



Trade Science Inc.

ISSN : 0974 - 7532

Volume 6 Issue 12

*Research & Reviews in*

**BioSciences**

*Short Communication*

RRBS, 6(12), 2012 [396-398]

## **A comparison of proteins in seminal plasma of buffalo bulls and cattle bulls**

**Veenu Minhas, Hari Mohan Saxena\***

Department of Veterinary Microbiology, College of Veterinary Science Guru Angad Dev Veterinary and Animal Sciences  
University, Ludhiana – 141004, (INDIA)  
E-mail : hmsaxena@yahoo.com

### **ABSTRACT**

The total protein concentration in seminal plasma from cattle bulls was found to be very significantly higher ( $p < 0.01$ ) than that of buffalo bulls. However, native – PAGE profiles of seminal plasma revealed two extra proteins (p16 and p14) in case of buffalo seminal plasma only, in addition to the three proteins (p43-45, p34 and p29) common to seminal plasma from both, buffalo and cattle bulls. Further studies are needed to understand the exact physiological roles and significance of the extra proteins in buffalo. © 2012 Trade Science Inc. - INDIA

### **KEYWORDS**

Seminal plasma;  
Protein profile;  
Protein concentration;  
Buffalo semen;  
Cattle semen.

### **INTRODUCTION**

Seminal plasma is the fluid in which spermatozoa are suspended and is composed of the secretions of male accessory sex glands. It has immunosuppressive properties<sup>[1]</sup>. Studies on seminal plasma proteins in bovines are scanty. They could help in understanding the immunochemical aspects of reproduction. The present studies were undertaken to determine the total protein concentration as well as to analyze by native PAGE the profiles of proteins in seminal plasma of cattle and buffalo bulls.

### **MATERIALS AND METHODS**

Fresh undiluted semen samples were collected from nine healthy cattle bulls and six healthy buffalo bulls from the Veterinary College Dairy Farm, Ludhiana. Semen was immediately centrifuged at 2500 rpm for 5 min.

Supernatant seminal plasma was collected and stored at  $-20^{\circ}\text{C}$  until used.

#### **Estimation of total proteins**

The method for protein estimation reported by Reinhold<sup>[2]</sup> was followed. The sample (0.1 ml) was mixed with 4.9 ml of 0.75N NaOH. A reagent blank tube containing 5 ml of 0.75N NaOH was also included. One ml of Biuret reagent was quickly added to each tube, mixed thoroughly and was allowed to stand at room temperature for 20 min. for development of color. The Optical Density of the unknown sample against the reagent blank was read at 545 nm.

#### **Native polyacrylamide gel electrophoresis (PAGE)**

Samples of seminal plasma were analyzed electrophoretically using Mini Slab Gel System (Genei, Bangalore). Discontinuous PAGE gels which consisted of resolving gels and stacking gels (7% and 2.5%, respectively) were prepared. After loading the samples in the

**Short Communication**

wells, they were subjected to electrophoresis for 15 min. at 120 V and one hour at 200 V. Along with the samples, low (43 kDa, 29 kDa, 20 kDa, 14.3 kDa, 6.5 kDa and 3 kDa) or medium (97.4 kDa, 68 kDa, 43 kDa, 29 kDa and 14.3 kDa) range standard molecular weight markers (Genei, Bangalore) were also run in each gel.

The gels were stained for one hour in staining solution (0.5% Coomassie Blue in 10% Acetic Acid and 40% Methanol) and then destained in the destaining solution (70 ml of Acetic Acid dissolved in distilled water to make the volume to one litre) till the background was clear.

**RESULTS AND DISCUSSION**

**Levels of total proteins in seminal plasma of cattle and buffaloes**

Total protein levels were estimated in samples of seminal plasma of nine normal healthy cattle bulls and six buffalo bulls. The mean value ( $\pm$  S. D.) of total proteins was  $5.97 \pm 0.668$  g/100 ml ( $59.7 \pm 6.68$  mg/ml) in case of cattle bulls and  $2.22 \pm 0.480$  g/100 ml ( $22.2 \pm 4.80$  mg/ml) in case of buffalo bulls (TABLE 1), respectively. The difference between the two values was very significant ( $p < 0.01$ ).

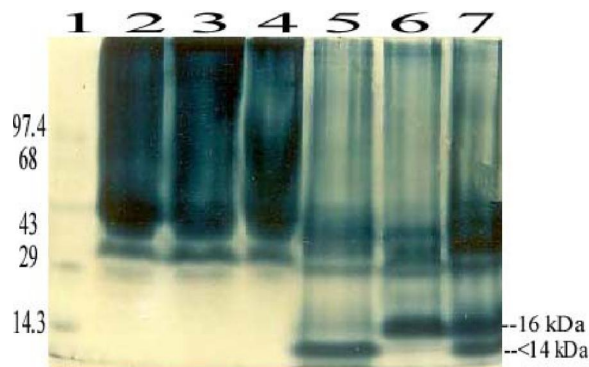
**Native – PAGE protein profiles**

Three main protein bands of 43 kDa, 34 kDa and 29 kDa, respectively, were detected in the Native – PAGE protein profiles of seminal plasma of cattle bulls.

Five main protein bands of molecular weights 43 kDa, 34 kDa, 29 kDa, 16 kDa and <14 kDa, respectively, were detected in the Native – PAGE protein profiles of seminal plasma of buffalo bulls (Figure 1, TABLE 2).

In our study, the mean value of total proteins in seminal plasma in cattle was found to be very significantly ( $p < 0.01$ ) higher than that of buffalo. We have not come across any report in the available literature on comparison of total proteins in seminal plasma of cattle and buffalo bulls. The level of total proteins in seminal plasma from normal healthy cattle bulls observed in our study was comparable to that reported earlier<sup>[3]</sup>. They found the total protein concentration to range from  $75.66 \pm 4.18$  to  $81.76 \pm 3.18$  mg/ml, variations were ascribed to

differences in age, breed or sperm concentration in the seminal plasma. However, the total protein levels observed in our study on indigenous bulls were comparatively lower than those reported<sup>[4]</sup> in cross-bred bulls. They reported an average value of 10.28 g/100 ml.



**Figure 1 :** Native – PAGE protein profiles of seminal plasma of cattle and buffalo bulls (from L to R) lane 1– molecular weight markers, lanes 2 to 4 – cattle seminal plasma, lanes 5 to 7 - buffalo seminal plasma.

**TABLE 1 :** Levels of proteins in seminal plasma of cattle bulls and buffalo bulls.

S. no.	Cattle bulls		Buffalo bulls	
	Animal no.	Protein concentration (mg/ml)	Animal no.	Protein concentration (mg/ml)
1	52	50.9	1413	15.8
2	812	62.0	1434	18.7
3	816	73.7	1437	23.9
4	831	65.6	1451	30.4
5	838	58.0	1556	25.0
6	847	50.7	1555	19.5
7	787	59.8		
8	727	57.9		
9	712	58.5		
<b>Mean <math>\pm</math> SD</b>		<b>59.7 <math>\pm</math> 6.6</b>	<b>Mean <math>\pm</math> SD</b>	<b>22.2 <math>\pm</math> 4.8</b>

**TABLE 2 :** Native – PAGE protein profiles of seminal plasma of bovines.

S. no.	Protein (kDa)	Presence in seminal plasma	
		Cattle	Buffaloes
1	43 – 45	+	+
2	34	+	+
3	29	+	+
4	16	-	+
5	<14	-	+

It was paradoxical to find in our study that although the total protein levels in seminal plasma of buffalo bulls were significantly lower than those in cattle bulls, the

## Short Communication

protein profiles of seminal plasma revealed two additional protein bands in buffaloes which were absent in cattle bulls. We are not aware of any such report in the available literature. A protein was purified and characterized from bovine seminal fluid and named seminal plasmin (SPLN)<sup>[5]</sup>. SPLN is present in the auxillary sex glands while its biosynthesis occurs in the seminal vesicle<sup>[6]</sup>. Proacrosin, the zymogen form of serine protease acrosin, is located within the acrosomal vesicle of mammalian spermatozoa and is involved in fertilization process<sup>[7]</sup>. A drosophila male accessory gland protein, peptide ACP/6A, which belongs to the serpin superfamily of proteins, was found in the female reproductive tract immediately after mating<sup>[8]</sup>. The exact significance of the additional proteins in seminal plasma of buffalo bulls identified in our study is not known. Further study and characterization could shed some light on their roles in the physiology of reproduction in buffaloes.

### CONCLUSION

The total protein concentration in seminal plasma from cattle bulls was found to be very significantly higher ( $p < 0.01$ ) than that of buffalo bulls. However, native – PAGE profiles of seminal plasma revealed two extra proteins (p16 and p14) in case of buffalo seminal plasma only. Three proteins (p43-45, p34 and p29) were found to be common to seminal plasma from both, buffalo and cattle bulls.

### ACKNOWLEDGEMENTS

We are thankful to the staff of the College Dairy Farm for providing the samples of bovine semen.

### REFERENCES

- [1] R.Rozansky; Lab.Clin.Med., **34**, 1526–1529 (1949).
- [2] J.G.Reinhold; In: Standard methods of clinical chemistry, M.Reiner (Ed); Academic Press, New York, **1**, 88 (1953).
- [3] R.A.Luthra; Ind.J.Dairy Sci., **47**, 524–526 (1994).
- [4] K.V.Patel; Indian J.Ani.Sci., **59**, 524–529 (1989).
- [5] E.S.P.Reddy, P.M.Bhargava; Nature, **279**, 725–728 (1979).
- [6] N.Sitaram, R.Nagraj; BioEssays, **17**, 415–421 (1995).
- [7] K.Nayernia; Biochem.Biophy.Res.Comm., **202**, 88–93 (1994).
- [8] S.Colman, B.Drahn, G.Petersen, J.Stolorov, K.Kraus; Insect Biochem.Mol.Biol., **25**, 203–207 (1995).