SEMINAL PLASMA AND SPERM MEMBRANE PROTEINS OF BUFFALO AND CATTLE BULLS: A COMPARATIVE STUDY

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ABSTRACT

The seminal plasma proteins and sperm membrane proteins of six each Bhadawari buffalo and Hariyana cattle were isolated by using the protein isolation kits and protein profiling were carried out by 1-D SDS-PAGE.

Keywords: buffalo, cattle, SDS-PAGE, seminal plasma protein, sperm membrane protein

INTRODUCTION

Seminal plasma is complex fluid containing a wide variety of both organic and inorganic components, among which proteins are an important part of the high-molecular-weight substances. The protein composition of mammalian seminal plasma varies in species and has important effect on sperm functions such as sperm motility (Henricks *et al*., 1998), viability (Brandon *et al*., 1999) and freezability (Asadpour *et al*., 2007), sperm capacitation and fertilization (Rodriguez *et al*.,1998) and also serve to protect sperm from damage or to maintain their longevity which were evident from the correlation observed among semen characteristic and seminal plasma proteins reported by Sharma *et al*. (2015).

Besides proteins of seminal plasma, sperm surface proteins reported to have important role in recognition of zona proteins for binding to zona and plasma lemma of ovum and also in acrosome reaction for successful fertilization (Jagadish *et al*., 2005). It has been reported that, the loss of integrity of the sperm plasma membrane is frequently associated with infertility in male, despite normal semen parameters (Rajeev and Reddy, 2004).

Looking to the importance of buffalo in India, it becomes necessary to understand the structural and functional attributes of the buffalo spermatozoa and its comparison with that of cattle which will help in better understanding of molecular features that make the buffalo sperm less fertile than cattle. In the scenario of climate change, indigenous animals are of choice for better dissemination of gemplasm and it urges the science to have a picture of the protein profiles of the semen as a whole to make it more suitable for artificial insemination. Keeping in this mind the present study was designed for comparative evaluation of protein profiles of seminal plasma and sperm membrane in buffalo and cattle bull semen.

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MATERIALS AND METHODS

Experimental animals

Semen samples were collected by an artificial vagina from six each sexually mature Haryana cattle and Bhadawari buffalo bulls (2 to 4 years old) from the District Dairy Demonstration farm of College of Veterinary Science and Animal Husbandry, Mathura. The animals were maintained in nearly identical nutritional and management conditions throughout the period of study. The animals utilized were clinically healthy and regularly dewormed and vaccinated against common ailments.

Semen evaluation

Ejaculates were collected twice a week from each bull in morning hours (8:00 to 9:00 AM) and total six ejaculates were collected from each bull during the study period. Immediately after collection, semen volume was determined with a graduated plastic tubes and concentration of spermatozoa (million/ml) was determined by automatic sperm counter.

Preparation of seminal plasma

The seminal plasma was prepared by centrifugation and protein extraction was carried out by using the Triprep extraction kit (Fisher Scientific). Fresh semen was centrifuged at 5000 rpm for 10 minutes. The total protein was estimated by spectrophotometric method at 280 nm (protein's absorbance at 280 nm). After the estimation of total protein, the protein extraction was carried out as per the protocol of the kit. Proteins were recovered by centrifugation at 10,000 rpm for 10 minutes, re-suspended in phosphate buffered saline (PBS) and stored at -20^{o}C until further analysis of seminal plasma proteins. The SDS-PAGE analysis was done after one day of sample processing.

Preparation of sperm membrane extract

The sperm membrane proteins were extracted by method described by Cheema and Babbar (2008) with some modification. The sperm pellets were washed three times in PBS (pH 7.4) and resuspended in lysis buffer containing 1.5% (w/v) Tris [hydroxylmethyl] aminomethane buffer (pH 6.8), 20% (w/v) sucrose, 10% (w/v) SDS, 5% (v/v) β-mercaptoethanol and 0.05% (w/v) bromophenol blue (3'3"5'5" tetrabromophenol-sulfonephthalein). The mixture was kept in boiling water bath for 5 minutes and centrifuged at 5000 rpm for 10 minutes and protein extraction was carried out by using the Triprep extraction kit (Fisher Scientific). Proteins were recovered by centrifugation at 10,000 rpm for 10 minutes, re-suspended in phosphate buffered saline (PBS) and stored at -20^{o}C until further analysis by SDS-PAGE.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed for separation and determination of molecular weight of seminal plasma and sperm membrane proteins. Proteins samples were subjected to SDS-PAGE as per method of Laemmli (1970), using a 12% polyacrylamide gel. The relative molecular weights were determined by using the (broad range molecular weight markers of Merck, Germany), Gel documentation and analysis system (Gel-doc. Model- Alpha imager TM1220, Alpha Innotech Corporation, USA).

Image analysis and statistical analysis

Gel images were analysed to determine molecular weight and relative protein content

using the Gel doc system. Data were analyzed using SPSS software program (SPSS version 16.0 for Windows). The results were presented as mean ± standard error of mean (S.E.M) and analysis of significance was attributed at P<0.05.

RESULTS AND DISCUSSION

The results of semen characteristics and protein concentration of seminal plasma and sperm membrane extract of buffalo and cattle bulls are depicted in Table 1 and 2, respectively. The statistical analysis of the result did not show significant difference in ejaculate volume and sperm concentration in buffalo and cattle bull semen while protein concentration of seminal plasma as well as sperm membrane extract showed significant difference (P<0.01) in buffalo and cattle bull semen. The lower ejaculate volume and sperm concentration was observed in buffalo compared to cattle bull in present study corroborate the reports of Khalek *et al*. (2008) in Nilliravi buffalo and Holestein cattle bulls. In spite of the insignificance of differences between the two species in semen characteristics, there was tendency of lower values of ejaculate volume and sperm concentration in buffalo than cattle bulls (Table 1). Variation in semen volume and sperm concentration might be due to differences in frequency of collection, season, nutrition, management, genetics, reproductive health status and age of bulls (Soderquist *et al*., 1992). Variations can also be due to skill of semen collector/attendant and temperature of AV.

The seminal plasma protein concentration in present investigation showed a significant difference (P<0.01) between buffalo and cattle bull semen. Comparable seminal plasma protein concentration was observed by Arangasamy *et al*. (2005) and Singh *et al*. (1995) in cattle and Nendre (2007) in Surti and Dhanju *et al*. (2001) in Murrah buffaloes. The concentration of sperm membrane extract protein showed significant difference (P<0.05) in buffalo and cattle bull spermatozoa. The concentration of membrane protein extracted from sperms of buffalo and cattle in present study was observed lower than the reports of Cheema and Babbar (2008) in crossbred cattle and Dhanju *et al*. (2001) in buffalo bulls. The variation in the concentration of extracted proteins may be due to genetic variability in the animals or may be due to variations in extraction methods used by different authors.

Table 2 shows the protein profile of seminal plasma and sperm membrane protein of buffalo and cattle bulls. The electrophoretogram of buffalo semen revealed 24 protein bands ranging between 6.0 to 200 kDa in seminal plasma and 14 protein bands ranging from 16.0 kDa to 205 kDa in sperm membrane extract proteins. Nine protein bands of molecular weight 20, 26.5, 36.5, 38, 44, 66, 70, 72 and 84 kDa were observed common in seminal plasma and sperm membrane of buffalo which indicated that these proteins are structural as well as secretory in nature. Asadpour *et al.* (2007) and Sharma *et al*. (2014) revealed 25 protein bands on SDS - PAGE analysis of seminal plasma in buffalo bulls, and most of the bands were observed to be comparable with results of present study. Selvaraju *et al*. (2010) and Dhanju *et al*. (2001) also reported protein bands of comparable molecular weight in seminal plasma and sperm membrane of buffalo spermatozoa, respectively. The reports of these authors simulate the findings of present study.

SDS-PAGE of cattle seminal plasma revealed 13 protein bands ranging from 6.5 kDa to 204 kDa. The proteins of comparable molecular weight were reported by Jobim *et al*. (2004) and Bellin *et al*. (2012) in seminal plasma of different

Table 1. Mean semen characteristics of Haryana cattle and Bhadawari buffalo bulls.

Semen attributes	Cattle	Buffalo
Semen volume (ml)	4.038±0.22[a]	2.958±0.18[a]
Sperm concentration (10^6/ml)	1736.944±60.46[a]	1678.05±86.68[a]
Seminal plasma protein (gm/dl)	7.86±0.34[a]	4.63±0.16[b]
Sperm membrane protein (mg/10^9sperms)	2.81±0.25[a]	4.42±0.63[b]

Means bearing at least one common superscript alphabet in one parameter did not differ significantly ($P \geq 0.05$), otherwise significant at 5% level ($P < 0.05$).

Table 2. SDS-PAGE protein profile of seminal plasma and sperm membrane of cattle and buffalo bull semen (kDa).

S. No.	Cattle		Buffalo	
	Seminal Plasma	Sperm Membrane	Seminal Plasma	Sperm Membrane
1	6.5	6.5	6.5	16
2	8.5	8.5	12.5	20
3	18.5	12	18.5	22.5
4	26.5	22.5	20	26.5
5	43	25	24	36.5
6	66	26.5	26.5	38
7	70	32.5	28	42
8	75	34.5	32	44
9	84	38	35	66
10	88	43	36.5	70
11	96	48	38	72
12	160	58	40.5	84
13	204	66	44	174
14		69	46	205
15		70	48	
16		84	60	
17		174	66	
18			70	
19			72	
20			84	
21			86	
22			96	
23			184	
24			200	

breeds of cattle. Bellin *et al*. (1996) reported proteins of 75, 84, 66 kDa molecular weight in seminal fluid of cattle bulls. Fernandez *et al*. (2009) reported eight bands ranging from 15 to 63kDa. Out of these eight bands, two similar proteins (22 and 25kDa) reported by Fernandez *et al*. (2009) in *Bos taurus taurus* bulls simulates the findings of present study. The cattle sperm membrane proteins revealed 17 protein bands ranging between 6.5 to 174 kDa. Five proteins of comparable molecular weight (84, 66, 48, 24 and 12 kDa) were reported by Bellin *et al*. (1996) in sperm membrane of vasectomised bulls. Cheema *et al*. (2011) reported proteins of comparable molecular weight (10, 25, 40, 65, and 70 kDa) in sperm membrane of buck. The reports of these authors simulate the findings of present study. Proteins observed other than these proteins in present study may be specific to cattle bulls. Seven protein bands of molecular weight 6.5, 8.5, 26.5, 43, 66, 70, and 84 kDa were observed common in seminal plasma and sperm membrane of cattle which indicated that these proteins are structural as well as secretory in nature.

The protein bands of molecular weight 6.5, 10 and 12 kDa in cattle and 26.5, 36.5 and 174 kDa in buffalo sperm membrane were observed to be most abundant proteins. Seven protein bands (6.5, 18.5, 26.5, 66, 70, 84 and 96 kDa) in seminal plasma and seven protein bands (22.5, 26.5, 38, 66, 70, 84, and 174) in sperm membrane were detected similar in both the species in present investigation. Proteins detected other than these proteins in present study may be said to be species specific proteins.

In conclusion, not all the seminal plasma and sperm membrane proteins are similar in buffalo and cattle only seven proteins are observed to be similar in seminal plasma and sperm membrane of buffalo and cattle semen. The difference in protein profile indicates species specific variations may account for their variable fertility and freezability of buffalo and cattle semen. Further studies may be carried out by employing the modern tools to characterize these proteins and their putative role in the regulation of variable fertility and freezability in buffalo and cattle bull semen. This will help in the formulation of extenders during semen processing so as to increase the post thaw quality of the buffalo semen.

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