

## POSSIBLE ASSOCIATION BETWEEN AGE AND REPRODUCTIVE PARAMETERS IN AKKARAMAN RAMS DURING BREEDING SEASON

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### Akkaraman Koçlarda Aşım Sezonu Esnasındaki Reprodüktif Parametrelerin Yaşla İlişkisi

#### ÖZET

Bu çalışma aşım sezonundaki 2 ve 4 yaşlarındaki 10 adet Akkaraman koç üzerinde yapılmıştır. Klinik olarak (testis uzunluğu ve kalınlığı, scrotum çevresi, scrotum hacim ve kalınlığı ve reaksiyon süresi), spermatolojik olarak (sperma hacmi, viskozitesi ve pH'sı, kitlesel hareket ve spermatozoa motilitesi, spermatozoa yoğunluğu ve anormal spermatozoa oranı) parametreleri ve testosteron düzeyleri araştırılmıştır.

Testisin morfometrik ölçümlerinde yaş grupları arasındaki fark önemli ( $P<0.05$ ) bulundu. Spermanın viskozite ve pH'sı, spermatozonların kitlesel hareketleri ve testosteron düzeyleri arasında fark gözlenmezken diğer parametreler yönünden yaş grupları arasındaki fark önemli ( $P<0.05$ ) bulundu. Scrotum çevresi ile reaksiyon süresi ( $r:-0.90$ ), sperma viskozitesi ( $r:-0.82$ ) ve spermatozoa motilitesi ( $r:-0.81$ ) arasında negatif ( $P<0.01$ ) yönde ilişki gözlenirken scrotum hacmi ( $r:0.65$ ) ile pozitif ( $P<0.1$ ) yönde ilişki gözlemlendi. Spermatozoa motilitesi ile sperma miktarı ( $r:-0.89$ ), pH ( $r:-0.85$ ) ( $P<0.05$ ), sağ testis kalınlığı ( $r:-0.73$ ) ( $P<0.01$ ) ile negatif yönde ilişki gözlenirken viskozite ( $r:0.93$ ) ( $P<0.01$ ) ile pozitif yönde ilişki tespit edildi.

Ayrıca sperma viskozitesi ile sol testis uzunluğu ( $r:-0.66$ ) ve sağ testis kalınlığı ( $r:-0.92$ ) arasında negatif yönde ilişki gözlemlendi. Anormal spermatozoa oranı ile scrotum kalınlığı ( $r:0.87$ ) ( $P<0.01$ ) arasında pozitif yönde ilişki gözlenirken scrotum hacmi ( $r:-0.81$ ) ile negatif ( $P<0.01$ ) yönde ilişki tespit edildi. Testosteron düzeylerinin sperma miktarı ( $r:0.82$ ) ile pozitif, kitlesel hareket ( $r:-0.81$ ) ve motilite ( $r:-0.66$ ) ile negatif yönde ilişkili olduğu belirlendi.

ANAHTAR KELİMELER: Yaş, koç, sperma, testis, testosteron

#### SUMMARY

The study was carried to investigate the possible association between age and reproductive parameters in rams. Ten Akkaraman rams aged 2-4 years were used during the breeding season. Animals were allocated into two groups (aged 2 and aged 4) and following parameters were examined: length and thickness of testes, scrotal circumference, volume and thickness of sac, and reaction time; volume, viscosity and pH values of semen, mass activity and motility of spermatozoa, sperm concentration and abnormal sperm rate, and testosterone levels.

Morphometric measurements of testes significantly differed between age groups ( $P<0.05$ ). Although the viscosity and pH of semen, mass activity from spermatological parameters and testosterone levels indicated no differences, other parameters were significantly differed between age groups ( $P<0.05$ ). Scrotal circumference was negatively associated with reaction time ( $r:-0.90$ ), semen viscosity ( $r:-0.82$ ), and motility ( $r:-0.81$ ) and positively associated with scrotal sac volume ( $r:0.65$ ). The motility was negatively correlated with ejaculate volume ( $r:-0.89$ ), pH ( $r:-0.85$ ), and thickness of the right testes ( $r:-0.73$ ), but positively associated with viscosity ( $r:0.93$ ).

It was also observed that the viscosity was negatively correlated with length of the left testes ( $r:-0.66$ ) and thickness of the right testes ( $r:-0.92$ ). Abnormal sperm rate was positively associated with scrotal thickness ( $r:0.87$ ) but was negatively correlated with scrotal sac volume ( $r:-0.81$ ). There was a positive correlation between ejaculate volume ( $r:0.82$ ) and testosterone levels. However negative correlation was seen between mass activity ( $r:-0.81$ ) and motility ( $r:-0.66$  with mass).

KEY WORDS: Age, ram, sperm, testis, testosteron.

## INTRODUCTION

Although there is considerable information available on the characteristics of ram semen and on factors affecting semen production in rams (Aral and Tekin 1996, Bielli et al 1997, Colas et al 1985, Gündoğan et al 1997, Kaya et al 1999, Piriñçi et al 2001b, Wiggins et al 1953), there appears to be very little information on the relationships among age, spermatological parameters, reaction time, serum testosterone levels and morphometric measurements of testes.

The rams of practically all breeds living above 40° latitude in the Northern hemisphere display seasonal variations in testes size, daily sperm production and testosterone levels (Dufour et al 1984, Gomes and Joice 1975, Pelletier et al 1988, Pérez et al 1997, Piriñçi et al 2001a, Taha et al 2000). The reproductive activity of rams are influenced by age (Fallah-Rad et al 2001, Hafez 1987, Sinha and Sahni 1985). The age of rams were also related with semen volume and scrotal sac volume (Demirci 1993).

Few studies have been reported that there was relationships between testes size, semen characteristics and testosterone levels of rams (Aksoy et al 1994, Ataman et al 1996, Foster et al 1989, Gündoğan 1999, Langford et al 1989, Nowakowski and Cwikla 1994, Rege et al 2000).

The objective of the present investigation was to describe the relationships of age between morphometric measurements of testes, reaction times, spermatological parameters and blood sera testosterone levels of the Akkaraman rams during the breeding season under Afyon continental climate conditions in Turkey.

## MATERIALS and METHODS

This work was carried out at the Research and Extension Farm of Faculty of Veterinary Medicine University of Afyon Kocatepe located Afyon Province (High latitue : 102°1m, 38° 45' N, 30° 32' W), Turkey.

This study was conducted on ten Akkaraman rams divided into two age groups with five rams in each group. The first two aged groups and average weight 70.0 (s.e. 5.69) kg and the second four aged groups and average weight 84.0 (s.e. 1.78) kg. All animals were kept outdoors with shelter during the daytime. Animals were offered roughage and concentrate supplement according to their body weight requirements.

The data of daily temperature, humidity and photoperiod length were obtained from the meteorological station in Afyon province. The mean values of ambient temperature is 10.2 °C, relative humidity 0.72 and photoperiod length is 4.54 h during breeding season in the same time of research period.

The morphometric measurement of testes, scrotal sac volume was measured by the water displacement method, circumference of scrotum was taken with a flexible meter, length and thickness of testes and scrotal were also measured by podany testimeter

described by Demirci (2002). Sexual behaviour of rams was recorded by using the reaction time criteria. The time elapsed between introducing the ram to an ewe at oestrus and semen collection was marked using a stopwatch.

Semen was collected from all rams early in the morning every other day by an artificial vagina. Total ten ejaculates were collected from each ram. The semen was brought to the laboratory immediately and was placed in a water bath at 37°C. The ejaculates were pooled and evaluated for total volume, viscosity, pH, mass activity, motility, sperm concentration and abnormal sperm rate. Ejaculate volume was recorded directly from graduated test tubes. The pH of semen was measured by pH test paper. The viscosity of semen was estimated (1-5) by Wiggins et al. (1953). The mass activity of spermatozoa was recorded immediately after semen collection by examining a drop of semen on a warm slide at 100X magnification under the microscope. The score was calculated from 0 to 5 (Demirci 2002). Motility, as a percentage of individually motile spermatozoa, was estimated by examining a drop of diluted fresh semen (with 2.9 % sodium citrate solution) under a microscope at 400X. Motility was scored on the basis of the percentage of spermatozoa with normal forward progressive movement, while those showing circling movements or those oscillating at one place were regarded immotile. Sperm concentration was measured by haemocytometric methode. Abnormal sperm rate were assessed according to Hafez (1987).

Blood was collected from the jugular vein of each animal five times with every other day in the afternoon during the breeding period. Blood samples were centrifuged, sera were collected and stored at -20°C until analysis. The concentration of Testosterone in the blood sera was measured using RIA (Coat-A-Count testosterone; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) according to Abraham (1981).

The data were analysed by using SPSS (Anonymous 1992) computer software package. The groups were compared by Mann-whitney U test and the partial correlation coefficients among different parameters were worked out by controlling for age (Anonymous 1992).

## RESULTS

Comparisons (means  $\pm$  SEM) of morphometric measurements of testes, reaction times, spermatological parameters and testosterone levels in different aged Akkaraman rams in the breeding season of Afyon province are presented in Table 1 and 2. Morphometric measurements of testes and reaction times showed significant ( $P<0.05$ ) differences between age groups. The viscosity and pH of semen, mass activity and testosterone levels showed no difference and other parameters showed significant ( $P<0.05$ ) differences in age groups. Partial correlation coefficients controlled for age among different parameters are presented in Table 3 to 5.

Table 1. Comparisons (means  $\pm$  SEM) of Morphometric Measurements of Testes and Reaction Times in Different Aged Akkaraman Rams in The Breeding Season of Afyon Province

Age	n	Testes lenght (cm)		Testes thickness (cm)		Scrotal circumference* (cm)	Scrotal sac volume* (ml)	Scrotal thickness* (cm)	Reaction time* (s)
		Right*	Left*	Right*	Left*				
2	5	9.60 $\pm$ 0.11	9.66 $\pm$ 0.11	4.71 $\pm$ 0.07	4.70 $\pm$ 0.10	581.25 $\pm$ 5.49	28.94 $\pm$ 0.22	0.40 $\pm$ 0.02	9.05 $\pm$ 0.29
4	5	10.75 $\pm$ 0.05	10.84 $\pm$ 0.03	7.06 $\pm$ 0.06	7.10 $\pm$ 0.08	898.75 $\pm$ 19.7	36.13 $\pm$ 0.79	0.51 $\pm$ 0.03	8.35 $\pm$ 0.18

\*: The groups are statistically different (P &lt; 0.05).

Table 2. Comparisons (means  $\pm$  SEM) of Spermatological Parameters and Blood Sera Testosterone Levels in Different Aged Akkaraman Rams in Breeding Season of Afyon Province

Age	n	Ejaculate volume* (ml)	Semen viscosity (1-5)	Semen pH (5.5-9.0)	Mass activity (1-5)	Sperm motility* (%)	Sperm concentration* (x10 <sup>9</sup> /ml)	Abnormal sperm rate* (%)	Testosterone levels (ng/ml)
2	50	0.89 $\pm$ 0.02	4.45 $\pm$ 0.09	6.58 $\pm$ 0.05	4.50 $\pm$ 0.08	82.25 $\pm$ 1.04	2.97 $\pm$ 0.03	4.54 $\pm$ 0.05	3.64 $\pm$ 0.18
4	50	1.01 $\pm$ 0.02	4.53 $\pm$ 0.08	6.50 $\pm$ 0.05	4.60 $\pm$ 0.08	84.50 $\pm$ 0.80	3.96 $\pm$ 0.02	3.99 $\pm$ 0.02	6.03 $\pm$ 0.24

\*: The groups are statistically different (P &lt; 0.05).

Table 3. Partial Correlation Coefficients Controlled for Age Among Reaction Times and Morphometric Measurements of Testes

	Testes lenght right	Testes lenght left	Testes thickness right	Testes thickness left	Scrotal circumfer.	Scrotal sac volume	Scrotal thickness	Reaction time
Testes lenght right	-							
Testes lenght left	0.29	--						
Testes thickness right	0.20	0.85**	--					
Testes thickness left	0.32	0.05	0.48	--				
Scrotal circumference	-0.42	0.42	0.61 <sup>†</sup>	0.37	--			
Scrotal sac volume	-0.31	-0.26	-0.02	0.45	0.65 <sup>†</sup>	--		
Scrotal thickness	0.31	0.17	0.16	-0.11	-0.58	-0.89**	--	
Reaction time	0.12	-0.35	-0.54	-0.54	-0.90**	-0.81**	0.72*	--

† : P &lt; 0.10 \* : P &lt; 0.05 \*\* : P &lt; 0.01

Table 4. Partial Correlation Coefficients Controlled for Age Among Spermatological Parameters and Testosterone Levels

	Volume	Viscosity	pH	Mass activity	Motility	Concen.	Abnormal	Testoster.
Volume	--							
Viscosity	-0.68 <sup>†</sup>	--						
pH	0.91**	-0.64 <sup>†</sup>	--					
Mass activity	0.49	0.16	-0.27	--				
Motility	-0.89**	0.93**	-0.85**	0.26	--			
Concentration	0.05	-0.41	-0.02	-0.48	-0.19	--		
Abnormal	0.30	-0.25	0.54	-0.17	-0.28	0.49	--	
Testosterone	0.82**	0.55	0.58	-0.81**	-0.66 <sup>†</sup>	0.38	-0.19	--

† : P &lt; 0.10 \* : P &lt; 0.05 \*\* : P &lt; 0.01

Table 5. Partial Correlation Coefficients Controlled for Age Among Morphometric Testes Measurements, Reaction Times, Spermatological Features and Testosterone Levels

	Testes lenght right	Testes lenght left	Testes thickness right	Testes thickness left	Scrotal circumfer.	Scrotal sac volume	Scrotal thickness	Reaction time
Volume	-0.68*	0.04	0.39	0.25	0.59	0.19	0.06	-0.27
Viscosity	0.16	-0.66 <sup>†</sup>	-0.92**	-0.51	-0.82**	-0.23	0.03	0.67 <sup>†</sup>
pH	-0.64 <sup>†</sup>	0.26	0.44	-0.03	0.47	-0.15	0.27	-0.07
Mass activity	0.12	0.44	-0.01	-0.50	0.11	-0.05	-0.38	-0.15
Motility	0.47	-0.44	-0.73*	-0.36	-0.81**	-0.23	0.03	0.54
Concentration	0.61 <sup>†</sup>	0.37	0.61 <sup>†</sup>	0.61 <sup>†</sup>	-0.10	-0.27	0.57	-0.02
Abnormal	0.11	0.40	0.39	-0.14	-0.25	-0.81**	0.87**	0.50
Testosterone	-0.36	-0.17	0.32	0.56	0.36	0.26	0.15	-0.23

† : P &lt; 0.10 \* : P &lt; 0.05 \*\* : P &lt; 0.01

## DISCUSSION

### Morphometric measurements of testes and reaction times

The testes length values were lower than the findings of Aral and Tekin (1996) (14.2 and 14.1 cm) in five aged rams and in consonant with the results of Gündoğan (1999) (9.05 cm) in 18 months old Akkaraman rams and Ataman et al (1996) (8-12.9 cm) in 15 months old Merino rams. The values of testes thick in two years old rams were in the range of 4.55-6.10 cm reported by different workers (Ataman et al 1996, Gündoğan 1999) the same values in four years old rams were lower than the findings of Aral and Tekin (1996) (6.2 and 6.1 cm) in the Akkaraman rams aged of 1 to 5 years.

Scrotal circumference values were lower than the results of Gündoğan (1999) and Pérez et al (1997), higher than Aral and Tekin (1996) and Kaya et al (1999) and similar with that of Ataman et al (1996).

Scrotal sac volume values were higher than the findings of Gündoğan (1999) in 18 months old Akkaraman rams and Ataman et al (1996) in 15 months old Merino rams and similar with Kaya et al (1999) in 3 to 4 years old Konya Merino rams.

The mean scrotal thickness values were lower than the value (0.7 cm) reported by Aral and Tekin (1996).

The mean reaction times values were lower than the findings of Taha et al (2000) in Barki rams (10.46 s) and similar with the value in the Awassi rams (8.43 s).

The morphometric measurements of testes and reaction times in all rams were in accordance with the references (Ataman et al 1996, Gündoğan 1999, Kaya et al 1999, Taha et al 2000). The differences might be due to breed, age, feeding and season. In addition because of the conditions of farm the rams and sheep are maintained closely therefore the effects of sheep could be concerned.

### Spermatological parameters and testosterone levels

The ejaculate volumes values were similar with the findings of Ataman et al (1996), Gündoğan et al (1997) (0.91ml), Gündoğan (1999) (0.87 ml) in Akkaramans and Taha et al (2000) (0.92 ml) in Awassies, lower than Aral and Tekin (1996) (1.1 ml), Kaya et al (1999) (0.9 ml) in 1 to 5 years old Akkaramans and 3 to 4 years old Merino rams and Piriñçi et al (2001b) 0.95 in two years old Akkaramans. Whereas Taha et al (2000) reported that this value as 0.72 in Barkies. The result of this study were higher than these values.

The viscosity and pH of semen values were similar with the findings of Gündoğan et al (1997), Gündoğan (1999) and Wiggins et al (1953) in both traits, the viscosity values were higher than the finding of Piriñçi et al (2001b) (4.3) in two years old Akkaraman rams.

The mean mass activity of spermatozoa values were higher than the values (4.1) of Aral and Tekin (1996) and similar with that of Ataman et al (1996),

Gündoğan et al (1997) and Gündoğan (1999) and lower than (4.7) that of Piriñçi et al (2001b).

The mean percentage of motile spermatozoa values were higher than the findings of Gündoğan et al (1997) (80.90 %) in 16 months old Akkaramans and Taha et al (2000) (68.91 and 64.3%) in sexually mature Awassi and Barkies, lower than that of Kaya et al (1996) (85.7%) and Piriñçi et al (2001b) (96%) and similar with the findings of Aral and Tekin (1996), Ataman et al (1996) and Gündoğan (1999).

The concentration of spermatozoa values were lower than the finding of Gündoğan et al (1997) ( $4.06 \times 10^9$ /ml for 16 months old Akkaramans), Piriñçi et al (2001b) ( $4.56 \times 10^9$ /ml for 2 years old Akkaramans) and Taha et al (2000) (5.16 and  $4.73 \times 10^9$ /ml in Awassi and Barkies), higher than Aral and Tekin (1996) ( $3.1 \times 10^9$ /ml), Gündoğan (1999) ( $3.8 \times 10^9$ /ml) and Kaya et al (1996) ( $3.6 \times 10^9$ /ml) and similar with Ataman et al (1996) in 15 months old Merino rams.

The mean abnormal spermatozoa rates values were similar with the findings of Aral and Tekin (1996) (3.3%), Gündoğan (1999) (3.48%) and Kaya et al (1996) (3.6%), lower than Pérez et al (1997) (9.4%) and Taha et al (2000) (14.2%) and higher Ataman et al (1996) (1.6%), Gündoğan et al (1997) (3.63% for 16 months old rams) and Piriñçi et al (2001b) (2.72%).

The spermatological parameters in all rams were in accordance with the references (Ataman et al 1996, Aral and Tekin 1996, Gündoğan et al 1997, Gündoğan 1999, Kaya et al 1999, Taha et al 2000, Wiggins et al 1953). The differences might be due to breed, age, feeding and management, semen collecting time and season, evaluation technique and semen diluters.

The mean blood sera testosterone levels values were similar with the results of Piriñçi et al (2001a) and Taha et al (2000), higher than Gomes and Joyce (1975) (0.76 ng/ml), Gündoğan (1999) (2.74 ng/ml) and lower than Dufour et al (1984) (6.6 ng/ml in blood plasm), Pérez et al (1997) (19.7 nmol/l =12.85 ng/ml) and Taha et al (2000) (14.41 ng/ml). The differences in blood sera testosterone levels might be due to breed, age, feeding and management, sample collection time and evaluation procedure.

### Correlation

Testes thickness right was correlated with testes length left ( $r:0.85$ ) positively ( $P<0.01$ ). Scrotal thickness and reaction time were negatively ( $P<0.01$ ) related with scrotal sac volume ( $r:-0.89$  and  $r:-0.81$ ). These results showed that the rams having thinner scrotal skin had higher scrotal sac volume. The reaction time was also correlated with scrotal circumference ( $r:-0.90$ ) negatively ( $P<0.01$ ). These results were in consonant with the findings of Aral and Tekin (1996), Ataman et al. (1996), Foster et al. (1989), Kaya et al. (1999) and Nowakowski and Cwikla (1994).

The correlations between spermatological parameters showed that the ejaculate volume was positively ( $P<0.01$ ) associated with pH ( $r:0.91$ ) and testosterone level ( $r:0.82$ ) whereas the correlation with motility ( $r:-0.89$ ) was negative ( $P<0.01$ ). The association

between viscosity and motility was positive ( $r:0.93$ ) and significant ( $P<0.01$ ). These findings were similar with the results of Aral and Tekin (1996), Ataman et al (1996), Kaya et al (1999), Langford et al (1989) and Rege et al (2000).

The viscosity of semen was negatively ( $P<0.01$ ) correlated with the testes thickness right ( $r:-0.92$ ) and scrotal circumference ( $r:-0.82$ ). The correlations between motility and testes thickness right ( $r:-0.73$ ) and scrotal circumference ( $r:-0.81$ ) were negative and significant ( $P<0.05$ ,  $P<0.01$ ). The abnormal sperm rate was negatively ( $P<0.01$ ) correlated with scrotal sac volume ( $r:-0.81$ ) and positively correlated ( $P<0.01$ ) with scrotal thick ( $r:0.87$ ). These results showed that rams having greater scrotal sac volume and less scrotal thickness had lower abnormal sperm rate. These findings were in consonant with that of Aksoy et al (1994), Aral and Tekin (1996), Ataman et al (1996), Demirci (1993), Gündoğan (1999), Kaya et al (1999) and Pérez et al (1997).

As a result, all the reproductive parameters studied were in the normal ranges. The effect of age on morphometric measurements of testes, reaction times, ejaculate volume, percentage of motile spermatozoa, sperm concentration and abnormal sperm rate were significant ( $P<0.05$ ). Therefore this factor must be considered in selecting rams. In addition testes thickness right, scrotal circumference, scrotal sac volume and scrotal thick from the morphometric measurements of testes could be used to evaluate the reproductive performance of a ram.

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