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Short communication

SDS-polyacrylamide gel electrophoresis of buffalo bulls seminal plasma proteins and their relation with semen freezability

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Abstract

The objective of this study was to evaluate the protein profiles of seminal plasma in buffalo bulls and to examine their correlation with semen characteristics. Semen of 10 buffalo bulls were collected by a bovine artificial vagina. Semen characteristics (motility, morphology, viability and concentration) were recorded. A part of the semen sample (1 ml) was diluted by tris-egg yolk-glycerol extender, packed in French straws and was frozen in liquid nitrogen. The straws were later thawed and semen characteristics were compared with those of the fresh semen.

Seminal plasma was harvested by centrifugation; treated with cold ethanol and then, underwent SDS-polyacrylamide gel electrophoresis (PAGE). Twenty five protein bands were identified on the gel, of which those of <35.5 kDa were prominent (72% of the bands). Of these protein fractions, 24.5 kDa was significantly correlated with sperm progressive motility in fresh and viability in frozen-thawed semen while 45 kDa bands were correlated with abnormal morphology in frozen-thawed semen; 55 kDa protein fractions were correlated with sperm viability of fresh semen.

Progressive motility, viability and abnormal sperm morphology of frozen-thawed semen were highly correlated with these parameters in the fresh semen.

In conclusion, seminal plasma protein fractions in buffalo bulls are similar to those reported in other animal species and have some correlations with semen characteristics before and after freezing.

Keywords: Buffalo; Seminal plasma proteins; Sperm freezability

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1. Introduction

Sperm morphology and motility, the number of sperm per insemination, percentage of acrosome reacted sperms and in vitro fertilization have been extensively evaluated as an indication of sperm's ability to fertilize an egg. Evidence suggests that 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).

In addition, the molecular composition of the seminal fluid is very complex, seminal plasma plays an important role in the fertilizing ability of sperm (Cross, 1993), maintains spermatozoa motility in the bull and ram, and improves ram sperm viability. This fluid may also influence bovine fertility and sperm storage; however, its role in storage remains controversial (Jobim et al., 2004).

Several studies provide direct evidence that some proteins of seminal plasma are adsorbed to the surface of ejaculated sperm (Desnoyers and Manjunath, 1992). 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 (Manjunath and Therien, 2002). Other studies have indicated that seminal plasma proteins reverts the cold-shock damage on ram sperm membrane (Jobim et al., 2004).

However, there is a little information available regarding to buffalo seminal plasma proteins. This study was carried out to assess the protein profile of the buffalo seminal plasma by using SDS-PAGE and to investigate a possible relationship between these proteins and the freezability of the spermatozoa in buffalo bulls.

2. Materials and methods

2.1. Animals

Semen samples were collected by a bovine artificial vagina from 10 sexually mature buffalo bulls (4–5 years old) from The Buffalo Breeding Center northwest of Iran, Urmia (37°33′N, 45°4′E). Four semen samples obtained from each buffalo bull at different seasons during a period of 2005–2006.

2.2. Semen evaluation

Immediately after collection, the ejaculate was placed in a 37 °C water bath and the volume was recorded. The percentage of progressively motile spermatozoa was estimated by microscopic examination at 400× magnification on a pre-warmed slide (37 °C), 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 (Hausser Scientific, Horsham, PA. USA), 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). One ml of the ejaculate of each bull was diluted in tris-egg yolk-glycerol extender, kept refrigerated for 18 h, packed in French straws, and the straws were frozen in liquid nitrogen (−196 °C) according to the routine methods in the center. The straws were later thawed in 37 °C water bath for 30 s. Semen characteristics were evaluated as mentioned before and the results compared with those of the

fresh semen. The rest of semen samples were cooled to room temperature and transported to the laboratory within 2 h.

2.3. Preparation of seminal plasma

Fresh semen was centrifuged at 5000 rpm for 10 min. (Clements, 2000, England). The supernatants were transferred into 1.5 ml tubes, re-centrifuged to eliminate the remaining cells. After total protein determination, nine volumes of cold ethanol (−20 °C) were added and left with constant stirring for 90 min at 4 °C 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 −24 °C until further analysis of seminal plasma proteins within a week time.

2.4. Molecular weight determinations (SDS-PAGE)

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 Gabriel (1971) by using a 10% polyacrylamide gel, pH 7.2. The apparent molecular mass was estimated by using the low molecular weight calibration kit (Pharmacia, USA). A volume of 0.1–0.2 ml of sample was mixed with 3 μl of 0.05 (w/v) bromophenol blue and 10 μl of glycerol then mounted to the gel.

Electrophoresis was run at 8 mA per tube until tracking dye was about two-thirds down the tube. After electrophoresis, gels were stained with Coomassie blue G-250 (Pharmacia Biotech), for 2 h, and then de-stained with 10% (v/v) acetic acid and 10% (v/v) methanol solutions.

Protein fractions were compared with the marker proteins by calculating R_f values according to the method recommended by Hames (1990).

2.5. Data analysis

Data analysis was performed using SPSS software (SPSS version 11.5 for Windows; SPSS Inc., Chicago, IL, USA) computer program. Results are quoted as arithmetic mean ± standard error of mean (S.E.M.) and significance was attributed at $p < 0.05$.

Pearson's correlation coefficient (two-tailed) test was used to examine the correlation between all the parameters of the semen, and the correlations of seminal plasma protein fractions with all the parameters of the semen were tested by multiple linear regression test.

3. Results

The results of the semen evaluation of 10 buffalo bulls are summarized in Table 1, and depicted as mean ± S.E.M. The mean values obtained for progressive motility, abnormal morphology and viability of fresh semen was highly significantly different with those of the frozen-thawed semen ($p < 0.001$).

Progressive motility ($r = 0.504$, $p < 0.01$) and viability ($r = 0.435$, $p < 0.01$) of frozen thawed semen were highly correlated with the motility and viability of the fresh semen, and, its abnormal morphology was correlated ($r = 0.611$, $p < 0.01$) with those of the fresh semen.

The seminal plasma protein fractionation on the SDS-polyacrylamide gel resulted in 25 bands (12% 14.4 kDa; 20% 24.5 kDa; 8% 31.3 kDa; 4% 35.2 kDa; 28% 35.5 kDa; 12% 45 kDa, 8% 55 kDa and 8% 80.5 kDa). Of these protein fractions, 24.5 kDa was significantly correlated

Table 1
Semen characteristics of the buffalo bulls

Number of bulls (n)	10
Ejaculate volume (ml)	3.7 ± 1.4
Sperm concentration ($\times 10^6$ cells/ml)	1121.0 ± 167.0
Progressive motility	
Fresh (%)	70.0 ± 8.5 a
Frozen-thawed (%)	59.6 ± 7.1 b
Abnormal morphology	
Fresh (%)	15.9 ± 4.7 a
Frozen-thawed (%)	23.6 ± 5.8 b
Viability	
Fresh (%)	75.8 ± 9.8 a
Frozen-thawed (%)	59.8 ± 6.2 b

Mean values denoted by letters (a and b) are different in paired groups ($p < 0.05$). All values are mean ± S.E.M.

with sperm progressive motility in fresh ($p < 0.002$) and viability in frozen-thawed semen ($p < 0.019$) while 45 kDa bands were correlated with abnormal morphology in frozen-thawed semen ($p < 0.009$); 55 kDa protein fractions were correlated with sperm viability of fresh semen ($p < 0.012$).

4. Discussion

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. This study was designed to get some information in this field. In this study 25 protein fractions were detected in buffalo seminal plasma with molecular masses ranging from 14.4 to 80.5 kDa by using SDS-PAGE technique. The protein fractions separated on the gel were mostly below 35.5 kDa, molecular weights and 35.5 kDa proteins was the most frequent band (28%). 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. Observation of three protein fractions of 14.4 kDa, five protein bands of 24.5 kDa and seven bands of 35.5 kDa in this study is in agreement with their results, but the highest molecular weight protein band recorded here was 80.5 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.

In this study the semen characteristics and the records of freezability of the spermatozoa obtained from four samplings in different seasons were available (Table 1); the seasonal variations in buffalo bull semen characteristics had been studied previously (Alavi-Shoushtari and Babazadeh-Habashi, 2006), so, were not mentioned here. Statistical analyses showed that 24.5 kDa protein fraction was significantly correlated with sperm progressive motility in fresh ($p < 0.002$) and viability in frozen-thawed semen ($p < 0.019$), while 45 kDa band was correlated

with abnormal morphology in frozen-thawed semen ($p < 0.009$); 55 kDa protein fraction was correlated with sperm viability of fresh semen ($p < 0.012$). This is in agreement with the report of Jobim et al. (2004) who found an 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. A 24.5 kDa protein band we observed here may be related to bull high fertility and semen freezability reported by Jobim et al. (2004). Nauk and Manjunath (2000) also reported that any correlation of fertility with the concentration of BSP proteins or each BSP protein could not be established because the number of bulls used in their study was limited to only five animals. To establish any such correlation would require screening several hundred ejaculates.

5. 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 (24.5, 45 and 55 kDa) 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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