

The Relationship Between Sexual Behaviors and Serum Testosterone Concentrations in Norduz Rams

Serhat Karaca^{1*}, Sibel Erdoğan¹, Ayhan Yılmaz²

¹YuzuncuYıl University, Agricultural Faculty, Department of Animal Science, 65200, Van, Turkey

²Siirt University, Agricultural Faculty, Department of Animal Science, 56000, Siirt, Turkey

*e-mail: skaraca@yyu.edu.tr; Phone: +90 (432) 225 1024/21652; Fax: +90 (432) 486 5413

Abstract

The aim of this study was to investigate the relationship between sexual behavior and serum testosterone concentrations in Norduz rams. Sexual behavior characteristics were tested by exposing rams individually to 3 estrous ewes for 15 min each and four sexual behavior tests were performed every other day for each ram. Neither sexual behavior characteristics nor serum testosterone concentrations (TC) of rams varied over the course of testing ($p>0.05$); whereas vocalization and TC varied significantly among rams ($P<0.05$). Moreover, significant correlations [flehmen response-ejaculation frequency (0.462); duration of mounting-ejaculation frequency (-0.494); mounting frequency- age (-0.458); TC (before test)-duration of ejaculation (-0.544)] were detected between sexual behaviors and physiological traits ($p<0.05$). However, the relationship between sexual behaviors and TC were found insignificant except the ejaculation duration. Finally, it can be suggested that the effect of serum testosterone concentrations on sexual behaviors of experienced ram are limited in mating season.

Key words: Norduz, reaction time, serum testosterone, mating season

Norduz Koçlarında Eşeyssel Davranım Özellikleri ve Serum Testosteron Konsantrasyonu ile İlişkisi

Özet

Bu çalışmanın amacı, Norduz koçlarında eşeyssel davranış özelliklerinin belirlenmesi ve bu özellikler ile serum testosteron konsantrasyonu arasındaki ilişkinin incelenmesi olmuştur. Eşeyssel davranış özellikleri, koçların bireysel olarak 15 dakika süreyle 3 kızgın koyuna maruz bırakılmasından oluşan ve birer gün ara ile gerçekleştirilen, toplam 4 test ile belirlenmiştir. Eşeyssel davranış özellikleri ve serum testosteron konsantrasyonu (TK) test günlerine göre değişim göstermezken ($p<0.05$); TK ve ses çıkartmanın koçlara göre önemli farklılıklar gösterdiği belirlenmiştir ($p<0.05$). Ayrıca bazı eşeyssel davranışlar ile fizyolojik özellikler arasında önemli korelasyonlar [flehmen-ejakülasyon sayısı (0.462), biniş süresi-ejakülasyon sayısı (-0.494), biniş sayısı-yaş (-0.458), TK (test öncesi)-ejakülasyon süresi (-0.544)] belirlenmiştir ($p<0.05$). Bununla birlikte, incelenen davranım özellikleri ile testosteron konsantrasyonu arasında ejakülasyon süresi ile olan negatif korelasyon dışında önemli bir ilişki saptanmamıştır. Sonuç olarak, koç katım döneminde serum testosteron konsantrasyonunun, deneyimli koçların eşeyssel davranış özelliklerine etkisinin sınırlı düzeyde olduğunu söylemek mümkündür.

Anahtar kelimeler: Norduz, reaksiyon süresi, serum testosteron, koç katımı

Introduction

Ram sexual behaviors are important for determining their reproduction potential. Males are known to show interest in females from shortly after birth, and this 'sexual play' is easily observed among flocks. Characteristics of male sexual behaviors have been reported to vary widely according to flock management and breeding practices. In order to ensure the healthy sexual development of males, it is important to identify appropriate management practices as well as relevant environmental factors (Price et al., 2001; Clemente et al., 2013; Souri and Mirmahmoudi, 2014). The male fertility is very important issue because numerous ewes is generally mated to a single ram. Thus, it is essential

to identify the best males for use as breeding stock. Sexual behavior or serving capacity tests are not common practice for small ruminants. However, these tests are an important part of the evaluation of the breeding soundness.

Important physiological and endocrinological parameters for ram reproduction are the concentration of peripheral Testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), which they can be criteria to definite the reproductive efficiency of rams (Lincoln et al., 1990; Langford et al., 1998; Kishk, 2008). The levels of these hormones in sheep are affected by factors that include genotype, age, nutritional status and season (Dufour et al., 1984;

Sanford et al., 2000; Fernandez et al., 2004). In order to improve reproductive efficiency of rams, it is important to evaluate the interaction of the different reproductive characteristics regarding with animal reproduction. Therefore, this study aimed to determine the relationships among serum testosterone concentrations and sexual behavior and also other some physiological traits.

Material and methods

This study was conducted with 5 male Norduz rams and 12 ewes at experimental farm of Animal Science Department of Yuzuncu Yil University in September 2013 (38°34'1"N 43°17'30"E and 1700 m above sea level). Fat-tailed Norduz sheep are native to the province of Van, located in the Eastern Anatolia region of Turkey. Animals were cared for in accordance with guidelines established by the Local Animal Studies Ethics Committee of Yuzuncu Yil University in Van, Turkey.

The rams used in the study were sexually experienced males with an average age of 3.2 ± 0.33 years and weighing between 80-95 kg. Visual and olfactory contact between rams and ewes was prevented prior to and during the experimental period. For this aim, rams were individually exposed to 3 estrous ewes for 15 minutes and four sexual behavior tests were performed every other day for each ram. Testing was conducted between 08.00 and 11.00 h, and the rams to be tested selected randomly in order to eliminate the effect of day. Ewes were induced into estrous using vaginal sponges impregnated with 60 mg medroxyprogesterone acetate for 14 days, followed by an intramuscular injection of 500 i.u. PMSG (PMSG, Intervet) upon sponge withdrawal. Teasing rams which they were prevented to mate were used to detect estrous ewes 24-72 hours after PMSG injection.

Sexual behavior characteristics were recorded as described by Price et al. (1988) and Price (1993) by a single observer located approximately 2 m from the site of action. The following sexual behavior characteristics were recorded:

FL: Flehmen response, i.e. the classic head-raised, lip-curling behavior of the ram as it detects the smell of estrous ewes;

AS: Anogenital sniffing, i.e. the ewe allows the ram to sniff her tail and genitalia;

V: Number of vocalizations from the time the ram enters the pen until the end of the test period;

MF: Frequency of mounting, i.e. number of mounts from the time the ram enters the pen until the end of the test period;

MD: Mounting duration (min), i.e. the length of time between the ram's introduction to the estrous ewes' pen and mounting;

ED: Ejaculation duration (min), i.e. the length of time between the ram's introduction into the pen and the end of copulation;

EF: Frequency of ejaculation, i.e. number of ejaculations from the time the ram enters the pen until the end of the test period;

DBE: Duration between ejaculations, i.e. the time interval between the first and second copulation;

EE: Ejaculation Efficiency, defined as the frequency of ejaculation divided by the frequency of mounting.

In addition, serum testosterone concentrations (TC) of rams were analyzed from blood samples taken immediately before and after each test period. On each test day, 10 ml of blood was collected from ram jugular veins into pastor tubes; samples were centrifuged at 3000xg for 15 min and stored at -20°C until measurement. The analysis of serum testosterone concentrations was performed by using chemiluminescent microparticle immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex (Architect system).

Statistical analysis was performed using the software SPSS (2013). Kruskal-Wallis test was used to examine the effects of ram and test on continuous phenotypes. The Dunnet test was used to detect differences among groups. Spearman Rank Correlation Coefficients were used to calculate correlations among variables.

Results

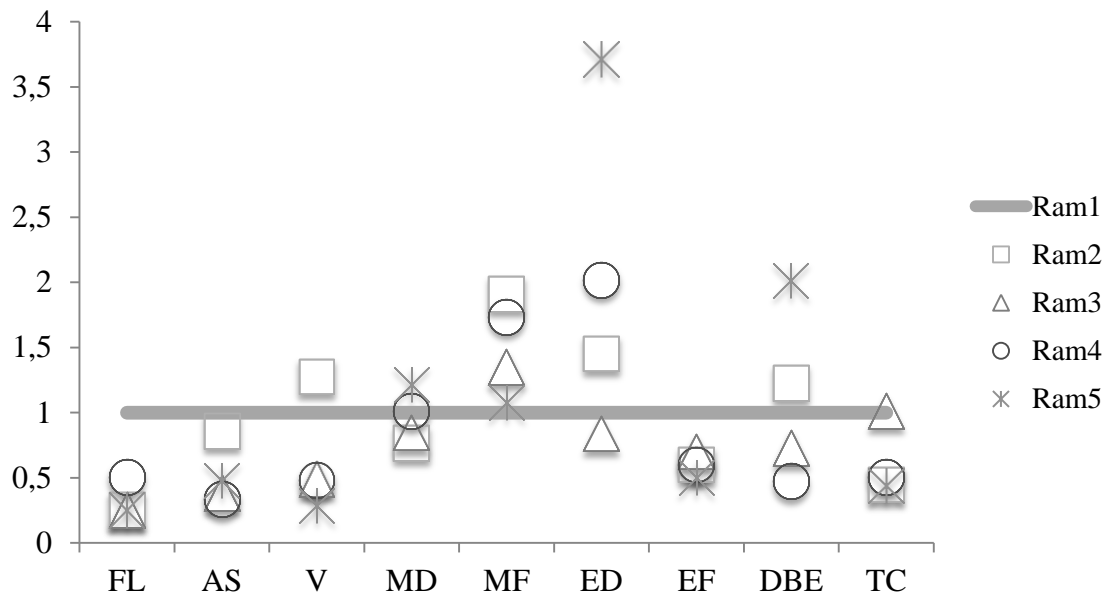
Findings for flehmen response (FL), anogenital sniffing (AS), vocalization (V), mounting duration (MD), frequency of mounting (MF), ejaculation duration (ED), frequency of ejaculation (EF), duration between ejaculations (DBE), ejaculation efficiency (EE) and serum testosterone concentrations (TC) are given in Table 1. As the table shows, no significant differences were found in serum testosterone concentrations or sexual behaviors between test days ($P > 0.05$). However, V and TC were significantly different between rams ($P < 0.05$). In addition to this, Figure 1 shows the differences among rams for sexual behaviors and serum testosterone concentrations.

Table 1. Sexual behaviors and serum testosterone concentrations in Norduz rams

Traits ¹	Tests				Overall	Significance		
	1	2	3	4		Test	Ram	Age
FL	0.4±0.24	0.2±0.20	0.6±0.40	0.6±0.40	0.4±0.15	ns	ns	ns
AS	4.2±1.32	3.0±1.05	3.0±0.77	2.6±0.74	3.2±0.47	ns	ns	*
V	21.8±3.93	40.0±8.76	42.2±12.90	22.4±9.81	31.6±4.84	ns	*	*
MD	0.7±0.17	0.9±0.06	1.0±0.08	1.0±0.13	0.9±0.06	ns	ns	ns
MF	22.4±5.49	28.4±6.22	22.8±4.88	36.6±10.60	27.5±3.54	ns	ns	ns
ED	4.2±2.69	1.9±0.44	4.7±1.25	1.8±0.34	3.4±0.87	ns	ns	ns
EF	2.0±0.31	1.4±0.67	1.6±0.24	1.8±0.49	1.7±0.21	ns	ns	ns
DBE	7.1±1.43	2.5±0.61	5.0±2.55	7.6±2.21	6.1±1.03	ns	ns	ns
EE	0.13±0.047	0.05±0.022	0.10±0.040	0.08±0.035	0.09±0.018	ns	ns	ns
Serum testosterone concentrations (TC) (ng/ml)								
Before test	4.33±0.715	3.99±0.85	5.69±1.43	5.59±2.24	4.90±0.68	ns	*	ns
After test	5.77±1.34	2.54±0.29	4.56±1.04	6.74±2.37	4.91±0.76	ns	ns	ns
Overall	5.08±0.86	3.51±0.32	5.76±1.07	6.17±1.90	4.90±0.60	ns	*	ns

¹ FL: flehmen response, AS: anogenital sniffing, V: vocalization, MD: mounting duration, MF: Frequency of mounting, ED: ejaculation duration, EF: frequency of ejaculation, DBE: duration between 1st-2nd ejaculations, EE: ejaculation efficiency

*p< 0.05; ns: not significant



¹ FL: flehmen response, AS: anogenital sniffing, V: vocalization, MD: mounting duration, MF: frequency of mounting, ED: ejaculation duration, EF: frequency of ejaculation, DBE: duration between 1st-2nd ejaculation, TC: serum testosterone concentration

Figure 1. The index with results for each ram comprised of average values for each test day and with the values for Ram1 used as the reference¹

Significant correlations were found among several of the sexual behavior characteristics investigated (Table 2). FL was positively correlated with EF (0.462). MD correlated positively with ED (0.483) and negatively with EF (-0.494), indicating that as the number of mounts increased, the frequency of ejaculation decreased. Body weight and age were negatively correlated with MF as -0.466 and -0.458, respectively.

In other words, as the increases in age of the rams to results in parallel increase both body weight and sexual experience. Thus, young males continue to exhibit mounting until ejaculation occurs. With the exception of ED (-0.544), no significant correlation was found between TC and sexual behavior characteristics. However, a positive correlation was established between TC and ram age (0.470).

Table 2. The Spearman rank coefficients among some sexual behaviors

Traits ¹	r
FL- EF	0.462*
MD-ED	0.483*
MD-EF	-0.494*
MF-age	-0.458*
TC (before testing)-ED	-0.544*
TC (overall)-age	0.535*

¹FL: flehmen response, MD: mounting duration, MF: frequency of mounting, ED: ejaculation duration, EF: frequency of ejaculation, TC: serum testosterone concentration

*P<0.05

Discussion

The main aim of this study was to test the hypothesis that a relationship exists between serum testosterone concentrations and sexual behaviors in rams. The findings regarding sexual behaviors and serum testosterone concentrations were generally in accordance with those reported by previous studies (Price et al., 2000; Kishk, 2008; Sogorescu et al., 2012; Benia et al., 2013; Clemente et al., 2013). Significant correlations were found between serum testosterone concentrations and some sexual behavior and physiological traits (Table 2).

No significant correlation was found between FL and ram body weight; however, FL had a positive correlation with EE. Bozkurt (2012) reported that when compared to young rams, FL occurred more frequently in mature rams that had learned to effectively detect estrous ewes from endocrinological concentrations. Although, rams have different ages, similar differences were not found in the current study in line with other studies (Price et al., 1988; Katz et al., 1988), those indicating that rams have no longer experiences needed for effectively mating activities. On the other hand, Yılmaz et al. (2009) reported that sexual behavior traits such as vocalization, ejaculation duration and ejaculation efficiency were significantly different among mature rams according to their ages and older rams have better performance than the younger ones. As for AS, V and MF, these characteristics had very high values compared to the other studies (Katz et al., 1988; Godfrey et al., 1998; Price et al., 2001). The earlier studies have also reported that high values of the sexual behaviors prior to mating, particularly recorded in inexperienced rams or bucks (Price et al., 1988; Kridli and Said, 1999; Darwish and Mahboub, 2011). However, Yılmaz et al. (2009) reported that Norduz rams had high values for courtship elements, such as vocalization (62.90), anogenital sniffing without

flehmen (4.65) and frequency of mounting without ejaculation (38.73) in accordance with our results. It may be suggested that breed and individual differences also play an important role in the expression of ram sexual behavior, particularly in courtship behavior.

In the current study, age of rams was negatively correlated with MF. This result is in line with Price et al. (1988) and Katz et al. (1988). Godfrey et al. (1998) reported that sexual behaviors in young rams developed with age. In the current study, no differences were observed between rams for MD in line with Yılmaz et al. (2009), who reported mounting duration was not significantly varied in mature rams according to age of them. This is partially supported by Benia et al. (2013), who observed no age-related improvements in sexual behaviors of Rebi rams after the age of 4. Moreover, according to the authors, differences in sexual behaviors could not be attributed to age only.

It is well known that serum testosterone level, semen characteristics and sexual activity varies depending on seasonal changes and significantly high in mating season (Souri and Mirmahmoudi, 2014). In the present study, the differences in TC were significant among rams prior to testing (P<0.05). Dufour et al. (1984) reported that although Suffolk rams that had high sexual activity, they did not have high plasma testosterone concentrations. Another study also found no direct relationship between ram sexual activity and plasma testosterone concentrations (Perkins et al., 1992). Although Davant et al. (1974) reported that serum testosterone concentration is not the only determining factor in explaining the social dominance of rams during mating. However, other studies have clearly shown a relationship between sexual behavior and serum testosterone concentrations in rams, especially during mating season (Gündoğan, 2007). Perkins et al. (1992) and Stellflug (2006) indicated that plasma testosterone concentrations did not vary between high and low-libido rams. The contradictory findings indicate that reproduction in rams is not determined solely by hormonal effects, but other characteristics are relevant to sheep reproductive characteristics. For this reason, it is necessary to examine a combination of reproductive characteristics in order to correctly estimate and control reproduction in rams (Moghaddam et al., 2012).

Testosterone concentrations are known to be affected by various factors such as body weight, season, genotype and age (Lincoln et al., 1990; Langford et al., 1998; Yılmaz, 2006). In contrast, Benia et al. (2013) found no differences in plasma testosterone concentrations of

Rembi rams by age. However, in the same study, it was stated that spermatological and sexual behavior characteristics were varied with levels of plasma testosterone concentrations.

As a result, significant differences in the sexual characteristics of rams are not expected after maturation. However, some recent studies have clearly shown different breeding systems to cause differences in rams, especially during the developmental stage. In the current study, for the most part, the relationships between TC and some sexual behaviors were not statistically significant, whereas there was high correlation coefficient between ED and TC (before test). It may be suggested that rams have higher concentration of testosterone, also have better ejaculation duration than lower ones. On the other hand, despite the role of testosterone concentration in regulating sexual activity in rams, other factors should also be involved. It was concluded that although identification of sexual behavioral characteristics is crucial to predict ram reproductive potential, evaluation of other aspects of reproductive management is also required for effective ram selection. It may be claimed that the effect of testosterone concentration on ram reproduction is related to certain gametogenic and somatic factors. This hypothesis may be investigated in further studies.

Finally, it was confirmed that ram sexual behaviors are a vital factor to completely predict the potential of reproduction in rams and it should be combined with other characteristics of ram reproduction to carry out effectively selection programs.

References

- Benia, A.R., Taibi, K., Ait-Amrane, A., Belhamiti, T., Hammoudi, S.M., Kaidi, R. 2013. Study of sexual activity variations in Algerian rams: Sexual behavior, testosterone concentration control and environmental factors. *Afr. J. Biotechnol.* 12(4): 6042-6048.
- Bozkurt, T. 2012. Sexual performance of White Akkaraman and Kıvrıkcık rams exposed to fat-tailed ewes. *J. Anim. and Vet. Adv.* 11(2): 271-275.
- Clemente, N., Orihuela, A., Flores-Perez, I., Aguirre, V., Valencia, J. 2013. Reproductive behavior of Saint Croix and Suffolk rams at medium latitudes (19 N) during long days while being exposed to Suffolk ewes in seasonal anestrus. *Arch. Med. Vet.* 45: 67-70.
- Darwish, R.A., Mahboub, H.D.H. 2011. Breed and experience effect on the sexual behaviors of Damascus and Egyptian-Nubian goat bucks. *Theriogenology* 76: 1386-1392.
- Davant, J.H., Han, D.K., Moody, E.L. 1974. Dominance in rams in relation to serum testosterone. *J. Anim. Sci.* 38: 1333-1334.
- Dufour, J.J., Fahmy, M.H., Minvielle, F. 1984. Seasonal changes in breeding activity, testicular size, testosterone concentration and seminal characteristics in rams with long or short breeding season. *J. Anim. Sci.* 58: 416-422.
- Fernandez, M., Ginaldez, F.J., Frutos, P., Lavin, P., Mantecon, A.R. 2004. Effect of undegradable protein supply on testicular size, spermogram parameters and sexual behavior of mature Assaf rams. *Theriogenology* 62(1-2): 299-310.
- Godfrey, R.W., Collins, J.R., Gray, M.L. 1998. Evaluation of sexual behavior of hair sheep rams in a tropical environment. *J. Anim. Sci.* 76: 714-717.
- Gündoğan, M. 2007. Seasonal variation in serum testosterone, T3 and andrological parameters of Turkish sheep breeds. *Small Rumin. Res.* 63(2-3): 312-316
- Katz, L.S., Price, E.O., Wallach, S.J.R., Zenchak, J.J. 1988. The relationship of male-male mounting to the sexual preferences of young rams. *J. Anim. Sci.* 66: 1166-1173.
- Kishk, W.H. 2008. Interrelationship between ram plasma testosterone level and some semen characteristics. *Slovak J. Anim. Sci.* 41(2): 67-71.
- Kridli, R.T., Said, S.I. 1999. Libido testing and the effect of exposing sexually Native Awassi rams to estrous ewes on sexual performance. *Small Rumin. Res.* 32:149-152.
- Langford, G.A., Shrestha, J.N.B., Sanford, L.M., Marcus, G.J. 1998. Reproductive hormone levels of early postpubertal ram lambs in relation to breed, adult testis size and semen quality. *Small. Rumin. Res.* 29: 225-231.
- Lincoln, G.A., Lincoln, C.E., McNeilly, A.S. 1990. Seasonal cycles in the blood plasma concentration of FSH, inhibin and testosterone, and testicular size in rams of wild, feral and domesticated breeds of sheep. *J. Reprod. Fert.* 88: 623-633.
- Moghaddam, G., Mohamad Reza, M.P., Seid, A., Abass, R., Raziallah, J.Z. 2012. Relationship between levels of peripheral blood testosterone, sexual behavior, scrotal circumference and seminal parameters in crossbred rams. *Acta Scientiae Veterinariae* 40(3): 1049.
- Perkins, A., Fitzgerald, J.A., Price, E.O. 1992. Luteinizing hormone and testosterone response of sexually active and inactive rams. *J. Anim. Sci.* 70: 2086-2093.
- Price, E.O., Katz, L.S., Wallach, S.J.R., Zenchak, J.J. 1988. The relationship of male-male mounting to the

- sexual preferences of young rams. *App. Anim. Behav. Sci.* 21: 347-355.
- Price, E.O. 1993. Practical considerations in the measurement of sexual behavior. *Methods in Neurosciences* 14: 16-31.
- Price, E.O., Bench, C.J., Borgwardt, R.E., Dally M.R. 2000. Sexual performance of twin ram lambs and the effect of number and sex of contemporary siblings. *App. Anim. Behav. Sci.* 68: 199-205.
- Price, E.O., Borgwardt, R.E., Dally, M.R. 2001. Male-male competition fails to sexually stimulate domestic rams. *App. Anim. Behav. Sci.* 74: 217-222.
- Sanford, L.M., Moore, C., Voglmayr, J.K., Fahmy, M.H. 2000. Sexual maturational changes in circulatory inhibin concentration in relation to FSH concentration and testicular size in Suffolk and DLS (1/2 Dorset, 1/4 Leicester, 1/4 Suffolk) rams. *Theriogenology* 54: 719-730.
- Sogorescu, E., Zamfirescu, S., Anghel, A.H., Nadolu, D., Dobrin N. 2012. Pattern of testosterone secretion, testicular volume and sperm production after applied the photoperiod treatments on Carpathian bucks. *Annals of RSCB* 17(1): 284-291.
- Souri, M., Mirmahmoudi, R. 2014. Effect of season on dry matter intake and reproductive activity of Merghoz buck goats in West of Iran. *Iranian Journal of Applied Animal Science* 4(2): 317-323.
- SPSS Inc. (2013). *SPSS for Windows. Version 22.0*, Chicago.
- Stellflug, J.N, Cockett N.E., Lewis, G.S. 2006. Relationship between sexual behavior classifications of rams and lambs sired in a competitive breeding environment. *J. Anim. Sci.* 84(2): 463-8.
- Yılmaz, A. 2006. Norduz Erkek Kuzularında Bazı Üreme Özelliklerinin Belirlenmesi. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü (Doktora tezi, basılmamış, 141 sayfa), Van.
- Yılmaz, A., Karakuş, F., Yeşilova, A. 2009. Norduz ve Karakaş koçlarında eşeysel davranış özellikleri ve yaşla değişimi. *Tarım Bilimleri Dergisi* 15(3): 270-276.