

Some Reproductive Parameters and Seminal Plasma Constituents in Relation to Season in Akkaraman and Awassi Rams

Mustafa GÜNDOĞAN

Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Afyon Kocatepe University,
ANS Campus, TR-03200, Afyonkarahisar-TURKEY
E-mail: mgundogan@aku.edu.tr

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Abstract: The aim of this study was to describe seasonal changes in scrotal circumference, sperm motility, sperm concentration, percentage of abnormal spermatozoa and biochemical and enzymatic properties of seminal plasma for 9 months in 10 mature fat-tailed Akkaraman and Awassi breed rams. Scrotal circumference and semen were evaluated monthly from rams and seminal plasma separated from semen to study it. Scrotal circumference, sperm motility and sperm concentration were higher during autumn months. Furthermore, autumn months showed moderate percentage of abnormal spermatozoa. The biochemical analyses of ram seminal plasma indicated that the highest values of total protein, albumin (A), globulin (G), total lipid and cholesterol were recorded in autumn while A/G ratio exhibited the lowest values. Alanine amino transferase (ALT) showed the lowest values in summer, while aspartate amino transferase (AST) and AST/ALT ratio exhibited the lowest values in autumn. The results suggest that rams show seasonality for some reproductive parameters and seminal plasma constituents; therefore Akkaraman and Awassi rams could be bred successfully in autumn months.

Key Words: Ram, season, semen, seminal plasma.

Akkaraman ve İvesi Koçlarda Bazı Reprodüktif Parametrelerin ve Seminal Plazma Bileşenlerinin Mevsimle İlişkisi

Özet: Bu çalışma, yağlı kuyruklu 5 Akkaraman ve 5 İvesi olmak üzere toplam 10 koçta dokuz ay süresince skrotum çevresi, spermatozoa motilitesi ve yoğunluğu, anormal spermatozoa oranı ile seminal plazmanın biyokimyasal ve enzimatik özelliklerindeki mevsimsel değişimleri araştırmak amacıyla yapıldı. Skrotum çevresi ve sperma aylık olarak alındı ve seminal plazma ayrılarak çalışma için değerlendirildi. Skrotum çevresi, spermatozoa motilitesi ve yoğunluğunun sonbahar aylarında yüksek olduğu gözlenirken anormal spermatozoa oranının bu dönemde makul seviyelerde olduğu gözlemlendi. Koçların seminal plazmalarının biyokimyasal analizlerinde sonbaharda toplam protein, albumin (A), globulin (G), toplam lipid ve kolesterol miktarlarının en yüksek değerleri gözlenirken A/G oranının en düşük değerde olduğu tespit edildi. Alanine amino transferase (ALT) değeri en düşük yazın, aspartate amino transferase (AST) değeri ve AST/ALT oranı en düşük sonbaharda gözlemlendi. Sonuç olarak, koçların bazı reprodüktif parametrelerinin ve seminal plazma bileşenlerinin mevsimsel olarak etkilendiği ve bu yüzden Akkaraman ve İvesi koçların yetiştirme için sonbahar aylarının tercih edilmesi yararlı olacaktır.

Anahtar Sözcükler: Koç, mevsim, sperma, seminal plazma.

Introduction

Small ruminants show a seasonal pattern in reproductive activity that is shaped by seasonal changes in their habitats. The seminal plasma, an extracellular fluid that provides the medium and vehicle for spermatozoa, is a composite mixture of secretions that come from the male accessory organs of reproduction. Biochemical estimates of seminal plasma are used for semen

evaluation, since using semen characteristics alone are not completely satisfactory for semen appraisal in the current practice of commercial artificial insemination (1-4). Few studies (3,4) have been carried out on males to investigate seasonal variations in their semen quality. However, the biochemical evaluation of ram semen and its relationship with physical characteristics are still completely unknown. With better knowledge of ram

reproductive physiology more accurate andrological evaluations could be conducted, which would improve reproductive efficiency and enhance breeding schemes and the rate of genetic gain. The present study is concerned with investigating the seasonal variations in some major enzymatic and biochemical constituents of seminal plasma and their relationship with some indicators of semen quality.

Materials and Methods

The work was carried out from June 2001 to February 2002 at the Veterinary Faculty of Afyon Kocatepe University.

Animals and management

In a factorial randomized complete-block design experiment, 10 sexually mature rams were used. Five Akkaramans of average weight 84.0 (s.e.m. 1.78) kg and 5 Awassis of average weight 86.5 (s.e.m. 4.41) kg were used to study the reproductive performance of these ram groups throughout 9 months. All animals were mean 48 ± 3 months old and were housed in a semi-open barn all day. Each animal received 500 g/day of a pelleted concentrate mixture and 1.1 kg/day of dry trefoil (5).

Andrological evaluation

In terms of andrological performance, scrotal circumference was measured monthly using a flexible meter described by Demirci (6). Semen was collected monthly using an artificial vagina. Ejaculates were placed in a water bath at 38 °C. Sperm motility (%) was subjectively estimated at 400 X magnification using a light microscope equipped with a warm stage and sperm concentration was measured by haemocytometric method (6). Percentages of abnormal spermatozoa were assessed according to Hafez (7).

Seminal plasma constituents

Seminal plasma was separated from ejaculates by centrifugation at 5000 r.p.m. for 10 min. The recovered seminal plasma fraction was further centrifuged at 10,000 r.p.m. for 15 min 4 °C and the supernatant was stored at -20 °C until analysis. Total seminal plasma protein was measured by the biuret method and total

albumin (A) concentration was determined by the method described by Doumas et al. (8). Total globulin (G) concentration was calculated as the difference between seminal plasma total protein and seminal plasma albumin; then A/G ratio was calculated. Total lipids and cholesterol concentrations were measured by a colorimetric method (9). Transaminase activities (aspartate amino transaminase, AST and alanine amino transaminase, ALT) were measured in a Hitachi 917 Clinical Chemistry Analyzer using commercially available diagnostic kits supplied by Roche Diagnostics GmbH (D-68298, Mannheim, Germany). Both enzyme activities were determined photometrically in which the decrease in NADH levels was directly proportional to enzyme activities and AST/ALT ratios were calculated (10).

Statistical analysis

According to the prevailing climatic conditions in Turkey, the months were gathered in 3 groups: Summer (June, July, August), Autumn (September, October, November) and Winter (December, January, February). Statistical analysis was performed using the general linear model procedure (11). A fixed effect model was assumed to underlay each observation in each trait studied. These effects were breed, season and the interaction between them. Significant differences among means were detected using Duncan's multiple range test of SAS (12).

Results

Climatological data during the period of 9 months are presented in the Figure. The seasonal values in scrotal circumference, sperm motility, sperm concentration and percentage of abnormal spermatozoa during the period of 9 months are presented in Table 1. These parameters varied significantly ($P < 0.01$) between seasons. Scrotal circumference significantly ($P < 0.01$) varied between breeds. Results for scrotal circumference, sperm motility and sperm concentration showed higher ($P < 0.01$) values during autumn than during the other seasons. Percentage of abnormal spermatozoa showed lower ($P < 0.01$) values during autumn and winter than during summer. The seasonal variations in seminal plasma total protein, albumin (A), globulin (G), A/G ratio, total lipid and cholesterol during the period of 9 months are presented in Table 2. Total protein concentration decreased in winter and summer and then increased again

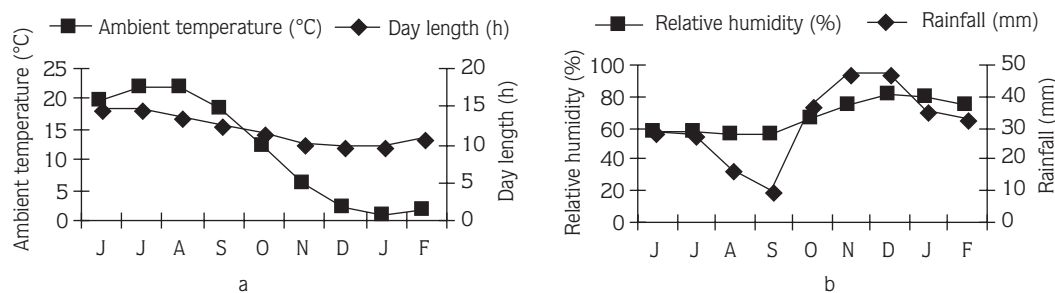


Figure. a; Mean values of ambient temperature (°C) and day length (h) during the period of 9 months. b; Mean values of relative humidity (%) and rainfall (mm) during the period of 9 months.

Table 1. Overall mean values of scrotal circumference, sperm motility, sperm concentration and percentage of abnormal spermatozoa in two ram breeds during the seasons studied ($X \pm s.e.m.$).

Season	n	Scrotal circumference (cm)	Sperm motility (%)	Sperm concentration ($\times 10^9/ml$)	Abnormal sperm (%)
Summer	30	31.42 ± 0.38^c	56.50 ± 2.05^c	3.36 ± 0.22^b	4.43 ± 0.14^a
Autumn	30	35.08 ± 0.94^a	78.58 ± 0.88^a	4.92 ± 0.13^a	3.27 ± 0.16^b
Winter	30	32.59 ± 0.72^b	67.17 ± 2.32^b	4.55 ± 0.11^a	3.42 ± 0.08^a

a-c : The different superscript lowercase letters in each column are statistically different ($P < 0.01$).

Breed	n	Scrotal circumference (cm)	Sperm motility (%)	Sperm concentration ($\times 10^9/ml$)	Abnormal sperm (%)
Akkaraman	45	31.72 ± 0.37	67.89 ± 3.36	4.17 ± 0.25	3.69 ± 0.21
Awassi	45	34.28 ± 0.82	66.88 ± 3.62	4.38 ± 0.28	3.72 ± 0.21
Degree of significance		*	-	-	

* : $P < 0.01$

Table 2. Overall mean values of some biochemical properties of seminal plasma in two ram breeds during the seasons studied ($X \pm s.e.m.$).

Season	n	Total protein (g/100 ml)	Albumin (A) (g/100 ml)	Globulin (G) (g/100 ml)	A / G	Total Lipid (mg/100 ml)	Cholesterol (mg/100 ml)
Summer	30	2.30 ± 0.04^b	1.12 ± 0.03^b	1.18 ± 0.02^b	0.96 ± 0.01^b	254.50 ± 12.86^c	111.0 ± 8.06^c
Autumn	30	2.50 ± 0.06^a	1.22 ± 0.03^a	1.28 ± 0.03^a	0.95 ± 0.04^b	396.17 ± 12.97^a	161.33 ± 9.89^a
Winter	30	2.28 ± 0.05^b	1.13 ± 0.04^b	1.15 ± 0.04^b	0.99 ± 0.05^a	346.83 ± 23.39^b	128.0 ± 10.92^b

a-c : The different superscript lowercase letters in each column are statistically different ($P < 0.01$).

Breed	n	Total protein (g/100 ml)	Albumin (A) (g/100 ml)	Globulin (G) (g/100 ml)	A / G	Total Lipid (mg/100 ml)	Cholesterol (mg/100 ml)
Akkaraman	45	2.40 ± 0.05	1.25 ± 0.05	1.17 ± 0.02	0.96 ± 0.06	328.11 ± 24.90	133.11 ± 11.72
Awassi	45	2.32 ± 0.05	1.18 ± 0.04	1.15 ± 0.03	0.97 ± 0.05	336.89 ± 24.58	133.78 ± 9.47
Degree of significance		-	-	-	-	-	

- : $P > 0.05$

in autumn. Breed had no significant effect on total protein concentration. Albumin and globulin concentrations increased during autumn and A/G ratio decreased at the same time. Total lipid and cholesterol decreased during summer and winter, and then started to increase, recording the highest values in autumn, and the effect of breed was not significant. There was a significant ($P < 0.01$) effect of season on activities of aspartate amino transferase (AST), alanine amino transferase (ALT) and AST/ALT ratio (Table 3). AST activity and AST/ALT ratio recorded the lowest values, while the highest values were recorded for ALT activity in autumn. There was no significant effect of breed on seminal plasma AST, ALT activity and AST/ALT ratio.

Discussion

Seminal plasma is synthesized and secreted by the testes and accessory sex glands in males and plays significant roles in the development of sperm motility and freezing ability. It is known that seminal plasma proteins coat and protect spermatozoa during ejaculation. Many studies have shown that low content of seminal plasma proteins is associated with poor semen quality (1,13). In the present study, a positive relationship was found between scrotal circumference, sperm motility, sperm concentration and level of total proteins in ram seminal plasma. Seminal plasma proteins are mainly composed of albumin and globulin in addition to small quantities of non-protein nitrogen, amino acids and peptides (14). These compounds make up the amphoteric property of seminal plasma proteins and, thus, low protein content in

seminal plasma reduces its buffering capacity and in turn semen quality (15). In addition, Barrios et al. (2) reported that the freezability of semen is positively correlated with total protein content in seminal plasma. In fact, the beneficial effects of seminal plasma proteins in improving sperm motility reside in their content of albumin or specific factors in seminal plasma (16). This suggestion is confirmed in the present study where the highest albumin level was noted in autumn (Table 2) and coincided with the highest sperm motility (Table 1) in the same season. The increase in protein content was due to the increase in globulin level as well. In the present study, A/G ratio was high (low globulin) during the season of poor semen quality and was low (high globulin) during the season of good semen quality. Taha et al. (17) reported similar findings in rams.

Seminal lipids play significant roles in the membrane structure of spermatozoa, sperm metabolism, sperm capacitation and fertilization of the female gamete (6,7). In addition, some researchers (17,18) reported that reductions in sperm concentration and motility were associated with a decrease in seminal plasma content of lipids and also with sperm aging (poor semen quality). In the present study, a low level of total lipid and cholesterol during the summer (Table 2), as well as low values of sperm motility and sperm concentration during the same period was observed (Table 1).

The transaminase activities (AST and ALT) in semen is a good indicator of semen quality because it measures sperm membrane stability (19). Thus, increasing the percentage of abnormal spermatozoa in ejaculate causes

Table 3. Overall mean values of some enzymatic properties of seminal plasma in two ram breeds during the seasons studied ($X \pm s.e.m.$).

Season	n	AST	ALT	AST / ALT
Summer	30	364.02 ± 12.06 ^a	6.63 ± 0.11 ^b	54.82 ± 1.04 ^a
Autumn	30	295.30 ± 18.16 ^b	7.27 ± 0.42 ^a	40.68 ± 1.26 ^b
Winter	30	388.33 ± 20.72 ^a	7.40 ± 0.52 ^a	52.77 ± 1.08 ^a

a,b : The different superscript lowercase letters in each column are statistically different ($P < 0.01$).

Breed	n	AST	ALT	AST / ALT
Akkaraman	45	347.78 ± 20.67	7.17 ± 0.34	48.85 ± 2.76
Awassi	45	350.67 ± 18.36	7.03 ± 0.31	49.99 ± 1.91
Degree of significance	-	-	-	-

- : $P > 0.05$

high concentration of transaminase enzyme in the extracellular fluid due to sperm membrane damage and ease of leakage of enzymes from spermatozoa. In the present study, positive relationships were found between the percentages of abnormal spermatozoa and AST level and AST/ALT ratio in ram seminal plasma. AST level and AST/ALT ratio were low (moderate percentage of abnormal spermatozoa) during the season of good semen quality (Tables 1-3). Furthermore, many studies have correlated the AST level in semen with sperm concentration (20). In the present study, negative correlations were observed with sperm concentration, AST level and AST/ALT ratio. Taha et al. (17) reported similar findings in rams. The highest seminal plasma level of (Table 3) ALT activity was in winter and coincided with the moderate sperm concentration (Table 1). Taha et al. (17) reported different findings in Barki and Awassi rams under subtropical conditions. This may be due to breed, season, latitude, age, feeding and management.

In conclusion, the biochemical analyses of rams' seminal plasma indicated that the highest values of total protein, albumin, globulin, total lipid, cholesterol and ALT level were recorded in autumn, while AST level and AST/ALT ratio exhibited the lowest values. In terms of transaminase activities, AST showed the highest activity in winter and the lowest activity in autumn, while ALT exhibited the highest activity in winter and the lowest activity in summer. The results suggest that rams show a reproductive seasonality for seminal plasma constituents. Further studies are needed to elucidate the roles of spring months in seminal plasma constituents. However, Akkaraman and Awassi rams could be bred successfully in autumn months.

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