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Research Article

Some Reproductive Parameters and Biochemical Properties in Akkaraman and Awassi Rams

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Abstract: The objective of the present study was to describe some reproductive parameters, hormonal levels and some biochemical properties of blood serum in rams under the Afyonkarahisar province conditions. Semen (via an artificial vagina) and blood were collected five times during the breeding season from a total of 10 mature and healthy fat-tailed rams (Akkaraman and Awassi) in Afyonkarahisar. Breed effects were significant for relative testes volume, sperm concentration, percentage of abnormal spermatozoa, triiodothyronine (T_3), albumin/globulin ratio, total lipid ($P < 0.05$) and cholesterol ($P < 0.01$). Some reproductive parameters and biochemical properties of Akkaraman and İvesi rams indicated that the values were recorded in the optimum range during the breeding season under Afyonkarahisar conditions. In addition, some reproductive parameters of rams show a correlation with some biochemical and enzymatic properties.

Key Words: Biochemical properties, hormone, ram, semen.

İvesi ve Akkaraman Koçlarda Bazı Reprodüktif Parametreler ve Biyokimyasal Özellikler

Özet: Afyonkarahisar koşullarında yetiştirilen koçlarda bazı reprodüktif parametreler ve hormon miktarları ile kan serumu biyokimyasal özelliklerin araştırılması amacıyla yapılan bu çalışmada, aşım sezonu esnasında, yağlı kuyruklu ve sağlıklı toplam 10 koçtan (Akkaraman ve İvesi) beşer numune kan ve sun'i vagen yardımıyla da sperma toplandı. Relatif testis hacmi, spermatozoa yoğunluğu, anormal spermatozoa oranı, triiodothyronine (T_3) miktarı, albumin/globulin oranı, toplam lipid miktarı ($P < 0,05$) ve kolesterol miktarı ($P < 0,01$) yönünden koçlar arasındaki farklılıkların önemli olduğu gözlendi. Afyonkarahisar koşullarında aşım sezonu esnasındaki İvesi ve Akkaraman koçların bazı reprodüktif değerlerinin ve bazı biyokimyasal özelliklerinin optimal sınırlar içerisinde olduğu ve ayrıca koçların bazı reprodüktif parametrelerinin bazı biyokimyasal ve enzimatik özellikler ile ilişkileri olduğu gözlendi.

Anahtar Sözcükler: Biyokimyasal özellik, hormon, koç, sperma.

Introduction

Most sheep show a seasonal pattern in reproductive activity that is shaped by seasonal changes in their habitats. Biochemical estimates of blood serum are used for semen evaluation, since using semen characteristics alone is not completely satisfactory for semen appraisal in the current practice of commercial artificial insemination (1-4). However, the biochemical evaluation of ram blood and its relationship with semen characteristics are completely unknown. With better knowledge of ram reproductive physiology a more accurate andrological evaluation 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 some major biochemical constituents of blood serum and their relationship with hormonal levels and some reproductive parameters in Akkaraman and Awassi rams during the breeding season under Afyonkarahisar province conditions.

Materials and Methods

This work was carried out in the breeding season (September-October 2001) at the Research and

Manipulation Livestock of the Faculty of Veterinary Medicine, University of Afyon Kocatepe located in Afyonkarahisar province (L: 1021 m, 38° 45´ N, 30° 32° W), Turkey. Semen evaluation was carried out at the Faculty of Veterinary Medicine, Afyon Kocatepe University. Hormonal and biochemical analyses were carried out at the Medicine School of Afyon Kocatepe University.

Animals and management

In a factorial randomized complete-block design experiment, 10 sexually mature and healthy rams were used. Each of five Akkaraman and Awassi were used to study the relationship between reproductive parameters and biochemical constituents of blood serum in rams groups during the breeding season. All animals were aged 3-4 years. Animals were offered rough and concentrate supplement according to their body weight requirements (5).

In terms of sexual performance, scrotal sac volume was measured by the water displacement method described by Demirci (6) and was then divided by body weight to calculate the relative testes volume. Sexual behavior of rams was recorded by using the reaction time criterion. The time elapsed between introducing the ram to a female ewe at estrous and semen collection was measured using a stopwatch.

Semen evaluation

Total 5 ejaculates were collected weekly by using an artificial vagina. Ejaculates were placed in a water bath at 38 °C. Progressive motility (%) was subjectively estimated at 400 X magnification using a light microscope. Sperm concentration was measured by a haemocytometer slide. Percentages of abnormal spermatozoa were assessed by fluid fixation method according to Demirci (6).

Blood Collection

Total 5 blood samples were collected weekly from each animal via the jugular vein. Serum was separated by centrifugation at 3000 r.p.m. for 15 min. and it was collected and stored at –20 °C until analysis.

Hormonal assay and determination of blood serum constituents

The concentration of triiodothyronine (T_3) and testosterone in the blood serum was measured by using commercial Chemiluminescent kits (ADVIA Centaur Competitive Immunoassay System, Bayer Diagnostic Corporation, U.S.) according to Fernandez-Ullao and Maxon (7) and Spratt et al. (8).

Total blood serum protein was measured by the Biuret method and total albumin (A) concentration was determined by the method of Doumas et al. (9). Total globulin (G) concentration was calculated as the difference between blood serum total protein and blood serum albumin, then A/G ratio was calculated. Total lipids and cholesterol concentrations were measured by a colorimetric method (10). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured in a Hitachi 917 Clinical Chemistry Analyzer by using commercially available diagnostic kits supplied from Roche Diagnostics GmbH (D-68298, Mannheim, Germany). Both enzyme activities were determined photometrically in which the decrease in NADH levels were directly proportional to enzyme activities and AST/ALT ratios were calculated (11).

Statistical analysis

The groups were compared by Student's t-test and the partial correlation coefficients and probabilities among different parameters were worked out (12).

Results

Overall mean values of some reproductive parameters and biochemical and enzymatic properties of blood serum in rams during the breeding season are presented in the Table. The partial correlation coefficients and probabilities among different parameters were worked out. Relative testes volume from reproductive parameters were negatively correlated with sperm concentration ($P < 0.01$) and sperm motility ($P < 0.05$) in all rams. Reaction times were positively correlated with testosterone levels in Akkaraman ($P < 0.01$) and Awassi ($P < 0.05$) rams. Percentages of abnormal spermatozoa were negatively ($P < 0.01$) correlated with testosterone levels and sperm motility but positively correlated ($P < 0.01$) with sperm concentration in all rams. Total protein from biochemical properties of blood serum were positively ($P < 0.01$) correlated with albumin, globulin and ALT activity but negatively ($P < 0.01$) correlated with total lipid, cholesterol and AST activity. Total lipids were positively ($P < 0.01$) correlated with cholesterol and ALT activity but negatively ($P < 0.01$) correlated with AST activity and AST/ALT ratio. AST activity was negatively (P

< 0.01) correlated with cholesterol and ALT activity and positively (P < 0.01) correlated with AST/ALT ratio in all rams.

Total protein and globulin were positively (P < 0.05) correlated with sperm motility, sperm concentration and testosterone level in all rams. Total lipid levels were negatively (P < 0.01) correlated with sperm motility and concentration and positively (P < 0.05) correlated with percentage of abnormal spermatozoa in all rams. In addition, total lipids and cholesterol were positively (P < 0.01) correlated with T_3 levels and negatively (P < 0.05) correlated with testosterone levels in all rams. Percentage of abnormal spermatozoa was negatively (P < 0.01) correlated with alanine amino transaminase (ALT) activity but were positively (P < 0.05) associated with aspartate amino transaminase (AST) activity and AST/ALT ratio in all rams.

Table. Overall mean values of some reproductive parameters, some biochemical and enzymatic properties of blood serum in rams during the breeding season in Afyon province conditions (X ± s.e.m.) (n:25).

	Akkaraman	Awassi
Rel. testes volume (ml/kg)	10.6 ± 0.34*	10.9 ± 0.32*
Reaction time (s)	6.8 ± 0.35	6.6 ± 0.40
Motility (%)	77.8 ± 0.73	75.4 ± 1.00
Sperm concentration (x10^9/ml)	4.7 ± 0.08*	5.0 ± 0.07*
Abnormal sperm (%)	3.9 ± 0.12*	3.5 ± 0.10*
Testosterone (ng/ml)	4.7 ± 0.46	4.8 ± 0.64
T_3 (µg/100ml)	1.22 ± 0.10*	0.87 ± 0.07*
Total protein (g/100ml)	7.2 ± 0.23	7.5 ± 0.08
Albumin (A) (g/100ml))	3.3 ± 0.05	3.3 ± 0.08
Globulin (G) (g/100ml)	3.9 ± 0.20	4.3 ± 0.19
A/G	0.85 ± 0.04*	0.77 ± 0.02*
Total lipid (mg/100ml)	69.7 ± 2.24*	73.7 ± 2.07*
Cholesterol (mg/100ml)	28.9 ± 1.41**	33.6 ± 1.24**
AST	116.7 ± 4.03	115.8 ± 4.11
ALT	15.7 ± 0.65	14.6 ± 1.89
AST/ALT	7.4 ± 0.62	7.9 ± 0.32

*P < 0.05
**P < 0.01

Discussion

Relative testes volumes were lower than the findings of Taha et al. (13) in Awassi rams and similar to those in Barki rams. The mean reaction times were lower than the findings of Taha et al. (13). The differences might be due to breed, age, feeding and season. In addition, because of the farm conditions the rams and sheep were maintained closely therefore the effects of sheep could be concerned. The percentage of motile spermatozoa were higher than the findings of Taha et al. (13) (68.91% and 64.3%) in sexually mature Awassi and Barkies, lower than that of Gündoğan et al. (14) (80.9%) in 16 month old Akkaramans, of Kaya et al. (15) (85.7%) and Pirinçci et al. (16) (96%) and similar to the findings of Aral and Tekin (17), Ataman et al. (18) and Gündoğan (19). The spermatozoa concentrations were lower than the findings of Taha et al. (13) (5.2 x10^9/ml in Awassi), higher than those of Aral and Tekin (17) (3.1x10^9/ml), Gündoğan (19) (3.8x10^9/ml) and Kaya et al. (15) (3.6x10^9/ml) and similar to those of Ataman et al. (18) in 15 month old Merino rams, Pirinçci et al. (16) (4.6x10^9/ml for 2 year old Akkaramans) and Taha et al. (13) (4.7 x10^9/ml in Barkies). Percentages of abnormal spermatozoa rates were similar to the findings of Aral and Tekin (17) (3.3%), Gündoğan (19) (3.5%) and Kaya et al. (15) (3.6%), lower than those of Pérez et al. (4) (9.4%) and Taha et al. (13) (14.2%) and higher than those of Ataman et al. (18) (1.6%) and Pirinçci et al. (16) (2.7%). Breed effect was significant (P < 0.05) for sperm concentration and percentage of abnormal spermatozoa. The differences in the spermatological parameters might be due to breed, age, feeding and management, evaluation technique, semen collection time and season.

Blood serum testosterone levels were similar to the results of Pirinçci et al. (20) and Taha et al. (13) in Awassi (Imported), higher than those of Gündoğan (19) (2.7 ng/ml) and lower than those of Pérez et al. (4) (19.7 nmol/l =12.85 ng/ml) and Taha et al. (13) (13.41 ng/ml) in Barki rams. Triiodothyronine levels were lower than the findings of Taha et al. (13) in Awassi (Imported) and Barki rams and similar to those in Awassi (locally born) rams. The differences in hormonal levels might be due to breed, age, feeding and management, sample collection time and evaluation procedure.

The biochemical and enzymatic properties of blood serum in rams were in the optimum range and in accordance with the references (21-24). In addition, breed effect was significant for A/G ratio, total lipid (P < 0.05) and cholesterol (P < 0.01) and relationships between some biochemical and enzymatic properties of rams' blood serum were observed.

Many studies have shown that the low content of seminal plasma proteins is associated with poor seminal quality (1,2,25-28). In the present study, it was found that there were positive relationships between sperm motility, sperm concentration, testosterone level and level of total proteins and globulin ($P < 0.05$) but there were negative relationships between percentage of abnormal spermatozoa and albumin and A/G ratio ($P < 0.05$) in all of rams' blood serum.

Kelso et al. (29) reported that reductions in sperm concentration and motility were associated with a decrease in seminal plasma lipids content and also with sperm aging (poor semen quality). In the present study, total lipid levels were negatively ($P < 0.01$) correlated with sperm motility and concentration and positively ($P < 0.05$) correlated with percentage of abnormal spermatozoa in all rams.

The blood cholesterol level drops before the metabolic rate rises (30,31). In the present study total lipids and cholesterol were positively ($P < 0.01$) correlated with triiodothyronine levels and negatively ($P < 0.05$) correlated with testosterone levels in all rams. The differences between rams may be due to breed and genetics.

The assay of transaminase enzyme activity (AST and ALT) is a good indicator of semen quality because it measures sperm membrane stability (32,33). In the present study, positive relationships were found between percentage of abnormal spermatozoa and AST activity and AST/ALT ratio ($P < 0.05$) and negative relationships between ALT activity ($P < 0.01$) in ram blood serum and negative correlations were observed with sperm concentration, AST activity and AST/ALT ratio ($P < 0.05$) in the two ram breeds.

In conclusion, some of the reproductive parameters and biochemical analyses of rams' blood serum indicated that the values were recorded in the normal range during the breeding season under Afyonkarahisar province conditions. Some reproductive parameters of rams show a correlation with some biochemical and enzymatic properties and could be used to evaluate the reproductive performance of a ram. In addition, total protein, total lipid, cholesterol, AST activity and AST / ALT ratio from biochemical and enzymatic properties of blood serum could be used to evaluate the reproductive performance of an Akkaraman and Awassi ram.

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