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Research Article

Comparison of Spermatological Characteristics and Biochemical Seminal Plasma Parameters of Normozoospermic and Oligoasthenozoospermic Bulls of Two Breeds

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Abstract: The aim of this study was to investigate the differences in spermatological and seminal plasma biochemical characteristics between normozoospermic and oligoasthenozoospermic bulls. A total of 8 bulls were used and they were divided into 4 groups: Holstein normozoospermic (HN) and oligoasthenozoospermic (HO), and Brown Swiss normozoospermic (BSN) and oligoasthenozoospermic (BSO). Spermatological parameters investigated included semen volume, color, concentration, motility, and percentage of abnormal sperm. Biochemical parameters measured in seminal plasma included pH, glucose, urea, creatinine, uric acid, cholesterol (CH), triglyceride, Mg, inorganic P (P_i), Ca, Na, K, and Cl ion concentrations. Colorimetric and ion selective electrode methods were used for these analyses. In general, Ca and P_i levels were lower in the oligoasthenozoospermic groups than in the normozoospermic groups of the 2 breeds. In Brown Swiss bulls, CH and triglyceride levels were higher in the BSN group than in the BSO group. In Holstein bulls, Na level was higher in the HO group than in the HN group. In contrast, K level was higher in the HN group than in the HO group.

Negative correlations were found between abnormal spermatozoa morphology and mass activity, motility, sperm concentration, and CH, P_i, Ca, and Na concentrations; however, there was a positive correlation between abnormal spermatozoa morphology and K^+ ion concentration.

Key Words: Bull semen, normozoospermia, oligoasthenozoospermia, seminal plasma

İki Farklı Irk Normozoospermik ve Oligoasthenozoospermik Boğaların Spermatolojik ve Seminal Plazma Biyokimyasal Parametrelerinin Karşılaştırılması

Özet: Bu çalışmanın amacı, normozoospermic ve oligoasthenozoospermic boğalar arasındaki spermatolojik ve seminal plazma biyokimyasal özelliklerindeki farklılıkların incelenmesidir. Çalışmada toplam 8 baş boğa kullanıldı ve boğalar; Holştayn normozoospermik (HN) ve oligoasthenozoospermik (HO) ile Brown Swiss normozoospermik (BSN) and oligoasthenozoospermik (BSO) olmak üzere 4 gruba ayrıldı. Spermatolojik parametreler olarak hacim, renk, yoğunluk, motilite ve anormal spermatozoa oranı incelendi. Seminal plazma biyokimyasal parametrelerinden ise pH, glikoz, üre, kreatin, ürik asit, kolesterol, trigliserid, magnezyum, inorganik fosfor (P_i), kalsiyum, sodyum, potasyum ve klor iyon konsantrasyonları ölçüldü. Bu analizlerde Kolorimetri ve İyon Selektif Elektrot Metotları kullanıldı. Genellikle, Ca ve P_i seviyeleri her iki ırkın oligoasthenozoospermik gruplarında diğer gruba oranla daha düşük bulundu. Brown Swiss boğalarda CH ve trigliserid seviyeleri BSN grubunda, BSO grubuna göre daha yüksek bulundu. Holştayn boğalarda Na seviyesi HO grubunda HN grubuna göre daha yüksek bulundu. Buna karşılık, K iyonu düzeyi HN grubunda HO gruptan daha yüksek saptandı.

Anormal spermatozoa morfolojisi ile mass aktivite, motilite, yoğunluk, CH, P_i, Ca ve Na konsantrasyonları arasında negatif korelasyonlar bulunurken, K konsantrasyonu ile anormal spermatozoa morfolojisi arasında pozitif korelasyon tespit edildi.

Anahtar Sözcükler: Boğa sperması, normozoospermi, oligoasthenozoospermi, seminal plazma

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Introduction

Classical methods of semen evaluation generally measure sperm concentration, progressive motility, the percentage of viable cells, and morphology. These assays are poor in predicting fertility outcome because only those samples with markedly poor quality can be identified. To solve this problem, new procedures of in vitro seminal analysis or multiple analysis of the same sample have been evaluated (1).

Seminal plasma is very important for sperm metabolism, function, survival, and transport in the female genital tract. Cations such as Na, K, Ca, and Mg in the seminal plasma establish osmotic balance, while essential trace elements are components of many important enzymes. Thus, biochemical evaluation of seminal plasma is an important criterion for assessing fertility and diagnosing male reproductive disorders (2-5).

Abnormal levels of Ca, Mg, Zn, and Cu in seminal plasma are correlated with infertility in humans. Ca is the trigger for the acrosome reaction in mammalian spermatozoa and there is substantial evidence that Ca is differentially involved in sperm motility, depending on the stage of sperm maturation. Mg may also play a role in spermatogenesis, in particular in sperm motility. At the same time, Mg is an important cation found in nearly all enzymatic systems (6-9).

The sugar composition of seminal plasma has also been correlated with fertility, mainly due to its importance to spermatozoa energy production. Fructose and glucose are essential for ATP production and motility of spermatozoa (6,10,11). A key feature in the function of spermatozoa is the lipid composition of seminal plasma and sperm membrane. Cholesterol has a special relevance since it is the most abundant lipid in the spermatozoa of all mammalian species (12). The main nitrogenous components of urine, such as ammonia, urea, uric acid, and creatinine, also exist in seminal plasma. In bulls uric acid is probably formed by oxidation of hypoxanthine and xanthine, both of which occur in the seminal vesicles (13,14).

We observed that there was an oligoasthenozoospermia problem in the Holstein and Brown Swiss breeds during routine evaluations of semen quality at the Lalahan Livestock Central Research Institute, Artificial Insemination Laboratory. After semen freezing, sperm motility was < 10%. The aim of the present study was to investigate whether there were any differences in spermatological and biochemical seminal plasma characteristics of normozoospermic and oligoasthenozoospermic Holstein and Brown Swiss bulls.

Materials And Methods

Materials

The investigation was conducted at the Lalahan Livestock Central Research Institute, Artificial Insemination Laboratory, Ankara, Turkey. A total of 8 bulls aged between 3 and 4 years were used. The animals were divided into 4 groups of 2 bulls. Semen samples were collected twice a week using a bovine artificial vagina. A total of 80 ejaculates were analyzed (10 from each bull). Semen samples were categorized as normozoospermic (sperm concentration $> 100 \times 10^6$/ml and > 50% motile sperm) and oligoasthenozoospermic (sperm concentration $< 100 \times 10^6$ ml and < 50% motile sperm) (1).

Methods

Following collection, the semen samples were placed in a 34 °C water bath and evaluated for volume (ml), color, sperm concentration ($\times 10^6$ ml^{-1}), and forward progressive motility (%) using traditional semen analysis methods (1,11). For analysis of abnormal spermatozoa morphology, the semen samples were fixed with Hancock's solution and stained with Giemsa. These pathological changes were classified as head, mid-piece, and tail abnormalities.

After the biophysical semen parameters were evaluated, the semen samples were centrifuged at 3000 × g for 20 min at 4 °C to separate out seminal plasma. The seminal plasma samples were kept frozen at –20 °C until analysis. The levels of glucose, urea, creatinine, uric acid, CH, triglyceride, Mg, P, and Ca of the samples were determined using the colorimetric method, while Na, K, and Cl were determined using the ion selective electrode method. All biochemical analyses were carried out using the Olympus AU 600 analyzer system (Olympus Systems, Mishima, Japan) (15).

Statistical Analysis

A factorial design with a one-way classification (ANOVA) model was used to analyze the effects of different factors. Duncan's multiple range test was used to investigate differences between the means of the groups, while Pearson's correlation analysis was used for determining relationships between groups.

Results

Data on semen parameters are presented in Table 1. There was no individual variation in the volume of semen among the groups ($P > 0.05$). There was a positive correlation between semen volume, and P and K levels, and a negative correlation with Na and Cl levels. The majority of the semen samples examined had a milky color. Within breeds, the semen mass activity and sperm motility were naturally significantly different between the normozoospermic and oligoasthenozoospermic groups ($P < 0.05$). There were no significant differences in pH values among the bulls ($P > 0.05$).

Comparing the 2 breeds, sperm concentration was significantly lower in Brown Swiss bulls (BSN and BSO) than in Holstein (HN and HO) bulls. Semen concentration was positively correlated with volume, mass activity, motility, CH, and P_i levels (r_s: 0.23^{*}; r_s: 0.63^{**}; r_s: 0.63^{**}; r_s: 0.65^{**}; r_s: 0.53^{**}), but it was negatively correlated with abnormal head (%) and mid-piece (%), and Cl levels (r_s: -0.57^{**}; r_s: -0.42^{**}; r_s: -0.41^{**}) ($P < 0.01$). No significant differences were detected among the groups, except in BSO bulls (25.75 ± 2.80) for abnormal head ratio (%) ($P > 0.05$).

For abnormal mid-piece and tail, while no significant differences were detected between the normozoospermic groups (1.80 ± 0.18 for HN and 3.10 ± 0.37 for BSN) ($P > 0.05$), there were significant differences between the oligoasthenozoospermic groups for both abnormal mid-piece (13.55 ± 2.74 and 30.55 ± 2.76 for HO and BSO, respectively) and abnormal tail (19.60 ± 3.59 and 14.40 ± 0.85 for HO and BSO, respectively) ($P < 0.01$). There were also positive correlations between abnormal head and K levels (r_s: 0.34^{**}) in the oligoasthenozoospermic groups.

Data on seminal plasma parameters are presented in Table 2. There were significant differences between creatinine, urea, and uric acid levels in the seminal fluids of the groups ($P < 0.05$). Although the glucose concentration of seminal plasma in Holstein bulls was significantly lower than in Brown Swiss bulls ($P < 0.01$), the seminal plasma CH and triglyceride levels in the BSO group were lower than those in the BSN group ($P < 0.01$). While CH level was positively correlated with P_i and Ca concentration (r_s: 0.84^{**}; r_s: 0.25^{*}), it was negatively correlated with Cl (r_s:-0.44^{**}). In contrast to the Holstein groups, the Mg level showed significant differences between both groups of Brown Swiss bulls (P

Table 1. Least square means, standard deviation, and Duncan's multiple range test results of the spermatological parameters according to breed and group.

Traits	Variance analysis		Holstein n = 40		Brown Swiss n = 40	
	Breed n = 80		Normal (HN) n = 20	Abnormal (HO) n = 20	Normal (BSN) n = 20	Abnormal (BSO) n = 20
	MS	F	$\bar{X} \pm \bar{x}$	$\bar{X} \pm \bar{x}$	$\bar{X} \pm \bar{x}$	$\bar{X} \pm \bar{x}$
Semen volume (ml)	4.51	0.96	8.55 ± 0.56^{a}	5.67 ± 0.45^{b}	6.30 ± 0.54	6.97 ± 0.34
Mass activity (0-5)	20.00	29.86**	4.70 ± 0.12^{a}	1.25 ± 0.26^{b}	3.95 ± 0.22^{a}	0.00 ± 0.00^{b}
Motility (%)	5297.51	32.99**	82.50 ± 1.51^{a}	33.75 ± 5.41^{b}	82.75 ± 0.57^{a}	0.95 ± 0.40^{b}
Conc. ($\times 10^6$ ml^{-1})	1818986.40	21.48**	1029.50 ± 74.31^{a}	92.63 ± 50.30^{b}	469.45 ± 90.94^{a}	49.52 ± 24.72^{b}
Sem. plasma vol. (ml)	9.11	4.93*	5.45 ± 0.30^{a}	3.70 ± 0.28^{b}	4.45 ± 0.37^{b}	6.05 ± 0.24^{a}
Head (%)	2322.80	56.87**	3.20 ± 0.35	3.85 ± 0.40	2.90 ± 0.20^{b}	25.75 ± 2.80^{a}
Mid-piece (%)	1674.45	21.79**	1.80 ± 0.18^{b}	13.55 ± 2.74^{a}	3.10 ± 0.37^{b}	30.55 ± 2.76^{a}
Tail (%)	82.01	1.14*	1.85 ± 0.23^{b}	19.60 ± 3.59^{a}	3.00 ± 0.82^{b}	14.40 ± 0.85^{a}

[a,b] Means within rows followed by different superscripts are significantly different ($P < 0.05$).
*$P < 0.05$.
**$P < 0.01$.

Table 2. Least square means, standard deviation, and Duncan's multiple range test results of the seminal plasma parameters according to breed and group.

Traits	Variance analysis		Holstein n = 40		Brown Swiss n = 40	
	Breed n = 80		Normal (HN) n = 20	Abnormal (HO) n = 20	Normal (BSN) n = 20	Abnormal (BSO) n = 20
	MS	F	$\bar{X} \pm \bar{x}$	$\bar{X} \pm \bar{x}$	$\bar{X} \pm \bar{x}$	$\bar{X} \pm \bar{x}$
Glucose (mg dl^{-1})	11.11	9.32**	128.10 ± 16.90[a]	115.70 ± 27.56[b]	183.15 ± 12.13[a]	140.30 ± 23.86[b]
Urea (mg dl^{-1})	0.01	0.11	45.85 ± 12.19	28.45 ± 1.00	29.85 ± 0.78	30.95 ± 0.69
Creatinine (mg dl^{-1})	0.05	0.38	2.37 ± 0.73	1.40 ± 0.07	1.44 ± 0.04	1.43 ± 0.03
Uric acid (mg dl^{-1})	44.91	7.83**	8.17 ± 0.90[a]	5.39 ± 0.50[b]	5.95 ± 0.15	4.62 ± 0.24
Cholesterol (mg dl^{-1})	1248.20	24.74**	24.75 ± 1.00[a]	21.75 ± 2.18[b]	18.80 ± 1.14[a]	11.90 ± 1.73[b]
Triglyceride (mg dl^{-1})	0.34	1.27*	75.40 ± 6.45	68.15 ± 9.71	64.20 ± 5.22	53.65 ± 4.77
Magnesium (mg dl^{-1})	10.85	4.48*	8.05 ± 0.28	8.52 ± 0.26	8.09 ± 0.14[a]	7.01 ± 0.55[b]
Phosphorus (mg dl^{-1})	17.86	5.66*	6.50 ± 0.36[a]	4.43 ± 0.51[b]	5.12 ± 0.35[a]	3.92 ± 0.32[b]
Calcium (mg dl^{-1})	1.17	0.17	30.96 ± 0.54[a]	28.69 ± 0.47[b]	30.64 ± 0.35[a]	28.53 ± 0.85[b]
Sodium (mmol l^{-1})	2868.01	10.28**	103.25 ± 4.32[b]	123.45 ± 2.21[a]	105.40 ± 5.00	97.35 ± 2.66
Potassium (mmol l^{-1})	438.98	8.58**	30.83 ± 2.13[a]	18.08 ± 0.53[b]	25.16 ± 2.24[b]	33.12 ± 0.59[a]
Chloride (mmol l^{-1})	61.25	0.54	58.40 ± 2.45	61.50 ± 2.27	59.40 ± 2.13	64.00 ± 2.64

[a,b] Means within rows followed by different superscripts are significantly different ($P < 0.05$).
*$P < 0.05$.
**$P < 0.01$.

< 0.05). There was no correlation between Mg concentration, and biochemical and biophysical semen parameters.

In general, P_i concentration of oligoasthenozoospermic bull semen was lower than that of normozoospermic bulls ($P < 0.01$). P_i concentration was positively correlated with mass activity, motility, and sperm concentration, and CH and K levels, but was negatively correlated with abnormal head and mid-piece, and Na and Cl levels (Table 3).

Although Ca concentration in seminal plasma was similar in the 2 breeds, there were significant differences between the 2 groups within each breed ($P < 0.01$). There were negative correlations between abnormal head, mid-piece, and tail, and Cl concentration (r_s: –0.24*; r_s: –0.40**; r_s: –0.36**; r_s: –0.24*). The seminal plasma Na ion level was significantly different between the 2 groups of Holstein bulls ($P < 0.01$), but was not different between the 2 Brown Swiss groups ($P > 0.05$). K concentration was higher in the HN and BSO groups and there were significant differences between both breeds ($P < 0.01$) and groups ($P < 0.05$). There were positive correlations between K level and semen volume, abnormal head and P concentration (r_s: 0.34**; r_s: 0.34**; r_s: 0.40**), and a strong negative correlation between K and Na concentrations (r_s: –0.89**). Although there were significant differences in Cl ion concentration between the groups ($P < 0.05$), no significant differences were observed between the 2 breeds ($P > 0.05$).

Discussion

Spermatozoa motility showed significant differences between the HN/SBN and HO/BSO groups ($P < 0.05$), and motility was generally very low in oligoasthenozoospermic bulls. There were significant differences in sperm concentration between the groups and breeds ($P < 0.05$). In the present study, spermatozoa concentrations measured in normozoospermic bulls

Table 3. Correlations between biochemical and spermatological parameters.

Parameters	Sperm conc.	Ab. Head	Ab. M. piece	Ab. Tail	CH	P_i	Ca	Na	K	Cl
Sperm conc.	**	–0.57**	–0.42**	0.05	0.65**	0.53**	0.11	–0.01	–0.01	–0.41**
Ab. Head		**	0.45**	0.24*	–0.40**	–0.23*	–0.24*	–0.28*	0.34**	0.09
Ab. M. piece			**	0.46**	–0.36**	–0.30**	–0.40**	–0.07	0.17	0.18
Ab. Tail				**	0.15	–0.01	–0.36**	0.15	–0.15	–0.09
CH					**	0.84**	0.25*	–0.13	0.02	–0.44**
Pi						**	0.11	–0.46**	0.40**	–0.39**
Ca							**	0.10	–0.15	–0.24*
Na								**	–0.89**	0.40**
K									**	–0.18
Cl										**

*Correlation significant at $P < 0.05$.
**Correlation significant at $P < 0.01$.

were lower than those reported by Vaisberg et al. (16) ($1594 \pm 14.5 \times 10^6$ ml^{-1}) and Garner et al. (11) (1160×10^6 ml^{-1}), whereas they were higher than those reported by Vera et al. (17) (739.16×10^6 ml^{-1}) and similar to those reported by other researchers (10,18,19).

For abnormal sperm morphology, there were positive correlations between abnormal head and K levels; however, there were negative correlations between abnormal head and mid-piece, and CH, Ca, Na, and P_i concentrations (Table 3). Percentage of sperm abnormality findings were similar to those reported by Assumpção et al. (10), Garner and Hafez (20), and Vaisberg et al. (16), and lower than those reported by Massányi et al. (4). Biochemical evaluation of seminal plasma is an important criterion in assessing fertility levels and diagnosing male reproductive disorders (2,4,5); therefore, we evaluated a wide range of trace elements (Ca, Mg, K, Na, P, and Cl) and other biochemical parameters in the seminal plasma of the 2 breeds. We detected significant differences between normozoospermic and oligoasthenozoospermic bulls within the 2 breeds according to the seminal plasma biochemical parameters and spermatological characteristics.

According to Wong et al. (9), Mg is regarded as a marker of seminal vesicle secretions. In the present study there were significant differences between the 2 Brown Swiss bull groups ($P < 0.05$). These findings are supported by those reported by Garner and Hafez (20). In contrast to the Brown Swiss groups, Mg concentration was higher in the HO group than in the HN group. Gür and Demirci (18) reported an average Mg value of 6.32 mg dl^{-1} in normozoospermic Holstein bulls. This value is lower than the concentration measured in the present study (7-8.5 mg dl^{-1}) and that reported by Garner and Hafez (20) (8.05 mg dl^{-1}), and is considerably lower than the 12 mg dl^{-1} reported by Özkoca (21). It is reasonable to assume that Mg concentration was affected by factors other than breed, for example feeding conditions and methods of analysis.

Various authors have reported seminal plasma Ca concentrations between 24 and 45 mg dl^{-1} (18,20,21). Ca values in the present study did not differ between the 2 breeds ($P > 0.05$); however, there were significant differences between the groups of each breed ($P < 0.01$). Generally, P_i value was higher in Holstein bulls than in Brown Swiss bulls ($P < 0.05$) and was higher in the normozoospermic groups than in the oligoasthenozoospermic groups ($P < 0.01$). Similarly, Machal et al. (22) reported that P_i levels in bulls were positively correlated with sperm concentration.

The Na ion is an important element for spermatozoa functioning (22,23). For the 2 breeds investigated in the present study Na concentration was significantly higher in the HO group than in other groups (HN, BSN, and BSO) ($P < 0.01$). Although there was no correlation detected

between Na and sperm motility, there was a negative correlation between Na, and abnormal head and P_i level. It is known that modulation of a variety of ion channels (like Cl) of spermatozoa is a characteristic event associated with capacitation and acrosome reaction of mammalian spermatozoa (5,24); hence, increasing the Cl level in seminal plasma may play a role in infertility. There were significant differences in Cl levels between the groups of the 2 breeds ($P < 0.05$).

K is a natural metabolic inhibitor and a higher K concentration in seminal plasma decreases the metabolic activity of spermatozoa. Therefore, there is generally a negative correlation between K and sperm motility (18,19,25). In the present study, similar results were observed between the groups of Brown Swiss bulls; however, Na and K levels were unexpectedly higher in the HO group than in the HN group, but the cause of this is not known. The differences in the concentrations of some elements in seminal plasma may have been due to variations in exposure from feeding, management, and different detection methods.

The sugar composition of seminal plasma has been related to fertility, mainly due to its importance to spermatozoa energy production (10). In the present study we measured only glucose, and it was detected at significantly higher levels in normozoospermic bulls than in oligoasthenozoospermic bulls ($P < 0.01$). CH and triglyceride levels were significantly higher in normozoospermic bulls in comparison to oligoasthenozoospermic bulls ($P < 0.01$). Our results indicated that the cause of oligoasthenozoospermia in the study bulls was due to elements in seminal plasma other than those mentioned above.

In conclusion, lower Ca and P_i levels were measured in seminal plasma of the oligoasthenozoospermic groups than in the normozoospermic groups of the 2 breeds studied. The distribution of major ions between semen and seminal plasma could provide the basis for variation in semen quality between breeds and should be considered in the interpretation of results obtained in the evaluation of bull fertility. Success in predicting fertility is limited by spermatozoa characteristics, the process of fertilization, and approaches used for in vitro evaluation of seminal quality. We conclude that analysis of both biophysical and biochemical characteristics of semen provide very useful information regarding semen freezability and male fertility.

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