RELATIONSHIPS BETWEEN PHOSPHATASE ACTIVITY AND OTHER CHARACTERISTICS IN BULL SEMEN

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Summary. Alkaline and acid phosphatase levels, and various other semen characteristics, have been examined in 149 semen samples from twelve Holstein–Friesian bulls. Alkaline phosphatase and acid phosphatase mean values were 26.1 ± 18.0 and 7.4 ± 7.8 Sigma units/ml for spermatozoa free of seminal plasma and 105.7 ± 42.5 and 24.7 ± 11.8 Sigma units/ml for seminal plasma. Alkaline phosphatase activity of spermatozoa was significantly correlated with the percentage of motile spermatozoa, and percentage of live spermatozoa. When acid phosphatase values were adjusted for concentration of spermatozoa per sample, highly significant negative correlations were obtained with the percentage of motile spermatozoa and the percentage of live spermatozoa, and a positive correlation with the percentage of abnormal spermatozoa. When alkaline phosphatase of seminal plasma and the alkaline phosphatase/acid phosphatase ratio in seminal plasma were adjusted for concentration of spermatozoa per sample, highly significant negative correlations were obtained with the percentage of motile spermatozoa and the percentage of live spermatozoa, and a positive correlation with the percentage of abnormal spermatozoa. The percentage of motile spermatozoa was significantly correlated with alkaline phosphatase and acid phosphatase in seminal plasma, whereas the percentage of abnormal spermatozoa was negatively correlated with alkaline phosphatase and acid phosphatase.

INTRODUCTION

Semen owes its considerable amount of phosphatase activity mainly to the seminal plasma which abounds in different dephosphorylating enzymes derived from the male accessory organs of reproduction. Among the most active and best known enzymes in this group are the so-called acid and alkaline phosphatases (Mann, 1964).

The presence of phosphatase in bovine semen was first demonstrated by Mann (1945) and confirmed by Reid, Ward & Salsbury (1948) and Haq & Mullen (1949). King & Delory (1945) and Engberg, Anderson, Sury & Raft (1947) demonstrated that the activity of acid phosphatase in man is influenced

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by androgens and Reid *et al.* (1948) reported that phosphatase activity may be influenced by feeding, by testicular degeneration and by testicular hypoplasia. In a recent study, Bell & Lake (1962) found that seminal acid phosphatase activity at pH 4.9 was higher in man, the cock and turkey tom than in the boar and rabbit, with the bull intermediate, whereas alkaline phosphatase activity was highest in the boar and rabbit, lowest in the cock, turkey tom and man, with the bull again intermediate. Lundquist (1946) found very small quantities of acid phosphatase in semen of the bull.

In the present paper, the enzymatic activities of alkaline phosphatase and acid phosphatase in seminal plasma and spermatozoa free of seminal plasma from Holstein-Friesian bulls have been investigated and correlated with various other semen characteristics. This study was undertaken in order to elucidate further the significance of alkaline and acid phosphatase in semen physiology.

MATERIALS AND METHODS

An artificial vagina was used to obtain semen samples from twelve healthy Holstein-Friesian bulls ranging in age from 16 to 22 months. Two ejaculates were taken from each bull once a week, with intervals of 5 min between collections. Immediately after collection, the semen samples were stoppered and placed in a water-bath at 27° C and thereafter placed in a styrofoam carrier maintained at 5° C to prevent temperature shock and to eliminate any influence of temperature on phosphatase activity. A total of 149 semen samples was selected at random and used in this study.

The time interval between the collection and the examination of the semen characteristics was not more than 1½ hr. The collection tubes were graduated in ml and semen volume was measured directly. Semen samples were examined under a magnification of 440, and the percentage of spermatozoa showing progressive forward movement was assessed in increments of 5%. The percentage of morphologically abnormal spermatozoa and the percentage of live spermatozoa were determined on samples stained according to the procedure outlined by Herman & Madden (1963). The concentration of spermatozoa was determined photometrically as outlined by Willet & Buckner (1951).

Immediately after these semen characteristics had been determined, a portion of the semen was transferred by means of a blood volume pipette into Wintrobe tubes and centrifuged at 2000 g for 15 min in a refrigerated centrifuge at 5° C. After centrifugation, the seminal plasma was removed from the Wintrobe tube by means of a blood volume pipette and assayed for phosphatase activity. A volume of 0.075 M phosphate buffer, pH 6.8 (Sigma Stock No. 105-9), equal to that of the removed seminal plasma, was added to the spermatozoa. The suspension of buffer and spermatozoa was then assayed for phosphatase activity. Since time did not permit the assay of these samples on the day of collection, samples were stored in a refrigerator at 5° C for a period of 18 hr before being assayed. Phosphatase was assayed by the method outlined by Sigma Technical Bulletin 104 (Sigma Chemical Company, 1963) and activity expressed as Sigma units/ml. One Sigma unit of phosphatase liberates 1 μ M of *p*-Nitrophenol/hr (1 μ M = 0.1391 mg). Acid phosphatase was assayed at

pH 4.8 and alkaline phosphatase at pH 10.5, at 38° C. It has been reported that alkaline phosphatase activity is stable, or possibly increases by 5 to 10% after 24-hr storage in a refrigerator (Sigma Technical Bulletin 104). Acid phosphatase is not as stable as alkaline phosphatase under the prevailing conditions and the acid phosphatase values reported herein are $25 \pm 5\%$ lower than for samples assayed immediately after collection. No attempt was made to adjust the acid phosphatase values for changes during storage of samples.

Statistical analysis of the data was in accordance with the procedures outlined by Snedecor (1956). Before analysis, the percentages of motile, live and abnormal spermatozoa were transformed to arcsins.

TABLE 1
MEANS AND STANDARD DEVIATIONS OF PHOSPHATASE ACTIVITY
AND OTHER SEMEN CHARACTERISTICS

	Mean	Standard deviation
Semen characteristics		
Volume (ml)	3.7	1.2
Motile spermatozoa (%)	34.7	4.1
Concentration of spermatozoa ($\times 10^6$ cells/ml)	667.86	340.50
Live spermatozoa (%)	52.2	3.4
Abnormal spermatozoa (%)	12.3	2.3
Spermatozoa free of seminal plasma		
Alkaline phosphatase (Sigma units/ml)	26.1	18.0
Acid phosphatase (Sigma units/ml)	7.4	7.8
Alkaline/acid phosphatase (ratio)	4.2	3.6
Alkaline phosphatase (Sigma units/ 500×10^6 cells)	22.1	16.5
Acid phosphatase (Sigma units/ 500×10^6 cells)	6.5	6.5
Alkaline/acid phosphatase/ 500×10^6 cells (ratio)	3.7	2.8
Seminal plasma		
Alkaline phosphatase (Sigma units/ml)	105.7	42.5
Acid phosphatase (Sigma units/ml)	24.7	11.8
Alkaline/acid phosphatase (ratio)	5.2	3.9
Alkaline phosphatase (Sigma units/ 500×10^6 cells)	126.34	77.11
Acid phosphatase (Sigma units/ 500×10^6 cells)	43.02	70.94
Alkaline/acid phosphatase/ 500×10^6 cells (ratio)	8.50	14.34

Mean data for 149 semen samples from twelve bulls.

RESULTS

The means and standard deviations of the alkaline and acid phosphatase activities found in spermatozoa and seminal plasma, and of other semen characteristics are shown in Table 1. These data were obtained from 149 semen samples collected at random from twelve healthy Holstein-Friesian bulls. The alkaline phosphatase activity was found to be four times higher in seminal plasma than in spermatozoa. A similar relationship was also observed in the activity of acid phosphatase.

The correlations of volume of semen, percentage of motile spermatozoa, concentration of spermatozoa, percentage of live spermatozoa, and percentage of morphologically abnormal spermatozoa with alkaline phosphatase and acid

phosphatase activity of spermatozoa free of seminal plasma are shown in Table 2. Correlations with the ratio of alkaline phosphatase/acid phosphatase, and with measures of phosphatase adjusted to a common basis of cell number (500×10^6 cells), are also shown. No significant correlations were observed between volume of semen and any of the measures of phosphatase activity. The percentage of motile spermatozoa was positively correlated with alkaline phosphatase ($P < 0.05$) and with the ratio of alkaline phosphatase/acid phosphatase ($P < 0.01$), and negatively correlated with acid phosphatase when adjusted for cell number ($P < 0.01$). Highly significant ($P < 0.01$) negative correlations were

TABLE 2

CORRELATIONS BETWEEN PHOSPHATASE ACTIVITY OF SPERMATOZOA FREE OF SEMINAL PLASMA AND OTHER SEMEN CHARACTERISTICS

		Volume	Percentage motile spermatozoa	Concentration of spermatozoa	Percentage live spermatozoa	Percentage abnormal spermatozoa
Alkaline phosphatase/ml	<i>r</i>	0.08	0.18*	0.12	0.18*	-0.03
	<i>b</i>	11.62	0.32	0.62	0.28	-0.06
Acid phosphatase/ml	<i>r</i>	0.07	0.08	0.15	0.11	-0.06
	<i>b</i>	3.96	0.06	0.34	0.08	-0.06
Alkaline/acid phosphatase (ratio)	<i>r</i>	0.10	0.22**	0.04	0.17*	-0.07
	<i>b</i>	2.67	0.07	0.00	0.05	-0.03
Alkaline phosphatase/ 500×10^6 cells	<i>r</i>	0.08	0.05	-0.38**	-0.04	0.12
	<i>b</i>	9.62	0.03	-1.84	-0.06	0.25
Acid phosphatase/ 500×10^6 cells	<i>r</i>	-0.08	-0.39**	-0.40**	-0.37**	0.39**
	<i>b</i>	-3.97	-0.25	-0.75	-0.21	0.31
Alkaline/acid phosphatase/ 500×10^6 cells (ratio)	<i>r</i>	0.08	0.12	0.29**	0.11	-0.02
	<i>b</i>	17.40	0.32	-2.38	0.29	-0.06

r, Simple correlation coefficient; *b*, simple regression coefficient.

* $P < 0.05$; ** $P < 0.01$.

attained between concentrations of spermatozoa and the adjusted alkaline phosphatase, and the adjusted acid phosphatase, and a positive correlation with the ratio of alkaline phosphatase/acid phosphatase. Alkaline phosphatase and the ratio of alkaline phosphatase/acid phosphatase were significantly ($P < 0.05$) positively correlated with the percentage of live spermatozoa, whereas adjusted acid phosphatase was negatively correlated ($P < 0.01$) with the percentage of live spermatozoa. On the other hand, the percentage of abnormal spermatozoa was positively correlated ($P < 0.01$) with the adjusted acid phosphatase activity.

Correlations between the phosphatase activity of seminal plasma and other semen characteristics are presented in Table 3. Alkaline phosphatase of seminal plasma was positively and significantly ($P < 0.05$) correlated with the percentage of motile spermatozoa and negatively correlated with the percentage of abnormal spermatozoa. Acid phosphatase activity in seminal plasma was negatively correlated with volume of semen and with the percentage of morphologically abnormal spermatozoa, and positively correlated with the percentage of motile spermatozoa and with concentration of spermatozoa. All semen characteristics

investigated were significantly correlated with the ratio of alkaline phosphatase/acid phosphatase.

When alkaline phosphatase activity in seminal plasma was adjusted for concentration of spermatozoa, highly significant ($P < 0.01$) negative correlations were observed with the percentage of motile spermatozoa, concentration of spermatozoa, and the percentage of live spermatozoa, and a positive correlation with the percentage of morphologically abnormal spermatozoa. No significant correlations were obtained between any of the semen characteristics investigated and the adjusted acid phosphatase activity in the seminal plasma. When the alkaline phosphatase/acid phosphatase ratio was adjusted for con-

TABLE 3
CORRELATIONS BETWEEN SEMINAL PLASMA PHOSPHATASE ACTIVITY AND OTHER SEMEN CHARACTERISTICS

		Volume	Percentage motile spermatozoa	Concentration of spermatozoa	Percentage live spermatozoa	Percentage abnormal spermatozoa
Alkaline phosphatase/ml	<i>r</i>	0.04	0.16*	0.06	0.09	-0.17*
	<i>b</i>	11.47	0.62	0.78	0.37	-0.87
Acid phosphatase/ml	<i>r</i>	-0.24**	0.21*	0.30**	0.13	-0.22**
	<i>b</i>	-22.05	0.25	1.04	0.13	-0.31
Alkaline/acid phosphatase (ratio)	<i>r</i>	0.16*	-0.28**	-0.24**	-0.19*	0.24**
	<i>b</i>	4.75	-0.10	-0.28	-0.07	0.12
Alkaline phosphatase/ 500×10^6 cells	<i>r</i>	0.15	-0.26**	-0.27**	-0.25**	0.26**
	<i>b</i>	93.03	-2.01	-6.30	-1.72	2.52
Acid phosphatase/ 500×10^6 cells	<i>r</i>	0.07	0.06	0.01	0.04	-0.07
	<i>b</i>	38.39	0.45	0.22	0.27	-0.62
Alkaline/acid phosphatase/ 500×10^6 cells (ratio)	<i>r</i>	0.04	-0.30**	-0.21**	-0.23**	0.28**
	<i>b</i>	5.50	-0.42	-0.91	-0.29	0.49

r, Simple correlation coefficient; *b*, simple regression coefficient.

* $P < 0.05$; ** $P < 0.01$.

centration of spermatozoa, significant negative correlations were obtained with the percentage of motile spermatozoa, concentration of spermatozoa, and the percentage of live spermatozoa, and a positive correlation with the percentage of morphologically abnormal spermatozoa.

DISCUSSION

Cytochemical studies by Bern (1949) and Rollinson (1954) have shown that alkaline phosphatase and acid phosphatase in mammalian semen are derived primarily from the secretions of the seminal vesicles and prostate gland. In the cock and turkey tom, the relatively high acid phosphatase activity of seminal plasma is derived mainly from the secretions of the vasa efferentia and vasa deferentia (Bell & Lake, 1962). Roussel & Stallcup (unpublished data) have demonstrated histochemically that alkaline phosphatase is secreted by the testis, caput epididymidis, corpus epididymidis and cauda epididymidis of Holstein-Friesian bulls: acid phosphatase was not present in the testis, but slight amounts observed in the caput epididymidis with increasingly larger

amounts in the corpus epididymidis and the cauda epididymidis. Bern (1949) demonstrated cytochemically that alkaline phosphatase is present in the testis, epididymis and vas deferens of the rabbit. Haq & Mullen (1949) found some biochemical evidence suggestive of the production of acid phosphatase by the testis and possibly by the epididymis, and they also found that bulls producing semen with low concentrations of spermatozoa also showed lowered seminal acid phosphatase activity. This is in agreement with the present observation of a positive correlation between acid phosphatase activity in seminal plasma and the concentration of spermatozoa.

Roussel & Stallcup (1965) found that alkaline phosphatase and acid phosphatase activities in blood serum of Holstein-Friesian bulls, ranging from 1 to 95 weeks of age, were 2.36 ± 0.67 and 0.29 ± 0.18 Sigma units/ml, respectively. It was further shown that alkaline phosphatase in blood serum is negatively correlated ($P < 0.05$) with age of bulls. It is unlikely, therefore, that the large amounts of alkaline phosphatase and acid phosphatase found in semen originate from blood, since the blood levels are very low. The functions of phosphatase in seminal plasma have been discussed by Rollinson (1954) and Mann (1964).

The alkaline phosphatase activity of semen is four times greater in the seminal plasma than in the spermatozoa. This was also essentially true for the acid phosphatase activities which are considerably lower. Bell & Lake (1962), however, reported that alkaline phosphatase activity in whole semen was only slightly higher than acid phosphatase.

In the present study, the phosphatase activities in seminal plasma provided more correlations with other semen characteristics than phosphatase activities found in spermatozoa free of seminal plasma. The alkaline phosphatase activity of seminal plasma adjusted for cell concentration and the ratio of alkaline phosphatase/acid phosphatase activities in seminal plasma adjusted for cell concentration appear to provide the best indices of semen quality as judged by the percentage of motile spermatozoa, the percentage of live spermatozoa and the percentage of morphologically abnormal spermatozoa. Perhaps the metabolic activity of spermatozoa is influenced by the presence of phosphatase in seminal plasma.

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