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Electrophoretic profile of sperm membrane proteins and correlation with fresh semen characteristics in Assam Hill goat

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ABSTRACT

An experiment was carried out to study the electrophoretic profile of sperm membrane proteins in Assam Hill goat (AHG) bucks and their correlation with fresh semen characteristics. Ejaculates (64) consisting of 8 each from 8 bucks were collected by artificial vagina method. Each ejaculate was split into 2 equal parts. First part was used for fresh semen evaluation and the other part for protein study. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE) indicated 20 different protein bands with molecular weight ranging from 10 kDa to 75 kDa. Among the 20 proteins, only 6 proteins bands were consistently present in all 8 bucks (100%). Protein bands with molecular weight 10, 14, 22, 28, 55, 57 and 60 kDa showed significant positive correlation with certain parameters of the fresh semen, while 47 kDa protein band presented negative correlation with fresh semen characteristics in AHG bucks. In conclusion, the proteins showing significant positive correlation with fresh semen characteristics might serve to screen semen of AHG bucks for breeding programmes.

Key words: Correlation, Electrophoresis, Goat, Semen characteristics, Sperm membrane protein

Assam Hill goat (AHG) is an important goat germplasm distributed in Asom (North Eastern region of India) and its adjoining areas. It is essentially meat type animal with high prolificacy, but a poor milker. Selective breeding of this breed is mostly suggested for its genetic conservation and economic upliftment of the marginal farmers. Proteomics approaches are promising in the identification of proteins associated with sperm fertility. Soluble and structural seminal proteins have an important role in the sperm metabolism, which can influence the fertility as reported in bull (Killian *et al*. 1993), buffalo bull (Asadpour *et al*. 2007), boar (Lovercamp *et al.* 2007) and ram (Yue *et al*. 2009). Seminal proteins mediate the binding of sperm to oviductal epithelium and preserve membrane integrity, exerting inhibiting effects on the mitochondrial activity and metabolism to conserve energy needed until fertilization as well as to minimize the production of reactive oxygen species and lipid peroxidation of sperm membrane (Schoneck *et al*. 1996). The proteins are topographically reorganized into specific regions of the sperm surface (Wolf and Voglmayr 1984) and change the properties of the sperm membrane by binding to it and/or modifying the structure or the arrangement of the existing membrane molecules. Therefore, these proteins in semen can serve as potential fertility markers for selection of high fertile animals and ensure quality frozen semen. Surface membrane proteins in sperm facilitate communication between the sperm and the egg, regulating a set of hormonal, enzymatic and surface modifying events required for successful fertilization and embryo development (Yanagimachi 1994). The present study was undertaken to correlate the protein profile of AHG bucks with certain fresh semen characteristics.

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MATERIALS AND METHODS

Experimental animals and semen processing: The study was conducted with 8 AHG bucks aged 2 to 2.5 years maintained at All India Coordinated Research Project on Goat Improvement (Assam Hill Goat Unit), Assam Agricultural University, Burnihat, India with approval from the Institutional Animal Ethics Committee. The bucks were selected randomly and maintained under uniform dietary and managerial condition under intensive system of management in well-ventilated sheds. Semen was collected from each buck twice a week during the period from September 2013 to February 2014 with standard artificial vagina using a restrained doe as a mount. Each ejaculate

was split into 2 equal parts. One part was used for fresh semen evaluation and the other part was centrifuged at 700 × g for 15 min and stored at –80ºC until used for sperm membrane protein extraction. Ejaculates (64) comprising 8 from each buck were used for the study. Ejaculates were evaluated for initial sperm motility, sperm concentration by Neubauer counting chamber, live sperm by Eosin-Nigrosin staining technique as described by Blom (1977), sperm abnormality by differential interference phase contrast microscopy, Hypo-osmotic swelling test (HOST)-reacted sperm (Jeyendran *et al*. 1984) and intact acrosome by Giemsa staining technique (Watson 1975).

Sperm membrane protein extraction and estimation: Sperm pellets were washed 3 times with 1 ml tri sodium citrate (TC) buffer (pH 7.3) to remove traces of seminal plasma by centrifuging at 7000 × g for 5 min at 4°C. Sperm pellet was resuspended in 1 ml of TC buffer with 0.1 M phenyl methyl sulfonyl fluoride (PMSF) and 0.1% Triton X–100 and homogenized by passing 4–5 times through 5 ml syringe with 20 guage needle. Tubes containing sperm suspension were incubated in 4°C for 1 h and vortexed for 5 min at every 10 min interval. After incubation, the tubes were centrifuged at 12000 × g for 30 min at 4°C. Supernatant was transferred to another tube and protein concentration was estimated using protein estimation kit by Bradford macro method (Bradford 1976).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE): SDS-PAGE was performed as per Laemmli *et al.* (1970). Linear gradient gel (6–16%) was prepared using gradient mixer. Three ejaculates from each buck were pooled together to load the gel. Sperm protein samples were mixed with 2 × sample buffer with 1:1 ratio. Equal quantity of samples was loaded to all wells (10 µg/well) along with protein molecular weight marker (6.5–212 kDa). Gel was subjected to pre run for 2–4 min at 50 V prior to loading samples. After loading of samples, gel was run at 40 V for 10 min followed by 100 V until the sample front reached the bottom using Mini PROTEAN tetra cell. After completion of the run, gel was left at room temperature for 2 min. Finally, gels transferred into a rectangular plastic container and incubated with fixing solution (acetic acid 12%, ethanol 50% and formalin 0.05%) for overnight under constant agitation. Gels were stained with silver stain. After overnight fixing with the fixing solution, the gel was washed with 20% ethanol for 20 min. The ethanol solution was removed and enough volume of sensitizing solution was added and incubated for 2 min. Finally, cold silver staining solution was added to the gel and shaked in dark for 20 min to allow the silver ions to bind with proteins. The gel was rinsed briefly with distilled water and poured with developing solution. The reaction was stopped as soon as the desired intensity of the bands reached by adding terminating solution. Images were captured on gel doc using Gene snap image acquisition software and relative volume of protein were analyzed by Gene tools gel analysis software. Pearson's correlation of relative volume of protein bands with fresh semen characteristics was done statistically (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

The mean values of fresh semen characteristics in AHG, viz. ejaculate volume (ml), initial sperm motility (%), sperm concentration ($\times 10^6$/ml), live sperm (%), sperm abnormality (%), HOST-reacted sperm (%) and intact acrosome (%) recorded were 0.39±0.01, 77.97±0.73, 3201.00±143.78, 83.02±0.65, 7.66±0.73, 66.95±0.74 and 93.34±0.51, respectively.

The total protein extracted from sperm membrane in different AHG bucks ranged from 1.53±0.92 to 2.89±0.72 mg/ml with an overall mean of 2.06±0.18 mg/ml. Pearson's correlation indicated a highly positive significant ($P<0.01$) correlation ($r=0.639$) between the sperm concentration and protein yield.

SDS–PAGE indicated 20 different protein bands with molecular weight ranging from 10 kDa to 75 kDa. They were 10, 14, 16, 22, 24, 28, 30, 34, 38, 45, 47, 49, 50, 55, 57, 59, 60, 62, 70 and 75 kDa. Among the total 20 protein bands identified, only 6 bands were found consistently in all 8 bucks. They were of molecular weight 10, 14, 16, 49, 57 and 60 kDa. The protein with molecular weight 22, 30 and 38 Da showed frequency distribution of 87.50%. The 28, 45 and 47 kDa protein had frequency distribution of 75.00%. Frequency distribution of 62.50% was showed by 24, 34, 55, 59 and 70 kDa proteins followed by 37.50% by 50, 62 and 75 kDa proteins.

The correlations between the different sperm membrane proteins and characteristics of fresh semen characteristics in AHG bucks are presented in Table 1. Among all the protein bands detected, only 10, 14, 22, 28, 55, 57 and 60 kDa molecular weight proteins showed significant positive correlation while 47 kDa protein showed significant negative correlation with some of the parameters of fresh semen. On the other hand, 16, 24, 30, 34, 38, 45, 49, 50, 59, 62, 70 and 75 kDa molecular weight proteins did not show any significant correlation with characteristics of fresh semen.

The highly positive significant correlation between sperm concentration and protein yield observed in the study was in conformity with the findings of Macanovic *et al*. (2015) who recorded a significant positive correlation ($r=0.843$, $P<0.01$) between sperm/ml and protein expression of magnesium superoxide dismutase in seminal plasma of corresponding samples. The amount of sperm membrane present per ml of semen is dependent on the number of sperm/ml of semen. So, protein yield from sperm membrane/ml of semen is positively correlated with the sperm concentration/ml of semen. de Souza *et al*. (2007) did not find any significant correlation between total protein concentration of seminal plasma and semen characteristics including sperm concentration in the ejaculates of adult dogs. This variation might be due to different species and the sample used.

Out of 20 protein bands detected by SDS-PAGE, more

Table 1. Correlation of relative quantity of different molecular weight proteins in sperm membrane with characteristics of fresh semen in Assam Hill goat bucks

Protein mol. wt. (kDa)	Volume	Initial motility	Per cent livability	Sperm concentration	Per cent abnormality	Per cent HOST- reacted	Per cent Intact acrosome
10	0.903**	0.803*	0.845*	0.849**	-0.658	0.866**	0.835**
14	0.613	0.786*	0.697	0.473	-0.560	0.740*	0.716*
16	0.453	0.637	0.588	0.278	-0.177	0.631	0.682
22	0.450	0.872*	0.807*	0.787*	-0.582	0.861*	0.875*
24	-0.682	–0.710	–0.069	–0.437	0.039	–0.232	–0.118
28	0.616	0.892*	0.952**	0.564	–0.749	0.839*	0.855*
30	–0.030	0.2297	0.208	0.022	–0.014	0.179	0.235
34	–0.400	–0.470	–0.544	–0.324	0.475	–0.431	–0.353
38	–0.502	–0.465	–0.511	–0.397	0.319	–0.503	–0.547
45	–0.226	–0.045	–0.127	0.313	0.509	0.118	0.065
47	–0.504	–0.963**	–0.933**	–0.527	0.635	–0.874*	–0.932**
49	0.570	0.648	0.483	0.516	–0.127	0.713	0.693
50	–0.956	–0.994	–0.233	0.046	0.805	–0.230	0.836
55	0.819	0.898*	0.054	–0.095	0.495	0.728	0.819
57	0.567	0.922**	0.934**	0.622	–0.798	0.848**	0.882**
59	0.296	0.047	0.348	0.738	0.440	0.859	0.679
60	0.439	0.739*	0.795*	0.681	–0.424	0.767*	0.744*
62	0.023	0.207	0.996	0.934	–0.816	–0.852	–0.783
70	0.060	–0.671	0.647	0.632	–0.772	–0.102	–0.553
75	0.945	0.989	0.268	–0.010	–0.826	0.194	0.856

*P<0.05; **P<0.01.

numbers of bands were less than of 50 kDa, which was in agreement with Teixeira *et al*. (2009). They reported 16 protein bands with molecular weight ranging from 14 to 97 kDa in seminal plasma proteins of Anglo-Nubian goats while studying month-wise distribution of proteins in the seminal plasma. Yue *et al*. (2009) reported a total of 15 protein bands in ram seminal plasma by SDS–PAGE with molecular weight ranging from 15.13 to 116.20 kDa and found that low molecular weight proteins were predominant. Teixeria *et al*. (2009) reported that 14, 22, 24, 40, 50 and 66 kDa molecular weight proteins were distributed throughout the year in seminal plasma in Anglo-Nubian goats. However, in the present study, although 14, 22, 24 and 50 kDa proteins were found in all the bucks but 40 and 66 kDa proteins were not detected. Villemure *et al.* (2003) isolated and characterized gelatin–binding proteins from goat seminal plasma and observed presence of proteins with molecular weights of 14, 15, 20 and 22 kDa, which was in accordance with the present findings except for 15 and 20 kDa proteins. Karunakaran *et al*. (2012) reported protein bands of molecular weight ranging from 3 to 205 kDa in the sperm membrane of bull. They observed that fertility related heparin–binding protein with molecular weight 15/14 kDa was present in all the bulls. In the present study, 14 kDa protein was found in the sperm membrane of all AHG bucks.

The significant positive correlation of 10 kDa molecular weight protein with almost all fresh semen parameters studied indicated that 10 kDa protein might serve to screen AHG bucks with optimal semen quality. The 14 kDa sperm membrane protein detected in the present study was found to have significant correlation with initial sperm motility, per cent HOST-reacted sperm and per cent intact acrosomes. Nandi *et al*. (2012) did partial characterization of 14 kDa protein detected in goat spermatozoa and indicated that it might play critical role in the acrosomal membrane fusion event. The 28 kDa protein in the present study showed significant positive correlation with initial sperm motility, per cent livesperm, per cent HOST–reacted sperm and per cent intact acrosomes in fresh semen. Karunakaran *et al*. (2012) reported that bulls positive for 28–30 kDa heparin binding proteins in sperm membrane had higher chance of fertility. Since semen with higher percentage of sperm motility, live sperm, HOST–reacted sperm and intact acrosome was considered to be more capable of fertilizing the ova, therefore expression of 28 kDa protein in sperm membrane might also be related to higher fertility. The 47 kDa protein showed a significant negative correlation with the initial sperm motility, per cent livability, per cent HOST–reacted sperm and per cent intact acrosome. Therefore, 47 kDa molecular weight protein might serve as a potential marker for screening AHG bucks for sub-optimal semen quality. The 55 kDa molecular weight protein showed significant positive correlation with sperm motility, which was consistent with the report of Asadpour *et al.* (2007) in buffalo bulls who reported that 55 kDa protein had positive correlation with sperm viability in fresh semen. Cancel *et al*. (1997) also reported that 55 kDa protein was prevalent

in seminal plasma of high fertility bulls and they further characterized and confirmed that the 55 kDa polypepetide was osteopontin. In the present study, the 57 kDa molecular weight protein showed significant positive correlation with some of the important fresh semen characteristics. The 60 kDa protein in sperm membrane of AHG bucks showed significant positive correlation with initial sperm motility, per cent live sperm, per cent HOST–reacted sperm and intact acrosome in the fresh semen. Soubeyrand *et al*. (1997) purified phospholipase A_2 (PLA_2) from bovine seminal plasma and found it to be a 60 KDa protein. PLA_2 was also found on the plasma membrane as well as in the acrosome and post-acrosomal substance of ejaculated bull sperm (Weinman *et al.* 1986). This protein was reported to play an important role in the late maturational events of spermatozoa, the acrosomal reaction and sperm-egg fusion (Yuan *et al*. 2003).

The present findings of association of certain seminal proteins with the fresh semen characteristics are in agreement with the findings of earlier workers, viz. de Souza *et al*. (2007) in canine seminal plasma, Asadpour *et al*. (2007) in buffalo seminal plasma, Yue *et al*. (2009) in ram seminal plasma, Cheema *et al.* (2011) in dog sperm membrane and Sharma *et al*. (2015) in buffalo seminal plasma. However, there is no literature available regarding correlation of sperm membrane proteins in goat bucks with semen characteristics worldwide to compare the present values.

In conclusion, SDS-PAGE revealed 20 different proteins in the sperm membrane of AHG bucks ranging from 10 to 75 kDa in molecular weights. Proteins with molecular weight 10, 14, 22, 28, 55, 57 and 60 kDa showed significant positive correlation with certain parameters of the fresh semen, while 47 kDa protein presented negative correlation. The proteins showing significant positive correlation with the fresh semen characteristics may serve as potential marker to screen AHG bucks with optimal semen quality while selecting breeding programmes.

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