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Relationships Between Seminal Plasma Proteins and Sperma Quality in Bulls*

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Abstract
In this study, seminal plasma proteins were seperated by sodium doedosil sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) and the relationship between obtained protein bands and motility, abnormal number of spermatozoa, abnormal number of dead spermatozoa and spermatozoa density indicating the quality of spermatozoa were examined before and after frozen samples of sperma. Ejaculate samples from 18 holstein bulls were taken and transported to the laboratory in cold conditions. After the examinations of the sperma samples, some of the samples were diluted and frozen. Some other sperma samples were centrifuged to obtain seminal plasma. Proteins of seminal plasma and frozen sperma samples were seperated by SDS-PAGE. Protein bands on the polyacrylamide gel from both seminal plasma and frozen sperma were evaluated as percentage distribution of proteins using densitometer. The percentage distribution of protein bands were compared with statistically the parameters of sperma. Results of this study showed that the protein band with 22 kDa has a relation with the motility and the number of dead spermatozoa. Moreover, the protein band with 29 kDa has a statistically significant relation with the number of abnormal spermatozoa. These results may suggest that seminal plasma proteins have a role on the functions of spermatozoa and its quality of life and on the physiologic role of seminal plasma.

Keywords: Seminal plasma proteins, SDS page electrophoresis, sperma, frozen sperma, bull.

INTRODUCTION

The proteins in the seminal plasma, which differ in animal species in terms of their variety and quantity, are separated into calcium-binding, zinc-binding, heparin-binding, iron-binding and CRISP (Cysteine-Rich Secretory Proteins). Apart from these, there are epididymis, seminiferous tubules of testis and leydig cells, vesiculo seminalis, proteins secreted from the prostate gland and peptides. 33.000 dalton glycoprotein of epididymis origin, alpha-lactalbumin, androgen binding protein (ABP), ABP from testes and P-mod-S protein increasing the transferrin level, plasminogen activator, inhibin, semenogelin for coagulation of ejaculate after ejaculation from vesiculo seminalis and in addition lactoferrin and fibronectin, examples of serine protease proteins of the glycoprotein structure known as 33 kDa prostate specific antigen are available (Hafez ESE, 1982; Anonim 1993; Günşen A, 1996).

Polyacrylamide gel electrophoresis technique has been used in experimental analysis of proteins and protein mixtures for many years and continues to play an important role in this field (Hames and Rickwood, 1990). The technique is highly sensitive and effective for characterizing small amounts of proteins (Bryan JK, 1977; Andrews A, 1985; Mashige et al., 1992; Ferron et al., 1993). Polyacrylamide gel electrophoresis technique has a high separation power for proteins and nucleic acids. It has advantages such as stability in the region of temperature and ionic strength and transparency of the gel. It is very suitable for the separation of proteins by their molecular size (Hames and Rickwood, 1990). In the present study it is aimed to determinate the differences between the protein fractions of the frozen and unfrozen samples of seminal plasma in the bulls.

MATERIALS AND METHODS

The samples of semen used in the study were taken from 18 months to 3 aged Holstein bulls reared under the same maintenance feeding conditions at the Lalahan Livestock Central Research Institute.

Spermatological parameters were determined by macroscopic, microscopic (motility, density, abnormal spermatozoa ratio, dead spermatozoa ratio) and chemical examinations (pH). A portion of the semen was diluted with dilution liquid according to the technique and stored in a liquid nitrogen tank in a straw, ready for artificial insemination.

The amount of semen was determined in ml after the semen collection by reading from the graduated sperm collection cup. Motility; after fresh, after reconstitution, after equilibration and in the dissolved sperm, a drop was taken on the slide and the coverslip was closed. The phase-contrast was determined in three different microscope fields with 200x magnification in the microscope. The number of spermatozoa in unit volume was determined by the hemacymetric method and was expressed as spermatozoa / ml. The spermatozoa detected in Hancock fixation solution were treated with 1000x magnification, and at least 200 spermatozoa were counted and the percentage of abnormal spermatozoa was determined. While determining the rate of abnormal spermatozoa, acrosome, head, middle and tail anomalies were evaluated separately. Samples taken from sperm were examined in a light microscope at 400x magnification by taking froth on a clean slide with 2% eosin dye prepared in % 3 sodium citrate solution. At least 200 spermatozoa were determined in different microscope fields and the percentage of dead was expressed as %. The pH of the semen was determined using indicator paper (5,5-9,0 Merck).

The semen, which was evaluated in terms of all the spermatological features, was diluted 1:10 with Tris extender. The semen diluted with two separate diluents taken in 0.5 ml straws in two different colors after the post-dilution motile were recorded and the open ends of the sequins were closed with polyvinyl alcohol. The semen drawn into the straws was then left to harvest for 2 hours at 40°C (equilibration). After the sperm sowing process was completed, the straws were immersed in liquid nitrogen at -196°C by freezing in liquid nitrogen vapor at -120°C for 7 minutes. The semen was then stored in the storage tank. For post-treatment examinations, the semen frozen in the straws were thawed in the water bath at 37.5°C for 30 seconds, and the after melting, spermatozoa motility, rate of abnormal spermatozoa, rate of dead spermatozoa, and pH were re-examined.

The remaining part of the semen was centrifuged at 4000 rpm for 20 minutes prior to electrophoresis. Spermatozoa were removed and seminal plasma was obtained. Electrophoresis of seminal plasma was performed by SDS-PAGE (Spectrophotometer; Schimadzu UV-1202 VIS) and densitometric evaluations of gels were determined by a densitometer (Junior 24 Helena, France).

In the evaluation of fresh and post-freezing distribution of seminal plasma protein bands, T-test was applied for dependent groups and Mann-Whitney U Test analysis was used to evaluate the relationship between spermatological parameters and protein bands. SPSS 11.5 computer program was used for statistical analysis.

RESULTS

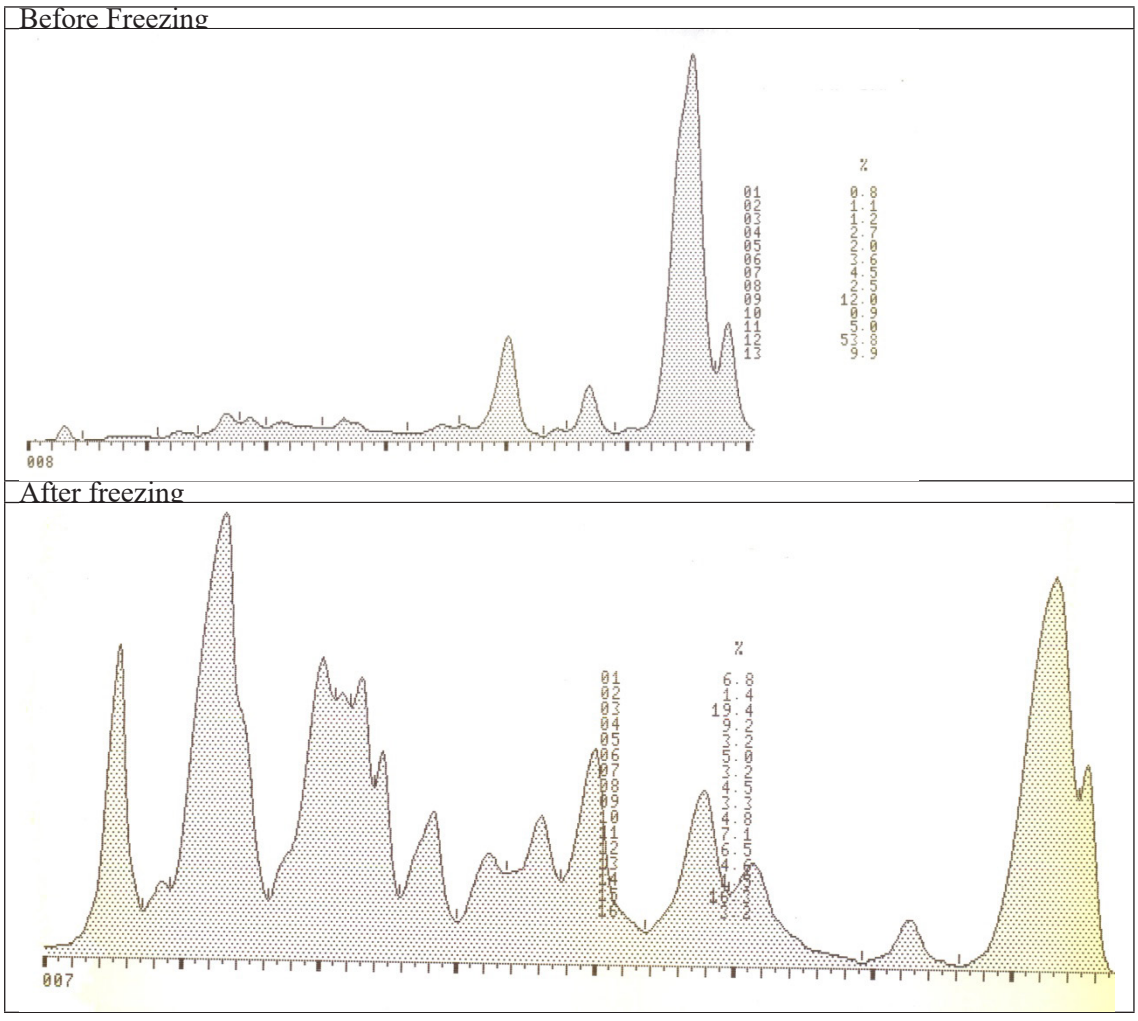
The densitometric distribution of the seminal plasma proteins in PAGE were given in Figure 1 before and after freezing respectively. The distribution of protein bands before and after freezing in seminal plasma and after thawing is given in Table 1. A statistically significant ($p<0.001$) decrease was observed in all bands before and after freezing.

Table 1. Seminal plasma densitometric distribution of 15, 16.5, 22 and 29 kDa protein bands before and after freezing (%).

Bands	N	X ± Sx	Minimum	Maximum
15 kDa Before Freezing *	18	10.79 ± 0.56	6.00	15.5
15 kDa After Freezing *	18	1.87 ± 0.33	0.0	4.70
16.5 kDa Before Freezing*	18	55.79 ± 1.24	45.00	63.90
16.5 kDa After Freezing *	18	16.98 ± 1.28	8.10	30.50
22 kDa Before Freezing *	18	5.55 ± 0.2	4.20	7.00
22 kDa After Freezing *	18	2.1 ± 0.18	1.40	4.10
29 kDa Before Freezing *	18	11.25 ± 0.48	6.00	14.70
29 kDa After Freezing *	18	6.96 ± 0.61	3.20	11.60

*: $p<0.001$

Figure 1. Densitometric distribution of seminal plasma proteins before and after freezing.



The values of the spermatological parameters are given in Table 2. The decrease in motility, abnormal rate of spermatozoa and increase in the rate of spermatozoa were observed in spermatological parameters before and after freezing (Table 2).

Table 2. Spermatological parameters in fresh and frozen sperm.

n=18	Fresh sperm		Frozen sperm	
	min-max	mean	min-max	mean
Quantity	1.5-8.5	4.63	-	-
Density	713-1575	1182.6	-	-
Motility	40-80	63.05	20-60	39.44
Abnormal	7-14	10	25-46	34.94
Acrosome	0-8	3.4	19-35	27.33
Head	0-3	0.7	1-3	1.1
Center	1-8	4.3	1-7	3.39
Tail	1-5	1.5	1-6	3.1
Dead	10-54	26.05	21-62	41.22
pH	6-7	6.34	5.8-6.2	6.09

The percentage distributions of the protein bands of seminal plasmas, grouped by the number of abnormal spermatozoa before and after freezing, are given in Table 4. While there was no statistically significant change in the bands with 15 kDa, 16.5 kDa and 22 kDa molecular weight ($p \geq 0.05$), there were significant differences in $p \leq 0.05$ in 29 kDa band.

The percentage distributions of the protein bands of seminal plasmas, grouped by motility before and after freezing, are given in Table 3. There was no statistically significant difference between the 60% and 60% of the patients whose motility was less than 60% ($p \geq 0.05$). Significant differences were found in the $p \leq 0.05$ level in the 22 kDa band after freezing.

Table3. Distribution of seminal plasma protein bands before and after freezing according to motility (%).

		n	Motility	X ± Sx	Min-Max
15 kDa	BF	9	≤ %60	10,64 ± 0,79	6,00-14,50
		9	> % 60	10,93 ± 0,83	7,80-15,50
	AF	11	≤ % 40	2,2 ± 0,40	0.0-4.70
		7	> % 40	1,36 ± 0,57	0.0-4.00
16.5 kDa	BF	9	≤ %60	53,98 ± 2,03	45,00-63,90
		9	> % 60	57,61 ± 1,24	53,70-62,60
	AF	11	≤ % 40	17,37 ± 1,1	11,20-22,80
		7	> % 40	16,37 ± 2,9	8.10-30.50
22 kDa	BF	9	≤ %60	5,93 ± 0,28	4,60-7,00
		9	> % 60	5,17 ± 0,24	4,20-6,20
	AF*	11	≤ % 40	1,88 ± 0,20	1.40-3.70
		7	> % 40	2,44 ± 0,31	1.70-4.10
29 kDa	BF	9	≤ %60	11,84 ± 0,63	9,10-14,70
		9	> % 60	10,66 ± 0,70	6,00-13,50
	AF	11	≤ % 40	6,86 ± 0,79	3,20-11,00
		7	> % 40	7,13 ± 1,03	4.20-11.60

BF: Before Freezing, AF: After freezing

*:p≤ 0.05

Table 4. Distribution of seminal plasma protein bands after freezing according to the number of abnormal spermatozoa (%).

		n	Number of Abnormal Spermatozoa	X ± Sx	Min-Max
15 kDa	BF	8	≤ 9	10.98 ± 0.88	7.80-14.50
		10	> 9	10.64 ± 0.76	6.00-15.50
	AF	8	≤ 35	1.86 ± 0.51	0.0-4.00
		10	> 35	1.87 ± 0.47	0.0-4.70
16.5 kDa	BF	8	≤ 9	55.46 ± 1.2	52.409-62.30
		10	> 9	56.06 ± 2.05	45.00-63.90
	AF	8	≤ 35	14.23 ± 1.68	8.10-20.40
		10	> 35	19.19 ± 1.60	11.90-30.50
22 kDa	BF	8	≤ 9	5.41 ± 0.25	4.50-7.00
		10	> 9	5.66 ± 0.31	4.20-6.90
	AF	8	≤ 35	2.38 ± 0.35	1.50-4.10
		10	> 35	1.88 ± 0.13	1.40-2.80
29 kDa	BF*	8	≤ 9	12.35 ± 0.62	9.80-14.70
		10	> 9	10.37 ± 0.59	6.00-12.60
	AF*	8	≤ 35	12.35 ± 0.62	4.80-11.60
		10	> 35	10.37 ± 0.59	3.20-9.70

BF: Before Freezing, AF: After freezing

*: p≤ 0.05

The percentage distribution of the protein bands of seminal plasmas, grouped by the number of dead spermatozoa before and after freezing, is shown in table 5, and a statistically significant change in sperm spermatozoa between samples with less than 30 spermatozoa is shown in samples with less than 30 spermatozoa ($p \geq 0.05$). After freezing, significant differences were found at the level of $p \leq 0.05$ in the 22 kDa band according to the number of dead spermatozoa.

Table 5. Distribution of seminal plasma protein bands before and after freezing according to the number of dead spermatozoa (%)

		n	Number of Abnormal Spermatozoa	X ± Sx	Min-Max
15 kDa	BF	11	≤ 30	10.76 ± 0.69	7.80-15.50
		7	> 30	10.83 ± 1.01	6.00-14.50
	AF	9	≤ 35	1.64 ± 0.58	0.0-4.70
		9	> 35	2.1 ± 0.35	0.0-3.40
16.5 kDa	BF	11	≤ 30	57.1 ± 1.62	45.70-63.90
		7	> 30	53.74 ± 1.76	45.00-59.00
	AF	9	≤ 35	16.47 ± 2.24	8.10-30.50
		9	> 35	17.5 ± 1.35	11.20-22.80
22 kDa	BF	11	≤ 30	5.27 ± 0.26	4.20-4.10
		7	> 30	5.99 ± 0.27	5.00-7.00
	AF*	9	≤ 35	2.33 ± 0.25	1.70-4.10
		9	> 35	1.87 ± 0.24	1.40-3.70
29 kDa	BF	11	≤ 30	10.76 ± 0.60	6.00-13.50
		7	> 30	12.03 ± 0.75	9.10-14.70
	AF	9	≤ 35	7.18 ± 0.88	4.20-11.60
		9	> 35	6.74 ± 0.88	3.20-11.0

BF: Before Freezing, AF: After freezing

*: p≤ 0.05

The percentage distribution of seminal plasmids and protein bands in Table 6 is given in the semen, divided into two groups with density less than 1200 and more than 1200. A statistically significant change was not found between those with a density greater than 1200 and less than 1200 (p ≥ 0.05).

Table6. Distribution of seminal plasma protein bands according to the intensity of spermatozoa (%).

	Spermatozoa density	n	X ± Sx	Minimum	Maximum
15 kDa	≤ 1200	10	11.33 ± 0.69	8.8	15.5
	> 1200	8	10.113 ± 0.91	6.0	13.9
16.5 kDa	≤ 1200	10	56.94 ± 1.056	52.8	62.6
	> 1200	8	54.37 ± 2.46	45.0	63.9
22 kDa	≤ 1200	10	5.44 ± 0.25	4.2	6.5
	> 1200	8	5.69 ± 0.35	4.5	7.0
29 kDa	≤ 1200	10	10.68 ± 0.65	6.0	13.7
	> 1200	8	11.96 ± 0.66	9.8	14.7

DISCUSSION

The importance of seminal plasma biochemistry is primarily due to the effect of seminal plasma proteins on fertility and early embryonic development with spermatozoa, and the fact that some proteins are very specific and may be used as indicators. Some proteins contained in semen are important in the life force and function of spermatozoa, and some in the function of male gland sex glands (Petersen et al., 1998). The numbers and concentrations of seminal plasma proteins were found to be specific to animal species and breeds. This suggests that seminal plasma proteins can be used in the genotypic polymorphism (Scheit et al., 1990;Tsuji et al., 1993). In the study, Seshagiri and Pattabiraman (1991) determined the total protein content of the seminal plasma of Sindhi, Jersey, Sindhi x Jersey and Sindhi x Friesian hybrids by Lowry method. Firstly, the semen was injected into the rabbits with adjuvant to obtain antiserum; the resulting antiserum polyacrylamide was subjected to gel electrophoresis. When the relative area of each fraction covered in the gel was examined as % in the densitometer, there were also differences between species and race between the percentages of protein fractions as well as differences in total protein concentrations (Seshagiri and Pattabiraman, 1991).

Desnoyers et al. (1994), who identified bovine seminal plasma proteins by two-dimensional polyacrylamide gel electrophoresis were determined the dominant concentration of protein according to the bands BSP-A1 (pI 4.7-5.0), BSP-A2 (pI 4.9-5.2), BSP-A3 (pI 4.8-5.2) and BSP 30 kDa (pI 3.9-4.6). They reported that the molecular weights of BSP-A1, A2 and A3 were between 15000 and 16500 dalton and the BSP 30 kDa protein was 28000 daltons. Protein bands found in our study were compatible with the first two bands (BSP-A1 and BSP-A2 bands) of the bands identified by Desnoyers et al. (1994).

Bovine seminal plasma may contain components that may be harmful to sperm. Damages occur in the form of motility drop and membrane damage. It has been reported that such proteins are isolated from rabbits, cattle, rams, human and swine spermatozoa [9].

In pigs, these components are simple proteins of 10-12 kDa and are separated by dialysis at pH 4.0. Low molecular weight toxic components can be detected by dialysis in seminal plasma. Antibacterial-effective and peptide-structured components of 5-7 kDa, which are effective in reducing the quality of spermatozoa have been isolated from pig seminal plasma. In light of this information, it has been reported that some components in seminal plasma have negative effects on motility and survival of sperm.

Killian et al. (1993) examined the relationship between infertility and seminal plasma proteins in holstein cattle and identified four specific proteins associated with fertility. Two of the proteins, 26 kDa (pI 6.2) and 55 kDa (pI 4.5) were higher in seminal plasma of bulls with higher fertility rate than the other proteins, and polyacrylamide was more broad band in electrophoresis than in the other, 16 kDa pI 4.1

and 16 kDa pI 6.7 found that the two proteins formed more broad bands in bulls with low fertility. The findings of this study are important in the determination of fertility in animal husbandry and veterinary medicine.

Roncoletta et al. (1997), after freezing and thawing, with more than 80% of the total number of live spermatozoa, less than 50% of those who have applied seminal plasma SDS-Page electrophoresis, between the samples have determined 20 protein bands determined. Spermatozoa have more than 80% survival in seminal plasmas, 51800 dalton protein band found more dominant than other bands.

As a result; It is possible to say that the proteins of seminal plasma, the functions of spermatozoa, quality of life and seminal plasma have an important role in the fulfillment of their physiological duties. In addition to the routine analyzes used to determine the quality of the semen to be used in sunflower insemination, electrophoretic protein bands were considered to be useful in detecting protein. In addition, since the numbers and concentrations of seminal plasma proteins show specific differences between species and the same species, it can be said that the species can be used in genotypic polymorphism by detecting species and inter-race differences, and also in determining which species they actually belong to.

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