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Research Article

ELECTROPHORETIC PROFILE OF GOAT SEMINAL PROTEINS

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This experiment was carried out to study the electrophoretic properties of seminal proteins of Black Bengal buck semen. Semen ejaculates from nine Black Bengal bucks were used in the study. Semen ejaculates (n=10/buck) were collected by artificial vagina. Seminal plasma and sperm were separated by centrifugation. Seminal plasma proteins were precipitated by ice cold ethanol method and sperm proteins were extracted by Triton X detergent extraction method. Discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis was performed to assess the molecular weight of seminal proteins. A total of 10 protein bands in the molecular weight ranging from 17 to 180 kDa were found in the SDS-PAGE of seminal plasma proteins while nine bands of 17 kDa to 134 kDa were observed in sperm proteins. Seminal plasma proteins of molecular weight 75, 62-49, 20, 17 kDa and sperm proteins of 75, 20, 17 kDa were present in all the 9 bucks (100%). In conclusion variation among the bucks were noticed for presence of seminal plasma and sperm proteins and role(s) of seminal proteins on fertility needs to be studied so that they could be used as a marker for selection of breeding bucks.

Key words: Buck, Electrophoretic profile, Proteins, Semen

Animal husbandry and dairying are integral part of human life since the process of civilization started. Black Bengal breed of goat is found in West Bengal, Bihar, Jharkhand, Odisha, North Eastern India and Bangladesh. Bengal goat is very popular for its meat and skin quality, good adaptability and high fecundity. Most of the goat keepers are small, marginal farmers and landless labourers having flock size of 3 to 5 animals. In recent times, the availability of breeding bucks had become scarce due

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to castration and slaughter of male kids at early age for meat purposes (Khandoker *et al*., 2011). Artificial insemination (AI) technology has made possible the safe use of semen from selected sires in a large breeding female population. AI in goat is gaining popularity in several states of the country such as Tamil Nadu, Kerala, West Bengal, Assam and others for the last few years (Karunakaran *et al*., 2017). AI costs is reduced when compared to keeping bucks for breeding purpose in small farms and adaption AI practice provides the opportunity to access wide variety of bucks at relatively low cost. While adapting AI technology, accurate evaluation of the male fertility is important because it influences the reproductive potential of herd. Currently breeding soundness examination (BSE) is carried out before introducing a male into the semen collection programme. The variations in the fertility rate among the males were not addressed by the routine semen evaluation parameters (Larson and Miller, 2000). Attention is now being directed towards the assessment of other aspects of semen quality as predictors of fertility. Proteins present in the seminal plasma and sperm have been reported as markers of fertility (Karunakaran *et al*., 2012a, b). Seminal plasma, a complex mixture of secretions from testis, epididymis and accessory sex glands contained factors that modulated the fertilizing ability of sperm (Krishnan *et al*., 2015). Proteins such as osteopontin, prostaglandin D synthase, bovine seminal plasma proteins (BSP A1, A2, A3) and heparin binding proteins have been reported as indicators of bull fertility (Moura *et al*., 2006; Karunakaran and Devanathan, 2016; Krishnan *et al*., 2016). Agarwal *et al*. (2016) found differences in the seminal plasma protein profile of bucks with different semen freezability. Deori *et al*. (2018) reported that 60 kDa seminal protein had huge positive connection with parameters of the fresh semen, while 47 kDa protein bands gave negative relationship in semen qualities of Assam Hill Goat (AHG) bucks. From the investigation they indicated that the proteins demonstrating noteworthy positive connection with fresh semen qualities might serve to screen semen of AHG bucks. AI in Black Bengal goat is gaining popularity in West Bengal and adjoining states, and it is essential to identify the bucks with better semen quality as a donor for semen collection. Presently only few studies were available on the characteristics of Black Bengal buck semen and its preservation (Konyak *et al*., 2018; Karunakaran *et al*., 2019) and there is no report available on electrophoretic properties of Black Bengal buck semen. Electrophoretic profile of seminal plasma and sperm might serve as a tool for identifying the buck semen quality and the current experiment was carried out to study the electrophoretic profile of seminal plasma and sperm proteins of Black Bengal buck semen.

MATERIALS AND METHODS

The present study was carried out at ICAR-

National Dairy Research Institute (NDRI), Eastern Regional Station, Kalyani, West Bengal, India. The work was carried out after due approval of Institute Research Committee (IRC) of ICAR-NDRI, Karnal. Nine Black Bengal bucks (*Capra hircus*) were used in the study. Bucks were given with the following identification numbers 46, 48, 51, 52, 53, 55, 57, 59 and 67. All the experimental animals were clinically normal and donated semen of acceptable quality. Semen ejaculates were collected twice a week using artificial vagina. A total of 10 ejaculates form each buck and a total of ninety ejaculates were used in the study.

The seminal plasma and sperm cells were separated immediately after collection by centrifugation (560g for 10 min at 5°C). The sperm cells were washed with 2 mL of Tris calcium chloride (TC) buffer (40 mM Tris, 2 mM $CaCl_2$ and 0.01% sodium azide, pH 7.3) by centrifugation (560g for 5 min at 5°C) to remove the left-over seminal plasma, if any. The sperm cells were re-suspended with 1 mL of TC buffer containing protease inhibitor (1 mM phenyl methyl sulfonyl fluoride) and washed thrice by centrifugation (560g for 10 min at 5°C). The sperm pellet and the seminal plasma were stored at -20°C until extraction of protein (Karunakaran and Devanathan, 2016). Proteins in the seminal plasma were precipitated by ice-cold ethanol method (Asadpour *et al*., 2007). Sperm proteins were extracted by Triton X detergent extraction method as described by Nass *et al*. (1990). Discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) with 12% resolving gel and 5% stacking gel to characterize the proteins based on molecular weight. 100 µg of seminal plasma and sperm protein samples were used to load the gel. The gels were stained with Coomassie brilliant blue and de-stained in a mixture of methanol (25%) and acetic acid (10%) in distilled water. The apparent molecular mass was determined by using molecular weight markers and Gel Documentation and Analysis System and the gels were stored in acetic acid (7%).

RESULTS

Electrophoretic profile of seminal plasma proteins: A total of 10 protein bands with molecular weight ranging from 17 kDa to 180 kDa were observed in the SDS-PAGE of seminal plasma proteins (Table 1 and Fig. 1). The protein bands noticed in the gel were 180-136 kDa, 134 kDa-01 kDa, 75 kDa, 62 kDa-49 kDa, 48 kDa, 47 kDa-36 kDa, 35 kDa, 34 kDa-25 kDa, 20 kDa and 17 kDa. Out of these 10 bands, proteins of 75 kDa, 62 kDa-49 kDa, 20 kDa and 17 kDa were present in all the 9 bucks (100%), while the other protein bands such as 180 kDa-136 kDa, 134 kDa-101kDa, 48kDa, 47 kDa-36 kDa, 35 kDa and 34 kDa-25 kDa were present only 55.55%, 55.55%, 33.33%, 44.44%, 44.44% and 44.44% of the bucks screened.

Electrophoretic profile of sperm proteins: Electrophoretic profile of sperm

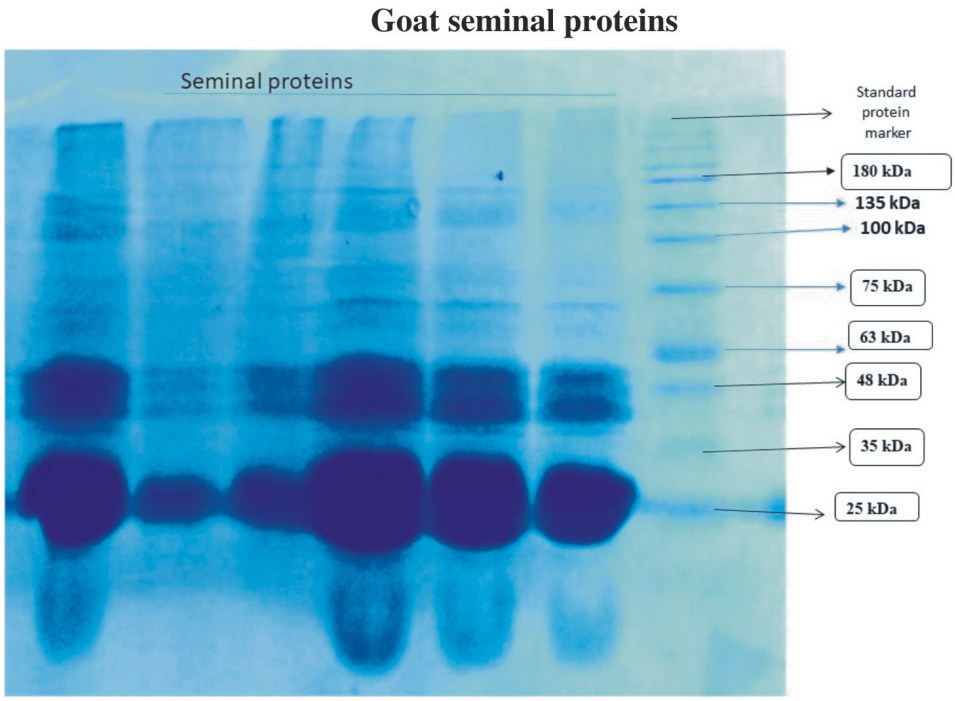

Fig. 1. Seminal plasma proteins of Black Bengal buck semen

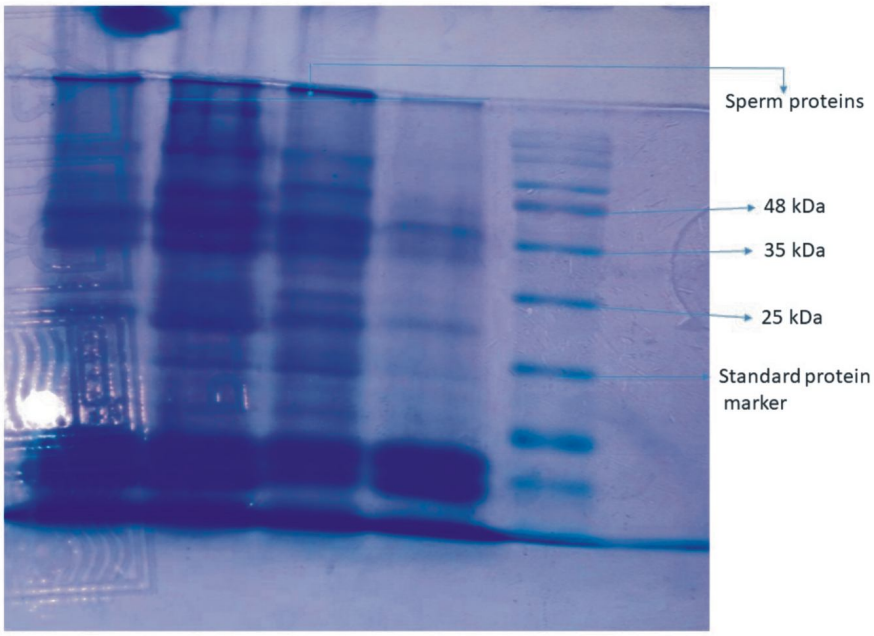

Fig. 2. Sperm proteins of Black Bengal buck semen

proteins of Black Bengal buck semen revealed presence of nine bands starting from 17 kDa to 134kDa (Table 2 and Fig. 2). Proteins with molecular weight 75 kDa, 20 kDa and 17 kDa were present in all the bucks screened (100%), while the other proteins such as 134 kDa-101 kDa, 100 kDa, 62 kDa-49 kDa, 63kDa, 47 kDa-36 kDa and 35 kDa were present only in 44.44%, 55.55%, 66.66%, 44.44%, 55.55% and 33.33% of the bucks. The protein band of molecular weight 134 kDa-101 kDa was present in buck number 46, 55, 57, 59 and absent in buck number 48, 51, 52, 53 and 67 with overall presence of 44.44%. The protein of molecular weight 100 kDa was present in bucks 46, 48, 51, 52, 53, 57, 67 and absent in buck 55 and 59 with overall presence of 77.77%. The protein band of molecular weight 75 kDa was present in all the bucks. The protein band of molecular weight 62 kDa-49 kDa was present in bucks 46, 48, 51, 52, 53, 67 and absent in bucks 55, 57, 59 with overall presence of 66.66%. The 63 kDa protein was present in buck number 52, 53, 55, 57, 59 and absent in 46, 48, 51, 67 with overall presence of 44.44%. The protein band of molecular weight 47 kDa-36 kDa was present in buck number 46, 51, 55, 57, 59 and absent in buck number 48, 52, 53, 67 with overall presence of 55.55%. The protein band of molecular weight 35 kDa was present in buck number 46, 51, 57, and absent in buck number 48, 52, 53, 55, 59, 67 with overall presence of 33.33%. The protein bands of molecular weight 20 kDa and 17 kDa were present in all the bucks.

Table 1. Electrophoretic profile of seminal plasma proteins of Black Bengal bucks assessed by SDS-PAGE

Protein molecular weight (kDa)	Buck number									Overall presence of protein no. (%)
	46	48	51	52	53	55	57	59	67	
180-136	-	+	+	+	-	-	+	-	+	5 (55.55%)
134-101	+	-	+	-	+	+	-	+	-	5 (55.55%)
75	+	+	+	+	+	+	+	+	+	9 (100%)
62-49	+	+	+	+	+	+	+	+	+	9 (100%)
48	+	-	-	-	-	+	-	+	-	3 (33.33%)
47-36	-	+	-	-	+	-	+	+	-	4 (44.44%)
35	+	-	+	-	-	+	-	+	-	4 (44.44%)
34-25	+	-	-	-	+	+	-	-	+	4 (44.44%)
20	+	+	+	+	+	+	+	+	+	9 (100%)
17	+	+	+	+	+	+	+	+	+	9 (100%)

(Figures in parentheses indicate percentage to total, - absence, + presence)

Table 2. Electrophoretic profile of sperm proteins of Black Bengal bucks assessed by SDS-PAGE

Protein molecular weight (kDa)	Buck number									Overall presence of protein no. (%)
	46	48	51	52	53	55	57	59	67	
134-101	+	-	-	-	-	+	+	+	-	4 (44.44%)
100	+	+	+	+	+	-	+	-	+	5 (55.55%)
75	+	+	+	+	+	+	+	+	+	9 (100%)
62-49	+	+	+	+	+	-	-	-	+	6 (66.66%)
63	-	-	-	+	+	+	+	+	-	4 (44.44%)
47-36	+	-	+	-	-	+	+	+	-	5 (55.55%)
35	+	-	+	-	-	-	+	-	-	3 (33.33%)
20	+	+	+	+	+	+	+	+	+	9 (100%)
17	+	+	+	+	+	+	+	+	+	9 (100%)

(Figures in parentheses indicate percentage to total, - absence, + presence)

DISCUSSION

Electrophoretic profile of seminal plasma proteins: SDS-PAGE of seminal plasma proteins revealed presence of 10 protein bands with molecular weight ranging from 17 kDa to 180 kDa in Black Bengal buck semen. Seshagiri and Pattabiraman (1991) observed 8 protein fractions in the seminal plasma of Jersey and Sindhi bulls, and 7 protein fractions in crossbred bulls. Kulkarni *et al*. (1996) observed protein fractions with molecular weight ranging from 11 kDa to 92 kDa in cattle seminal plasma. Arangasamy *et al*. (2005) and Harshan *et al*. (2006) reported a total of 18 and 19 protein bands respectively, in buffalo seminal plasma with molecular weight ranging from 3 kDa to 205 kDa. Teixeira *et al*. (2002) reported 16 protein bands with molecular weight ranging from 14 kDa to 97 kDa in seminal plasma proteins of Anglo-Nubian goats while studying month-wise distribution of proteins in the seminal plasma. Further they found that, the protein bands of 14 kDa, 22 kDa, 24 kDa, 40 kDa, 50 kDa and 66 kDa were distributed throughout the year in seminal plasma in Anglo-Nubian goats. Yue *et al*. (2009) reported a total of 15 protein bands in ram seminal plasma by SDS-PAGE with molecular weight ranging from 13 kDa to 116.20 kDa and found that low molecular weight proteins were predominant. The reason for the difference in the number of protein bands in the

present study when compared with other reports might be the species/breed differences and method of protein isolation. In the present study, the seminal plasma proteins were precipitated by ice cold ethanol method while Arangasamy *et al*. (2005) and Harshan *et al*. (2006) used the seminal plasma directly for SDS- PAGE.

Electrophoretic profile of sperm proteins: Electrophoretic profile of sperm proteins of Black Bengal buck semen revealed presence of nine bands starting from 17 kDa to 134 kDa. Some of the proteins (17 kDa, 20 kDa and 75 kDa) were present in all the bucks while variations among the bucks were noticed for the presence of other proteins. Karunakaran (2011) recorded sperm proteins such as 15/14 kDa, 28 kDa, 26 kDa and 55 kDa in 100%, 95.45%, 63.64% and 50.00% of the dairy bulls screened. Nauc and Manjunath (2000) reported 3 protein fractions of BSP- A1/ A2, BSP A3 and BSP 30 in the sperm membrane by SDS-PAGE and radio immuno-assay. They also reported a reduction in the concentration of these membrane proteins during cryopreservation. Deori *et al*. (2018) screened the sperm membrane proteins in Assam Hill Goat (AHG) and they had observed 20 different protein bands with molecular weight ranging from 10 kDa to 75 kDa. Among the total 20 protein bands identified, they found only 6 bands such as 10 kDa, 14 kDa, 16 kDa, 49 kDa, 57 kDa and 60 kDa were consistently present in all 8 bucks. Further, they observed that the protein with molecular weight 22 kDa, 30 kDa and 38 kDa showed frequency distribution of 87.50%, 28 kDa, 45 kDa and 47 kDa proteins had frequency distribution of 75.00%. The frequency distribution of 62.50% was showed by 24 kDa, 34 kDa, 55 kDa, 59 kDa and 70 kDa proteins followed by 37.50% by 50 kDa, 62 kDa and 75 kDa proteins in AHG in that study. Soubeyrand *et al*. (1997) purified phospholipase A2 (PLA2) from bovine seminal plasma and found it to be a 60 KDa protein. PLA2 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).

As like that of in bovine and other species, exploration on the influence of seminal proteins on sperm functions and fertilizing ability and identification of fertility associated proteins in the buck semen will be useful for selection of breeding bucks for AI purpose.

Conflict of interest: Authors declare that there is no conflict of interest regarding the present research work.

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