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Correlations between seminal plasma enzyme activities and semen parameters in seminal fluid of Arabian horses

Dogan, I.[1*]; Polat, U.[2] and Nur, Z.[1]

[1]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Uludag University, 16059 Gorukle, Bursa, Turkey; [2]Department of Biochemistry, Faculty of Veterinary Medicine, Uludag University, 16059 Gorukle, Bursa, Turkey

[*]**Correspondence:** I. Dogan, Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Uludag University, 16059 Gorukle, Bursa, Turkey. E-mail: idogan@uludag.edu.tr

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Summary

The objective of this study was to investigate aspartate-amino-transferase (AST), γ-glutamyl-transferase (GGT), alkaline phosphatase (ALP), lactate-dehydrogenase (LDH) and acid phosphatase (AcP) activities and semen parameters (volume, pH, concentration, total sperm number (TSN), progressive motility, dead sperm, total morphological defect (TMD) and hypo-osmotic swelling test (HOST)) in seminal plasma of Arabian horses. Furthermore, correlations between enzyme activities and semen parameters were examined. The study was performed using seven healthy Arabian stallions of proven fertility, between 11 and 17 years of age, from the Karacabey Stud Farm in Bursa, Turkey. Overall, 21 semen samples were collected from stallions during the breeding season from March to May. A significant negative correlation was observed between semen volume and concentration of TMD, AST, ALP ($P<0.05$) and LDH ($P<0.01$). pH showed a significant correlation with live:dead ratio, GGT activity ($P<0.05$) and progressive motility ($P<0.01$). All semen concentrations correlated significantly with TSN, TMD, ALP, AcP ($P<0.01$). Furthermore, significant correlations were found between live:dead ratio and TSN, HOST ($P<0.05$); TSN and ALP, AcP ($P<0.01$); progressive motility and HOST ($P<0.01$), GGT ($P<0.001$); AST and ALP, LDH, AcP ($P<0.001$); GGT and LDH ($P<0.05$); ALP and LDH, AcP ($P<0.01$) and LDH and AcP ($P<0.001$). No significant correlation was found between enzyme activities in stallion seminal plasma and semen parameters in different months, except for pH and HOST.

Key words: Stallion, Seminal plasma, Semen parameters, Enzyme activity

Introduction

In mammals, seminal plasma is a complex mixture of secretion from the epididymis and various accessory sex glands. The anatomy of accessory glands, as well as their chemical composition and the functions of their secretions vary among species (La Falci *et al*., 2002). A large variety of enzymes is present in seminal plasma, but in many instances the gland responsible for their production has not been identified. The enzyme levels of seminal plasma are very important for sperm metabolism as well as sperm function (Brooks, 1990). However, not many studies have been conducted in horses yet. Therefore, estimates of these enzymes have been recommended as markers for semen quality since they indicate sperm damage (Singh *et al*., 1996; Pesch *et al*., 2006). For example, ALP is primarily of testicular and epididymal origin and can be used as a clinical ejaculatory marker to differentiate azoospermia or oligospermia from ejaculatory failure (Turner and McDonnell, 2003). Ciereszko *et al*. (1992) reported a high correlation between ALP activity released by spermatozoa and semen quality when sperm cells were subjected to minimal and maximal stress. Likewise, ALP, AST and LDH are essential for metabolic

processes which provide energy for survival, motility and fertility of spermatozoa. In addition, LDH concentration in stallion seminal plasma, its importance and correlations to other semen parameters had not been published earlier. The objective of this study was to investigate the enzyme activities in seminal plasma of Arabian horses during the breeding season. Furthermore, correlations between enzyme activities and semen parameters were examined.

Materials and Methods

The study was performed using seven healthy Arabian stallions of proven fertility, between 11 and 17 years of age, from the Karacabey Stud Farm in Bursa, Turkey. During the experiment, the stallions were kept in boxes on straw and fed oats and hay three times daily. Water was freely available. The animals were fed hay, oats and pellets supplemented with minerals. During the breeding season (March to May) the stallions were used in natural service. All animals were exercised daily for at least 1 h. Overall, 21 semen samples were collected from stallions during the breeding season from March to May, on a monthly basis.

Ejaculate was collected with an artificial vagina on an oestrous mare. Immediately after collection, each ejaculate was subjected to conventional prebreeding examination [volume, pH, progressive motility, concentration, total sperm count, live:dead ratio, acrosome and total morphological defect and plasma membrane integrity (HOS test)] according to Davies-Morel (1999). The gel-fraction was removed after semen collection and then semen was filtered with sterile gauze. The volume was measured in a graduated cylinder. pH value was determined using pH indicator paper (Merck, Darmstadt, Germany). Motility was estimated using phase contrast microscopy (Nikon, Japan). Sperm concentration was determined with a haemocytometer. Total sperm count was calculated from ejaculate volume and sperm concentration. Sperm smears were eosin-nigrosin stained and live:dead ratio was examined for 200 spermatozoa. Giemsa-stained smears were used for morphological examination and abnormal sperm cell ratio was calculated for 200 spermatozoa. The morphological characteristics included abnormal acrosome, heads, mid-pieces and tail. The functional membrane integrity of fresh semen was assessed with the HOS test. 100 µl of fresh semen were added to 1 ml of a fructose-sodium citrate hypo-osmotic solution (100 mOsm/I) and incubated at 37°C for 30 min. At least 200 spermatozoa were observed at magnification of ×400 and classified by the presence or absence of a swollen tail (curled/coiled principle or end piece). The percentage of HOS-positive spermatozoa (number of spermatozoa with swollen tails per total number of spermatozoa ×100) was recorded for each sample.

After spermatological examination, a total of 21 semen samples were centrifuged (5000 g × 10 min) and seminal plasma were separated at room temperature and stored at -20°C until biochemical analysis. Activities of AST (IFCC-20649491 322), GGT (SZASZ-PERSIJN-03004732 122), ALP (IFCC-03333701 190), LDH (IFCC-03002721 122) and AcP (HILMANN-20737321 322) in seminal plasma were determined by using Roche Cobas Integra 400 Plus Analyzer.

Collected data are presented in Table 1 as means ± SD and minimum-maximum levels. The collected data were subjected to Friedman analysis, and Wilcoxon rank sum test was used to examine the statistical significance of the differences. Correlation analysis (two-tailed Pearson's correlation test) was used to assess the relationship between enzyme activities and semen parameters in seminal fluid in Arabian horses (Table 2). SPSS program package (SPSS 10.01 for Windows; SPSS Inc., Headquarters, Chicago, IL USA) was used for all statistical analysis. In all analysis, a p-value less than 0.05 was considered as statistically significant.

Results

The mean, minimum and maximum

Table 1: The enzyme activities and semen parameters in seminal fluid of Arabian horses

	March (n = 7)		April (n = 7)		May (n = 7)		Total (n = 21)	
	Mean ± SD	Min-Max	Mean ± SD	Min-Max	Mean ± SD	Min-Max	Mean ± SD	Min-Max
Volume (ml)	43.71±24.29	10.00-75.00	61.57±29.05	18.00-102.00	49.57±22.71	19.00-81.00	51.62±25.35	10.00-102.00
pH	6.74±0.13[a]	6.60-7.00	6.76±0.10[a]	6.60-6.90	6.60±0.10[b]	6.50-6.70	6.70±0.13	7.00-7.00
Concentration ($\times10^6$/ml)	322.86±314.04	70.00-830.00	130.00±77.03	50.00-280.00	241.43±152.47	110.00-540.00	231.43±211.90	50.00-830.00
Total sperm ($\times10^9$)	9641.4±8231.6	3420.0-26220.0	7680.0±4392.9	1260.0-14280.0	10745.7±6174.7	4080.0-20440.0	9355.7±6264.4	1260-26220
Dead sperms (%)	16.93±3.99	10.50-24.00	14.79±7.15	7.50-28.00	11.43±3.09	7.50-16.50	14.38±5.33	7.50-28.00
Progressive motility (%)	78.21±5.90	67.50-82.50	73.93±7.20	60.00-82.50	76.07±7.75	60.00-82.50	76.07±6.87	60.00-82.50
TMD (%)	31.07±10.47	19.50-47.50	25.93±7.63	15.50-38.00	27.57±6.52	18.00-37.50	28.19±8.25	15.50-47.50
HOST (%)	60.79±6.76[ab]	51.00-72.00	57.64±7.36[b]	48.50-68.00	61.43±6.39[a]	50.50-69.00	59.95±6.72	48.50-72.00
AST (IU/L)	105.09±93.86	25.62-253.93	145.27±154.52	27-388.70	187.36±193.87	20.79-423.30	145.90±149.22	20.79-423.30
GGT (IU/L)	10690.7±5055	5435-18123	17043±12969	6327-45204	13484-7682	3560-21377	13739±9106	3560-45204
ALP (IU/L)	17303±17889	2515-52915	15482±16881	4367-51735	6231.7±16881.8	1846-50206	15715±16267	1846-52915
LDH (IU/L)	787.5±524.0	98.92-1605	785.10±729.95	191-1984	810.06±805.66	77.30-1881.30	794.22±661.13	77.30-1984.90
AcP (IU/L)	32.52±26.24	8.45-82.21	33.46±30.71	9.08-98.70	34.18±28.79	6.57-76.20	33.39±27.18	6.57-98.70

[a, b] Values with different superscript in the same row for different month are significantly different $p<0.05$. TMD: Total morphological defect, HOST: Hypo-osmotic swelling test, AST: Aspartate-amino-transferase, GGT: γ-glutamyl-transferase, ALP: Alkaline phosphatase, LDH: Lactate-dehydrogenase and AcP: Acid phosphatase

Table 2: The correlation levels between enzyme activities and semen parameters in seminal fluid of Arabian horses

	pH	Concentration ($\times10^6$/ml)	Dead sperms (%)	Total sperm ($\times10^9$)	Motility (%)	TMD (%)	HOST (%)	AST (IU/L)	GGT (IU/L)	ALP (IU/L)	LDH (IU/L)	AcP (IU/L)
Volume (ml)	-0.36	-0.50*	-0.43	0.17	0.42	-0.47*	0.39	-0.52*	-0.32	-0.47*	-0.65**	-0.36
pH	-	0.09	0.45*	-0.43	-0.56**	0.36	-0.21	-0.05	0.52*	-0.11	0.11	-0.11
Concentration ($\times10^6$)		-	-0.10	0.59**	-0.02	0.61**	-0.18	0.36	-0.01	0.68**	0.40	0.58**
Dead sperms (%)			-	-0.44*	-0.22	0.10	-0.46*	-0.10	0.33	-0.07	0.19	-0.11
Total sperm ($\times10^9$)				-	0.40	0.13	0.07	0.25	-0.20	0.58**	0.16	0.61**
Progressive motility (%)					-	-0.18	0.56**	-0.34	-0.72***	0.15	-0.39	0.10
TMD (%)						-	-0.08	0.15	0.14	0.33	0.26	0.17
HOST (%)							-	-0.31	-0.33	-0.17	-0.39	-0.16
AST (IU/L)								-	0.42	0.70***	0.95***	0.79***
GGT (IU/L)									-	0.04	0.48*	0.18
ALP (IU/L)										-	0.70**	0.92**
LDH (IU/L)											-	0.75***

$^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$

results of enzyme activities (AST, GGT, ALP, LDH and AcP) and the mean semen volume levels, pH, concentration, live:dead ratio, total sperm number, progressive motility, TMD and HOST are presented in Table 1. No significant association was found between semen parameters and the activities of seminal plasma AST, GGT, ALP, LDH and AcP according to the results of two-tailed Pearson correlation test and Friedman analysis in repeated groups, except for pH and HOST in different months. However, significant correlation was found between enzyme activities and semen parameters in seminal fluid of Arabian horses (Table 2). A significant negative correlation was observed between semen volume and concentration ($r = -0.50$, $P<0.05$), TMD and volume ($r = -0.47$, $P<0.05$), AST ($r = -0.52$, $P<0.05$), ALP ($r = -0.47$, $P<0.05$) and LDH ($r = -0.65$, $P<0.01$). pH correlated with live:dead ratio ($r = 0.45$, $P<0.05$), progressive motility ($r = -0.56$, $P<0.01$) and GGT ($r = 0.52$, $P<0.05$). All semen concentrations correlated significantly with TSN ($r = 0.59$), TMD ($r = 0.61$), ALP ($r = 0.68$) and AcP ($r = 0.58$) ($P<0.01$). Furthermore, significant correlations were found between live:dead ratio and TSN ($r = -0.44$), HOST ($r = -0.46$) ($P<0.05$); TSN and ALP ($r = 0.58$), AcP ($r = 0.61$) ($P<0.01$); progressive motility and HOST ($r = 0.56$, $P<0.01$), GGT ($r = -0.72$, $P<0.001$); AST and ALP ($r = 0.70$), LDH ($r = 0.95$), AcP ($r = 0.79$) ($P<0.001$); GGT and LDH ($r = 0.48$, $P<0.05$); ALP and LDH ($r = 0.70$), AcP ($r = 0.92$) ($P<0.01$) and LDH and AcP ($r = 0.75$, $P<0.001$).

Discussion

To the best of our knowledge, very little information is available about the correlation between semen parameters and enzyme activities of stallion seminal plasma. The transaminase activities (AST-ALT) in semen are good indicators of semen quality because they measure sperm membrane stability (Corteel, 1980). Thus, increasing the percentage of abnormal spermatozoa in ejaculate causes high concentration of transaminase enzyme in the extra cellular fluid due to sperm membrane damage and ease of leakage of enzymes from spermatozoa (Gundogan, 2006). Negative correlation was reported by Pesch *et al*. (2006) between AST enzyme and sperm volume. While they reported AST enzyme of 26-400 IU/L for minimum and maximum levels, respectively which was similar to the finding of our study (20.79-423.30 IU/L). It was reported by Pesch *et al*. (2006) that GGT activity correlated significantly with motility and progressive motility and varied between 1300 and 19000 IU/L with median activity of 7500 IU/L. However, we found a significant negative correlation between GGT enzyme and progressive motility ($r = -0.72$, $P<0.001$). GGT plays an important role in the protection of spermatozoa from oxidative stress and provides an indicator of a primary testicular and epididymal origin of this enzyme in stallion (Kohdaira *et al*., 1986; Hinton *et al*., 1998). ALP is a dephosphorylating enzyme that is active in many tissues including bone, liver, kidney, intestine, lung and placenta. The majority of ALP in bulls originates from the seminal vesicles and, to a lesser extent, from the testes and epididymides. Little information is available on ALP activity in the seminal plasma of stallions (Turner and McDonnell, 2003). It was reported by Turner and McDonnell (2003) that the mean concentration of ALP activity in the 11 unprocessed ejaculates was 15.443 ± 6391 IU/L and the values ranged from 22.180 to 3574 IU/L for stallions. This information is consistent with our findings. ALP showed a significant positive correlation with concentration, TSN, AST, LDH and AcP, but not with volume in our study. Most of the energy needed by spermatozoa for motility comes from fructose oxidation in the process of anaerobic glycolysis, whose product is lactic acid, and in its passage through the cell membrane, lactate dehydrogenase plays a role (Bogin *et al*., 1976). LDH activities in stallion spermatozoa were measured 81.0 IU/L by Pesch *et al*. (2006), 13 ± 3 μmol/min.g by Kamp *et al*. (1996) and 1.1 μmol/ml by Brooks (1990), whereas it was found 787.50-810.06 IU/L in our study (Table 1). Pesch *et al*. (2006) reported the correlation

between LDH and motility, progressive motility and living sperm, which may indicates that extracellular LDH ensures metabolism of spermatozoa. In contrast, we found the correlation between LDH and volume ($r = -0.65$, $P<0.01$), AST ($r = 0.95$, $P<0.001$) and ALP ($r = 0.70$, $P<0.01$). Brooks (1990) reported the activity of ACP about 11.1 IU/L in stallions, which was lower than that recorded in this study. Zakrzewska *et al*. (2002) discovered that seminal plasma exhibits high activities of acid and alkaline phosphatase, the acid form being predominant. AcP in stallion is especially localized in corpus epididymidis, ductus epididymidis and vas deferens, but it is thought to be an indicator for the secretory function of prostate in man (Comhaire *et al*., 1989; López *et al*., 1989). An inverse correlation was found between AcP activity and semen concentration. Statistical analysis revealed that AcP activity was maximal in the azoospermic group, and decreased as the sperm concentration increased (Dave and Rindani, 1988). In fertile stallions, there were significant differences at the levels of pH between May and other months, and HOST between April and May ($P<0.05$). In general, these results are almost similar to the findings of Davies-Morel (1999). The reason for this differences is not clear, however it may be explained by the differences in breed, age, nutrition, season, method and frequency of semen collection that are known to influence this parameter (Davies-Morel, 1999).

In conclusion, it can be said that the correlation was found between enzyme activities of seminal plasma and semen parameters in stallions, but there was no significant difference between enzyme activities in stallion seminal plasma and semen parameters in different months, except for pH and HOST. Considering the clinical significance of seminal enzymes and, very little information about this subject in stallions, it is necessary to determine both enzyme activities and their influence on sperm metabolism.

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