Immunol 1992;2:89-92.

26. Weinberg JB, Doty E, Bonaventura J, Haney AF. Nitric oxide inhibition of human sperm motility. Fertil. Steril 1995;64:408-413.

27. Hellstrom WJ, Bell GM, Wang R, Sikka SC. Effect of sodium nitroprusside on sperm motility, viability and lipid peroxidation. Fertil. Steril 1994;61:1117-1122.

28. Roselli M, Keller PJ, Dubey RK. Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. Hum. Reprod. Update 1998;4:3-24.

29. Denhardt DT, Guo X. Osteopontin: a protein with diverse functions. The FASEB journal 1993;7:1476-1482.

30. Mazzali M, Kipari T, Ophascharoensuk V, Wesson JA, Johnson R, Hughes J. Osteopontin: a molecule for all seasons. Q. J. Med 2002;95:3-13.

31. Balercia G, Moretti S, Vignini A, Magagnini M, Mantero F, Boscaro M, Lamonica GR et al. Role of nitric oxide concentrations on human sperm motility. J Androl 2004;25:245-249.

32. Preedaa MG, Loganuthasamy K, Leela V, Pandiyan V. Study on correlation between expression levels of osteopontin gene and in vitro sperm characteristics in bovine semen. Journal of Entomology and Zoology Studies 2020;8:626-630.

33. Eriksson DW, Way AL, Chapman DA, Killian GJ. Detection of osteopontin on Holstein bull spermatozoa, in cauda epididymal fluid and testis homogenates, and its potential role in bovine fertilization. Reproduction 2007;133:909-917.

34. Arpita B, Saha S, Majumder GC, Dungdung SR. Optimum calcium concentration: a crucial factor in regulating sperm motility in vitro. Cell Biochemistry and Biophysics 2014;70(2):1177-1183.

35. Waheed MM, El-Bahr SM, Al-haidar AK. Influence of seminal plasma antioxidants and osteopontin on fertility of the Arabian horse. J Eq. Vet. Sci 2013;33:705-709.

36. Ruffolo Jr. RR, Giora ZF, Jacqueline AH, George P, Brain WM. Inflammatory cells and mediators in CNS disease. Taylor and Francis e-library, (Ed.) 2004.97.