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Original Research Article

Relationship of Seminal Plasma Protein with Semen Characteristics of Murrah Buffalo Bulls

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Buffalo, Proteins, Seminal plasma, Sperm characteristics

A B S T R A C T

Present study was carried out to assess the protein profile of Murrah buffalo seminal plasma by using SDS-PAGE and their relationship with the semen characteristics. Semen samples were collected by a bovine artificial vagina from Murrah buffalo bulls maintained at College of Veterinary Science, N. D. University of Agriculture & Technology, Kumarganj, Faizabad. Semen characteristics were measured using standard procedures. Seminal plasma was separated after centrifugation of semen at 5000 rpm for 10 min. The supernatants were collected and precipitated using nine volumes of cold ethanol, and the proteins recovered. These seminal plasma proteins were separated on a 12% SDS-PAGE using standard procedure. A total of 22 protein bands could be identified by the fractionation of seminal plasma protein on the SDS-PAGE; ranging from 14.1 kDa to 88 kDa. Some of these bands were more prominent in some samples. A protein fraction of 25.8 kDa was significantly correlated with progressive motility of sperm in frozen samples. The semen samples that had greater sperm viability showed a prominent fraction of 58 kDa protein in both fresh and frozen samples.

Introduction

Artificial insemination in cattle and buffalo is the common practice; and to keep the success at high, the quality of the semen must be good. Though efforts have been given by using different extenders to improve keeping qualities of frozen semen, still success is not satisfactory. Seminal plasma, which is a complex mixture of secretions from testis, epididymis and accessory glands, contains factors that modulate the fertilizing ability of sperm (Henalut et al, 1995). Bovine seminal plasma contains a family of major proteins, designated BSP-A1/-A2 and BSP-A3, with apparent molecular masses ranging from 15 to 17 kDa, and the BSP-30 kDa protein with molecular mass of 28–30 kDa, collectively called BSP proteins (Manjunath, 1984). The biological properties of BSP proteins have been extensively studied and it have been indicated that seminal plasma proteins reverts the cold-shock damage on ram sperm membrane (Jobim et al, 2004).

A correlation between seminal plasma proteins and fertility of the male has been reported in some species of domestic animals such as bull (Killian et al, 1993),

ram (Jobim et al, 2005), goat (Villemure et al, 2003), stallion and boar (Calvete et al, 1997). However, a little information is available regarding the buffalo seminal plasma proteins. Thus the present study was carried out to assess the protein profile of the Murrah buffalo seminal plasma by using SDS-PAGE and to correlate them with the semen characteristics.

Materials and Methods

Collection and preparation of seminal plasma

Semen samples were collected by a bovine artificial vagina from 8 Murrah buffalo bulls maintained at Network Project on Buffalo, N. D. University of Agriculture & Technology, Kumarganj, Faizabad. Immediately after collection, the ejaculate was placed in a 37^0 C water bath and the volume was recorded. The percentage of progressively motile spermatozoa was estimated by microscopic examination, and a subjective assessment of the progressive status was recorded according to procedure of Ax et al. (2000). Sperm concentration was measured using standard hemocytometer methods, the percentage of viable spermatozoa was estimated by viewing 200 spermatozoa under 400× magnification using eosin-aniline blue staining method of Ax et al. (2000).

For preparation of seminal plasma, fresh semen was centrifuged at 5000 rpm for 10 min. The supernatants were collected and re-centrifuged to eliminate any cells. Total protein in the seminal plasma was estimated by standard method (Lowry, 1951). After total protein determination, nine volumes of cold ethanol (-20^0C) were added and left with constant stirring for 90 min at 4^0C to precipitate the proteins. Proteins were then recovered by centrifugation at 10,000 rpm for 10 min, re-suspended in phosphate buffered saline (PBS) and stored at -20^0C until further analysis of seminal plasma proteins within a week time.

SDS-PAGE of seminal plasma

SDS-PAGE was used for separation and determination of molecular weights (MW) of seminal plasma proteins. Seminal plasma samples were subjected to the SDS-PAGE described by Laemmli (1970). Approximately 50 µg of seminal plasma were subjected to SDS-PAGE using a 10% polyacrylamide gel, pH 7.2. Electrophoresis was run at constant voltage until tracking dye was about two-thirds down thegel. After electrophoresis, gels were stained with Coomassie blue G-250 for 2 h, and then de-stained with 10% (v/v) acetic acid and 10% (v/v) methanol solutions. The gels were photographed and the molecular weights of protein bands were calculated using a gel documentation software (BioRad).

Results and Discussion

Efficacy of fungicides against *A. alternata* evaluated under *in vitro* condition presented in Table 1 indicated that all fungicides significantly decreased mycelial growth as compared to control. Higher concentration (500 ppm) of each fungicide was more effective in inhibition of growth as compared to lower concentration (100 ppm). Rate of growth among treatment ranged from 0.7 to 8.9 mm/day as compared to growth of control. Maximum growth inhibition occurred in Carbendazim and Chlorothalonil (0.0 mm) at 500 ppm concentration followed by Copper oxychloride (22.2 mm) and minimum was recorded in Carbendazim (79.9 mm) at 100 ppm concentration after eight days. The data presented in Table 1 indicated that days of incubation was proportional to mycelial

growth. Hundred percent inhibition zone was observed in Carbendazim and Chlorothalonil followed by 75.1 percent in Copper oxychloride at 500 ppm concentration and minimum percent zone inhibition 10.4 percent in Carbendazim at 100ppm concentration. It is evident from the data that there was a significant increase in mycelial growth of *A. alternata* with increase in incubation period. Rate of growth among treatment ranged from 2.4 to 8.1 mm/day as compared to growth of control.

Table.1 Sperm characteristics in fresh and frozen thawed samples (N=50)

Semen Characteristics	Semen sample	
	Fresh	Frozen thawed
Concentration / ejaculate (10^{6})	5315.95 ±195.75	-
Progressive motility (%)	65.25 ± 1.07	49.23 ± 0.83
Abnormal Morphology (%)	14.2 ± 0.35	23.58 ± 0.51
Viability (%)	84.18 ± 0.56	59.2 ± 0.89

Table.2 Molecular weight (kDa) and Percent distribution of protein bands in seminal plasma

Protein band (kDa)	Percent distribution
88	2
76.8	3
71.2	4
68.7	6
65.4	5
60.1	4
58	12
55.7	4
52.3	3
47.1	7
43.3	4
45.8	2
42	7
39.4	6
37.1	3
35.6	4
32.4	2
25.8	8
24.5	5
22.8	3
16.5	4
14.1	2

The results of the semen evaluation of buffalo bulls are summarized in Table 1, whereas number of protein bands in seminal plasma on SDS-PAGE and their distribution (%) is presented in Table 2.

The mean and SE of concentration of sperm (10^6) per ejaculate was found to be 5315.95 ±195.75. Progressive motility (%) in frozen thawed semen decreased from 65.25 ± 1.07 to 49.23 ± 0.83. Abnormal morphology (%) in frozen thawed semen increased from 14.2 ± 0.35 to 23.58 ± 0.51. The viability (%) also decreased in frozen thawed semen from 84.18 ± 0.56 to 59.2 ± 0.89.

A total of 22 protein bands could be identified by the fractionation of seminal plasma protein on the SDS-polyacrylamide gels. The size of these protein bands ranged from 14.1 kDa to 88 kDa. The percentage of their distribution ranged from 2% to 12%. Some of these bands were more prominent in some samples. This is in agreement with those reported in the ram (15–108 kDa, Jobim et al., 2005), bull (15–30 kDa, Manjunath, 1984), and stallion (14–30 kDa, Topfer-Petersen et al, 2005). Arangasamy et al (2005) found 18 protein bands in buffalo seminal plasma with molecular weights of ranging from 12 to 127 kDa with the majority being <25 kDa.

The difference between the number of protein fractions found in this study and the report of Arangasamy et al (2005) may be attributed to procedure of separating proteins before SDS-PAGE fractionation. They divided buffalo seminal plasma (BuSP) proteins into two groups of heparin and gelatin binding proteins while we precipitated proteins by cold ethanol before SDS-PAGE separation.

The protein fraction of 25.8 kDa was more prominent in semen samples showing a good progressive motility of sperm, whereas, the semen samples that had greater sperm viability showed a prominent fraction of 58 kDa protein in the SDS-PAGE.

Statistical analyses showed that 25.8 kDa protein fraction was significantly correlated with sperm progressive motility in fresh and frozen-thawed semen, while 58 kDa band was correlated with sperm viability of fresh semen. This result is in agreement with the report of Jobim et al (2004) who found a significantly higher 24.5 kDa proteins in bulls with high freezability semen and, partially agrees with the report of Nauk and Manjunath (2000) that two proteins of 26 and 55 kDa predominate in higher fertility bulls. The difference with the study of Nauk and Manjunath (2000) with present study could be due to difference in the number of ejaculate studied.

In conclusion differences in the seminal plasma protein profile of individual buffalo bulls with high and low semen quality were detected and seminal plasma proteins in buffalo bulls are similar to those reported in other animal species; some of the seminal plasma protein fractions are correlated with semen characteristics before and after freezing. This fact could support the hypothesis that seminal plasma proteins act on sperm physiology in different ways. Additional studies are necessary to define the types of proteins affecting sperm viability and the mechanisms of their actions.

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