RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

An Investigation on Certain Andrological and Biochemical Parameters in Rams under Afyonkarahisar Conditions

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SUMMARY

The objective of the present study was to describe some reproductive parameters, hormonal levels and some biochemical properties of blood serum of rams. Semen (via an artificial vagina) and blood were collected five times during the breeding season from a total of 5 mature and healthy Pirlak rams in Afyonkarahisar conditions. The values of testes length and thick, scrotal circumference and thick and relative testes volume were ranged from 8.5 to 12.9 cm, from 5.9 to 8.5 cm, from 30.5 to 40 cm, from 4 to 5 mm and from 10.35 to 18.50 ml/kg, respectively, and the mean values of the reaction times were found as 5.14 ± 0.21 s. The overall mean values of spermatological features were found as 1.21 ± 0.06 ml, 6.59 ± 0.07, 3.40 ± 0.09, 4.57 ± 0.09, 86.85 ± 0.79 %, 4.19 ± 0.08 x 10^9 spermatozoa/ml, 6.41 ± 0.27 %, 4.14 ± 0.21 % and 71.09 ± 1.13 % for volume, pH and viscosity of semen, mass activity, sperm motility and concentration, live-dead and abnormal sperm rate and HOS test value, respectively. In addition, some blood serum hormonal concentrations, biochemical and enzymatic properties of Pirlak rams indicated that the values were recorded in the optimum range, and also no difference was found individually for both certain andrological and biochemical parameters. However, some reproductive parameters of rams show a correlation with some biochemical parameters. In conclusion, Pirlak rams have reproductive capacity during the breeding season under the Afyonkarahisar province conditions.

Key Words
Biochemical properties
Hormone
Ram
Semen

Anahtar Kelimeler
Biyokimyasal özellik
Hormon
Koç
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Afyonkarahisar Koşullarında Koçlarda Bazı Reprodüktif Parametreler ve Biyokimyasal Özellikler Üzerine Bir Araştırma

ÖZET

Afyonkarahisar koşullarında yetiştirilen koçlarda bazı reprodüktif parametreler ve hormon miktarları ile kan serumu biyokimyasal özelliklerin araştırılması amacıyla yapılan bu çalışmada, aşım sezonu esnasında, sağlıklı toplam 5 Pırlak koçtan beşer numune kan ve sun'i vagen yardımıyla da sperma toplandı. Testis uzunluğu ve kalınlığı, scrotum çevresi ve kalınlığı ile relative testis hacmi değerleri sırasıyla 8.5 ile 12.9 cm, 5.9 ile 8.5 cm, 30.5 ile 40 cm, 4 ile 5 mm ve 10.35 ile 18.50 ml/kg arasında değişti ve ortalama reaksiyon süresi 5.14 ± 0.21 s olarak bulundu. Spermatlojik özelliklerden spermanın miktarı, pH'sı ile viskozitesi, spermatozoitlerin kitle hareketi, motilitesi ve yoğunluğu, ölü-canlı ve anormal spermatozoid oranları ile HOS test ortalama değerleri sırasıyla 1.21 ± 0.06 ml, 6.59 ± 0.07, 3.40 ± 0.09, 4.57 ± 0.09, % 86.85 ± 0.79, 4.19 ± 0.08 x 10^9 spermatozoa/ml, % 6.41 ± 0.27, % 4.14 ± 0.21 ve % 71.09 ± 1.13 olarak bulundu. Bunun yanında, Pırlak koçların bazı kan serumu hormonal düzeylerinin, biyokimyasal ve enzimatik özelliklerinin optimal sınırlar içerisinde olduğu ve aynı zamanda hem androlojikal parametreler hem de biyokimyasal özellikler bakımından koçlar arasında fark olmadığı gözlendi. Ayrıca koçlarda bazı reprodüktif parametreler bazı biyokimyasal parametreler ile ilişkiler gösterdi. Sonuç olarak, Afyonkarahisar koşullarında aşım sezonu esnasındaki Pırlak koçların reprodüktif kapasiteye sahip oldukları kanaatine varılmıştır.

INTRODUCTION

Andrological evaluations are important in management practice, especially for artificial insemination in a breeding program. Genetic improvements of sheep rely on the intensive use of a few superior rams either for natural mating, or in artificial insemination programs. The production of meat and milk may be increased through selective breeding of ewes with rams exhibiting desirable genetic combinations. 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.[1-5] But 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. Previous studies have been carried out on different breeds rams to investigate the semen quality, biochemical and enzymatic properties of blood serum.[6-9] None of the studies considered the Pirlak rams under similar conditions as in Turkey. In the present study, Pirlak breed that were crossbreed of the exotic (Kivircik, from a moderate zone) and the fat-tailed (Daglic, local) rams were used. Therefore, the first study in Pirlak rams during the breeding season under Afyonkarahisar province conditions is concerned with investigating references values of some andrological parameters, some hormonal concentrations and some major biochemical constituents of blood serum, and researching the their correlations.

MATERIALS and METHODS

Animals and management

The study was performed at the Research and Manipulation Farm of the Faculty of Veterinary Medicine of Afyon Kocatepe University located Afyonkarahisar Province (L: 1021 m, 38^0 45´ N, 30º 32º W), Turkey during breeding season (September-October). The study was conducted on 5 sexually mature Pirlak (Daglic x Kivircik) rams of average weight 62.8 (S.E.M. 2.71) kg. All animals were around 2 years of age and were kept outdoors with shelter during the daytime and housed in a semiopen barn at night. Animals were fed roughage and concentrate supplement that each animal also received 500 g/day of concentrate mixture and 1.0 kg/day of dry alfalfa.

Andrological evaluation

In terms of sexual performance, Testes length and thick and scrotal thick were measured via composing stick, and scrotal circumference was measured via tape measure. Scrotal sac volume was measured by the water displacement method 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.

Total 5 ejaculates were collected every other day by using an artificial vagina. Immediately after collection, the ejaculates were immersed in a warm water bath at 37 ºC until their assessment in the laboratory. Semen assessment was performed in approximately within 10 minutes after collection at the Artificial Insemination Laboratory of Department of the Reproduction and Artificial Insemination within Farm.

Semen evaluation

The volume of ejaculates was measured in a conical tube graduated at 0.1 ml intervals. Semen pH was estimated via pH test paper (range from 5.5 to 9.0). Semen viscosity and mass activity of spermatozoa were scored 1 to 5 according to Ax et al. [10]. Percentage of motile sperm was subjectively estimated using a phase contrast microscope (Olympus CX31, Olympus Optical Co., Ltd., Japan) with warm stage at magnification 400 × according to the standard method. It was performed from five different microscopic fields in each sample at 37 ºC. The mean of the five estimations was used as the final motility score. The sperm concentration was determined by means of a haemocytometer.[10]

The proportion of live-dead sperms was estimated via means of a stain mixture nigrosin-eosin.[11] The stain was separately prepared as eosin-Y 1.67 g and sodium citrate 2.9 g dissolved in 100 ml distilled water and as nigrosin 10 g and sodium citrate 2.9 g dissolved in 100 ml distilled water. The sperm suspension smears were prepared by mixing a drop of the semen sample with one drop eosin and two drops nigrosin of the stain on warm slide and spreading the stain with a second slide immediately. The viability was assessed by counting 400 cells under phase-contrast microscope (400 × magnification). The cells that were alive when the stain was applied exclude the stain, and the dead cells stain red with eosin against the dark nigrosin background.

The abnormal sperm rates were assessment according to liquid fixation method at least three drops of each sample added to Eppendorf tubes containing 1 ml of Hancock solution.[12] One drop of this mixture was put on a slide and covered with a cover slip. The percentage of total sperm abnormality (acrosomal abnormality, detached heads, abnormal mid-pieces and tail defects) was determined by counting a total of 400 spermatozoa under phase contrast microscope (magnification 400 ×; in needed 1000 ×, oil immersion).

Osmotic resistance test was performed according to Buckett et al.[13] and Revell and Mrode,[14] and it was evaluated according to Jeyendran et al.[15] The hypoosmotic swelling test (HOST) was used as a complementary test to the viability assessment protocol. The assay was performed by mixing 0.2 ml of semen with a 1 ml hypoosmotic solution (9 g fructose, 4.9 g trisodium citrate, distilled water to 1000 ml, 100 mOsm). This mixture was incubated at 37 °C for 60 minutes. After incubation, 0.2 ml of the mixture was spread with a cover slip on a warm slide. A total of 400 spermatozoa were counted with a phase contrast microscope (1000× magnification, oil immersion). The percentages of spermatozoa with swollen and coiled tails were assessment.

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 and biochemical analyses were carried out at the Medicine School of Afyon Kocatepe University.

Hormonal assay and determination of blood serum constituents

The concentration of thyroxine (T_4), triiodothyronine (T_3), thyrotropine (TSH) and testosterone in the blood serum were measured by using commercial Chemiluminescent kits (Roche-Hitachi Diagnostic Modular Analytics E-170 Immunoassay System, Roche Diagnostics GmbH, D-68298, Mannheim, Germany) according to Fernandez and Maxon[16] and Spratt et al.[17]

Total blood serum protein was measured by the Biuret method and total Albumin (A) concentration was determined by the method of Doumas et al.[18] 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 glucose, triglycerides, cholesterol, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) concentrations were measured by a colorimetric method.[19] Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were measured in a Roche Hitachi Diagnostic Modular Analyzer P-800 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.[20]

Statistical analysis

Results are presented as mean ± standard error of the mean (S.E.M.). Statistical analyses were performed using the SYSTAT package, version 5.01 (SYSTAT Inc., IL, USA). Means were examined by analysis of variance (ANOVA). The rams were compared by Student's t-test and Pearson's correlation coefficients were worked out to evaluate the correlations among different parameters. Differences were considered as statistically significant at the $P < 0.05$ level.[21]

RESULTS

Overall mean values of some reproductive parameters in rams during the breeding season are presented in the Table 1 and 2. The values of testes length and thick, scrotal circumference and thick and relative testes volume were changed in 8.5-12.9 cm, 5.9-8.5 cm, 30.5-40 cm, 4-5 mm and 10.35-18.50 ml/kg, respectively. The spermatological features of Pirlak rams were ranged from 4 to 9 s, from 0.6 to 1.9 ml, from 6.0 to 7.5, from 2.0 to 4.0, from 4.0 to 5.0, from 80 to 90 %, from 3.18 to 5.05 x 10^9 spermatozoa/ml, from 4.0 to 9.0 %, from 2.0 to 7.0 % and from 54.25 to 81.50 % for reaction time, volume, pH and viscosity of semen, mass activity, sperm motility and concentration, live-dead and abnormal sperm rate and HOS test, respectively, and difference among rams in both testes measures and spermatological features was found non-significant. Overall mean values of some hormonal, biochemical and enzymatic properties of blood serum in rams during the breeding season are presented in Table 3 to 5. In addition, correlations among different parameters are worked out.

Relative testes volume from reproductive parameters were negatively correlated with testosterone levels ($p<0.01$) (r: -0.967) and positively correlated with sperm motility ($p<0.05$) (r: 0.946) in all rams. And testosterone levels were negatively correlated with testes and scrotal thick and testes lenghth

($p<0.05$). Thyroxine levels were correlated with live-dead sperm rate ($p<0.05$) positively and mass activity of spermatozoa ($p<0.01$) negatively. Reaction times were positively correlated with both testosterone and albumine levels in rams ($p<0.05$). Sperm motility was negatively correlated with blodd serum globulin levels (r:-0.946, $p<0.05$). Scrotal thick were negatively correlated with cholesterol, LDL and AST activity ($P<0.05$). ALT activity was positively correlated with sperm concentration (r:0.884, $p<0.05$). AST/ALT ratio was negatively correlated with HOS test and sperm concentration ($p<0.05$).

DISCUSSION

Extensive studies on evaluating andrological parameters were conducted in rams of different breeds. In this study, testicle sizes were measured in Pirlak rams. Testes lengths were higher than the findings of Gundogan et al.[7] in Chios rams and similar to those in Akkaraman, Awassi, and Daglic rams. Testes thicks and scrotal circumferences were similar to those of Fourie et al.[22] in Dorper rams, Kafi et al.[4] in Persian Karakul rams and in Akkaraman, Awassi, Chios and Daglic rams, and scrotal thicks were lower than of the Akkaraman rams and similar to in Awassi, Chios and Daglic rams according to the findings of Gundogan et al.[7] But scrotal circumferences were higher than the findings of Kheradmand et al.[23] in Bakhtiary rams and Kridli and Al-Yacoub[24] in 8 months aged Awassi ram lambs. Relative testes volumes were lower than the findings of Gundogan[6] in Chios rams and Taha et al.[9] in Awassi rams, and higher than the findings of Gundogan[6] in Daglic rams, and similar to the findings of Gundogan and Serteser[3] in Akkaraman and Awassi rams and Taha et al.[9] in Barki rams. The reaction times were lower than the findings of the Taha et al.[9] in Awassi and Barki rams, the Gundogan et al.[7] in Akkaraman, Awassi, Chios and Daglic rams and the Gundogan[6] in Chios and Daglic rams and similar to the findings of Gundogan and Serteser[3] in Akkaraman and Awassi rams . The differences in the testes measurements and the reaction times 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.

In this study, generally, semen characteristics were shown by Pirlak native breeds agreed with those reported for rams.[10] The overall mean of semen volumes found were in agreement with that recorded in Persian Karakul rams by Kafi et al.[4] and in Akkaraman, Awassi, Chios and Daglic rams by Gundogan et al.[7] but lower than in Chios and Daglic rams by Gundogan[6] and in Chios and Friesian rams during autumn by Karagiannidis et al.[25], and higher than in Bakhtiary rams by Kheradmand et al.[23] and Katahdin rams by Lezama et al.[26] Semen viscosity values were lower than, and semen pH and mass activity of spermatozoa were similar to the findings of Gundogan et al.[7] in Akkaraman, Awassi, Chios and Daglic rams. In addition, the mass activity of spermatozoa was higher than the reports of Fouire et al.[22] in Dorper rams, and similar to the findings of Kafi et al.[4] in Persian Karakul rams. The percentage of motile spermatozoa were higher than the findings of Abdel-Rahman et al.[1] in Najdi and Naemi rams semen collected via electroejaculation, Taha et al.[9] in sexually mature Awassi and Barkies, Gundogan and Serteser[3] in Akkaraman and Awassi rams and Gundogan[6] in Chios and Daglic rams and were similar to the findings Abdel-Rahman et al. [1] in Merino rams, Gundogan et al.[7] in Awassi and Chios rams and Fourie et al.[22] in Dorper rams, and higher than in 2-5 years aged Barbari and Sawakni rams by Abdel-Rahman et al.[1] The spermatozoa concentrations were lower than the findings of Taha et al.[9] in Awassi and Barki rams, and Gundogan and Serteser[3] in 3-4 years aged Akkaraman and Awassi rams, and were higher than the findings of Gundogan et al.[7] in Akkaraman, Awassi, Chios and Daglic rams, and similar to the findings of Karagiannidis et al.[25] during spring in Chios and Friesian rams, of Kheradmand et al.[23] in Bakhtiary rams and in Katahdin yearling rams for first ejaculation by Lezama et al.[26] Live-dead spermatozoa rates were higher than the findings of Abdel-Rahman et al.[1] in 2-5 years aged Barbari, Merino, Naemi, Najdi and Sawakni rams semen collected via electroejaculation and Kheradmand et al.[23] in Bakhtiary rams. Percentages of abnormal spermatozoa were similar to the findings of Gundogan[6] and Gundogan et al.[7] in Chios rams, higher than those of Gundogan and Serteser[3] in Akkaraman and Awassi rams and Gundogan et al.[7] in Akkaraman, Awassi, and Daglic rams, and lower than those of Pérez et al.[8] in Corriedale rams, Karagiannidis et al.[25] in Chios and Friesian rams and Taha et al.[9] in Awassi and Barki rams. The differences in the spermatological features might be due to breed, age, feeding and management, semen collection time and technique, evaluation technique and season.

Blood serum testosterone levels were similar to the results of Pérez et al.[8] (19.7 nmol/l =12.85 ng/ml) and Taha et al. [9] (13.41 ng/ml) in Barki rams and higher than those of Taha et al.[9] in Awassi (Imported). Triiodothyronine levels were lower than the findings of Taha et al.[9] in Awassi (Imported) and Barki rams and similar to those in Awassi (locally born) rams and to findings of Gundogan and Serteser[3] in Akkaraman rams. Thyroxine and thyrotropine levels were higher than

the findings of Zamiri and Khodaei[27] in Iranian fat-tailed Ghezel and Mehreban rams. The differences in hormonal levels might be due to breed, age, feeding and management, sample collection time and evaluation procedures.

The biochemical and enzymatic properties of blood serum in rams were in the optimum range and in accordance with the references.[28-33] 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.[2,34,35] 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.[36] 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 and live-dead spermatozoa in all rams.

The blood cholesterol level drops before the metabolic rate rises.[37,38] 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.[39,40] In the present study, negative relationships were found between sperm concentration and AST/ALT ratio ($p<0.05$) in ram blood serum and positive correlations were observed with sperm concentration and ALT activity ($p<0.05$) in rams.

In conclusion, some of the reproductive parameters and biochemical analyses of rams' blood serum indicated that the values were recorded in the normal range, and also the study notified Pirlak rams to have reproductive capacity 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 Daglic x Kivircik ram ■

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Tablo 1. Afyonkarahisar koşulları altında aşım mevsimi süresince Pırlak koçların bazı testis ölçülerinin tüm ortalama değerleri (X± S.E.M.) (n: 5).

	Testes Length (cm)		Testes Thick (cm)		Scrotal Circumference (cm)	Scrotal Thick (mm)	Relative Testes Volume (ml/kg)
	Right	*Left*	*Right*	*Left*			
Rams	10.67 ± 0.21	9.96 ± 0.19	7.07 ± 0.11	6.88 ± 0.09	33.73 ± 0.41	4.46 ± 0.01	13.18 ± 0.45

Tablo 2. Afyonkarahisar koşulları altında aşım mevsimi süresince Pırlak koçların bazı spermatolojikal özelliklerinin tüm otalama değerleri (X± S.E.M.) (n: 25).

	Reaction Time (s)	Semen Volume (ml)	Semen pH	Semen Viscosity (1-5)	Mass Activity (1-5)	Sperm Motility (%)	Sperm Concentration ($x10^9$/ml)	Live-dead Sperm Rate (%)	Abnormal Sperm Rate (%)	HOST (%)
Rams	5.14 ± 0.21	1.21 ± 0.0.6	6.59 ± 0.07	3.40 ± 0.09	4.57 ± 0.09	86.85 ± 0.79	4.19 ± 0.08	6.41 ± 0.27	4.14 ± 0.21	71.09 ± 1.13

Tablo 3. Afyonkarahisar koşulları altında aşım mevsimi süresince Pırlak koçların kan serumunun bazı hormonal düzeylerinin tüm ortalama değerleri (X± S.E.M.) (n: 25).

	Testosterone (ng/ml)	T_3 (ng/dl)	T_4 (µg/dl)	TSH (µlU/ml))
Rams	11.97 ± 2.43	1.93 ± 0.15	8.76 ± 0.59	0.01 ± 0.01

Tablo 4. Afyonkarahisar koşulları altında aşım mevsimi süresince Pırlak koçların kan serumunun bazı biyokimyasal özelliklerinin tüm ortalama değerleri (X± S.E.M.) (n: 25).

	Glucose (mg/dl)	T. Protein (g/dl)	Albumin (A)(g/dl))	Globulin (G)(g/dl)	A/G ratio	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VDLD (mg/dl)
Rams	75.0 ± 0.89	8.8 ± 0.34	3.66 ± 0.19	5.14 ± 0.39	0.77 ± 0.07	16.8 ± 1.83	42.6 ± 3.96	21.2 ± 1.88	18.04 ± 1.95	3.36 ± 0.37

Table 5. Afyonkarahisar koşulları altında aşım mevsimi süresince Pırlak koçların kan serumunun bazı enzimatik özelliklerinin tüm ortalama değerleri (X± S.E.M.) (n: 25).

	AST (U/L)	ALT (U/L)	AST/ALT
Rams	95.6 ± 6.03	22.0 ± 2.07	4.49 ± 0.48

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