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SEXUAL BEHAVIOUR, TESTOSTERONE CONCENTRATION, SEMEN CHARACTERISTICS AND TESTES SIZE OF OSSIMI RAMS AS AFFECTED BY AGE AND SCROTAL CIRCUMFERENCE

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SUMMARY

The objective of this study was to determine size of testes, sexual behaviour, semen quality and testosterone concentration on 40 Ossimi rams in relation to scrotal circumference and age. Rams were divided into two groups similar in age and body weight. Group A with age of 1-1.5 years old and group B with 2-2.5 years old. Each group was divided into subgroups according to the scrotal circumference (≤ 25 cm and > 25 cm) at the beginning of the study. Pre-copulation behaviour, body weight and testicular measurements for each ram were recorded weekly for one month before starting the experiment. Semen was collected to define its characteristics in addition to collect blood samples to determine testosterone concentration in the peripheral blood before copulation and after ejaculation.

Results revealed that there were significant differences in body weight, number of mounting, testicular measurements, semen characteristics and testosterone concentration between rams under two age groups. In contrast, there were no significant differences in reaction time and pre-copulation behaviour. Rams had scrotal circumference > 25 cm showed stronger sexual behavior, testosterone concentration and semen characteristics (P<0.001) compared to rams had scrotal circumference ≤ 25 cm. There were positive correlations between number of mounting, scrotal circumference and testosterone concentration. On the other hand, there were negative correlations between reaction time and each of number of mounting, scrotal circumference and testosterone concentration.

It could be concluded that the sexual activity and semen characteristics improved with age progress and increasing of scrotal circumference.

Keywords: Rams, sexual behaviour, testes, testosterone, semen characteristics

INTRODUCTION

Natural mating is commonly used for breeding sheep under the prevailing system of sheep production in Egypt. So, studying the factors control semen quality and sexual behavior as well as testing rams' reproductive performance is required (Sarlós, 2006).

Sexual behaviour of the rams is affected by breed and age (Simitzis *et al.,* 2005). Previous results indicated positive association between rams with high scores for sexual behaviour and fertility (Matos and Thomas, 1991; Perkins *et al.,* 1992 and Kilgour, 1993). Frequency of ano-genital sniffs, foreleg kicks, nudges, vocalizations, flehmens and mount attempts were reported to be displayed as pre-copulatory behavior after exposure to ewes (Perkins and Charles, 2007), which underlying rams' sexual motivation (Price *et al.,* 1992 and Kridli *et al.,* 2007).

Close relationship among testicular size, sperm production and fertility was reported by Toe *et al.* (2000). This is most probably due to the interrelationship between sexual hormones (testosterone and LH, FSH) and testes diameter as well as libido (Dufour *et al.,* 1984; Kilgour *et al.,* 1985 and Matos and Thomas, 1991).

Peripheral testosterone concentration affects ram behaviour (Charles *et al.,* 2002), since testosterone concentration was found to be higher in sexually active rams than inactive ones (Stellflug *et al.,* 2004). Perkins and Charles (2007) reported that a minimum threshold of testosterone concentration is required for the acquisition and display of adult sexual behaviour as well as sex organs differentiation.

Up to the knowledge of the author, rare works were conducted to study the relationship between scrotal circumference and semen quality and sexual libido of the native sheep breeds.

In the light of the previous results, this study was planned to determine the effect of age and scrotal circumference on sexual behaviour, semen characteristics and development of sexual organs of Ossimi rams.

MATERIAL AND METHODS

Experimental animals and management

The present study was carried out in the Experimental Farm of Animal Production Department, Faculty of Agriculture, Assiut University, Egypt. The study was started at the 1st

of September 2011 and extended to the 30th October, 2011.

A total number of 40 Ossimi rams were selected to be used in this study. Rams were divided into two groups, the 1st (A) was of 1-1.5 years old and the 2nd one (B) was of 2-2.5 years. Rams of group A (n= 18) and B (n= 22) had a body weight of 36.9 ± 1.6 kg and 56.1 ± 1.4 kg, respectively. Each group was divided into two subgroups according to the scrotal circumference (≤ 25 cm and > 25 cm) at the beginning of the study. Rams were kept in semi open pens under the normal environmental conditions. Rams were fed on concentrate feed mixture, contained 2.33 Mcal/kg metabolizable energy, 13.66% crude protein, 1.97% crude fat, 11.3% crude fiber, 1.2% limestone, 0.51% calcium and 0.3% phosphorous. Beside the concentrate feed mixture, rams were fed on wheat straw *ad libtum*. Water was made available alls day time.

Measurements

Pre-copulationy behaviour, body weight and testes measurements (scrotal circumference, testis diameter and length) for each ram were recorded weekly for one month before measuring sexual traits. Scrotal circumference was measured using a flexible tape at the widest scrotal diameter (Oberst *et al.,* 2011), while diameter of each testis was measured using a pair of dial calipers between two lateral surfaces of the testis plus scrotal skin**.** Testis length was measured by the metal calipers from top of the tail to the head of the epididymis for each testis.

Sexual behaviour evaluation

Rams were kept isolated away from ewes except during the sexual behaviour test, which conducted by exposing each ram to a restrained ewe for 10 minutes. Reaction time (time from entry into the pen to the first mount (when the front legs left the ground and the brisket of the ram made contact with the rump of the restrained ewe) and/or ejaculation was recorded (Tulley and Burfening, 1983). Frequency of mounts without ejaculation before sexual exhaustion (loss of libido), pre-copulation behaviour (bouts of leg kicking, when the foreleg of the ram is repeatedly directed toward the ewe in rapid succession), bouts of ano-genital sniffing and flehmens (Price *et al.,* 1994a,b) were recorded as sexual behaviour, which taken place between 08:00 and 12:00 hr.

Semen collection and semen physical characteristics

Rams were allowed to run with ewes to observe their sexual behaviour. Rams achieved erection and extrusions of the penis out of the sheath were trained for semen collection. Semen was collected during the second mount using artificial vagina once weekly.

After collection, semen volume (ml), pH, mass motility, sperm concentration / ml, total sperm per ejaculate, live and dead sperm and abnormal spermatozoa of each ejaculate were recorded and assessed as described by Greyling and Grobbelaar (1983) and Mahmoud (2002). Semen volume was measured using a graduated collection tube to the nearest 0.1 ml. Initial pH of semen samples was measured using comparative nitrating pH paper. Mass movement of sperm was assessed on a scale from 0 (no motility) to five (excellent motility) (Martínez-Rodríguez *et al.,* 2012). The percentages of live and dead sperms were determined from unfixed-smear stained with eosin. Two hundred sperms were calculated from different fields in the stained smear to determine live sperm percentage. Sperm concentration (X 10^9) was determined using Haemocytometer (Martínez-Rodríguez *et al.,* 2012). Total number of spermatozoa per ejaculate was calculated by multiplying the ejaculate volume by sperm concentration per ml. Sperms abnormalities included abnormalities in the sperm head; mid-piece and tail of sperm were also recorded (Hulet *et al.,* 1965).

Collection of blood samples

Blood samples were collected from the jugular vein between 08:00 and 12:00 hr before feeding. Two blood samples were collected from each animal; the first was collected pre, while the second was collected after copulation behaviour and ejaculation. Blood samples were allowed to clot at 4 °C for 10 hours before centrifugation at 4000 rpm for 20 minutes for serum harvesting. Serum was decanted into dry eppendorf tubes and stored at -20 °C until assessing testosterone concentration using ELISA kits (Bio Check, Foster City, CA 94404, USA). The standard curve was performed before samples running at 450 nm optical density with different concentration of the standard curve, which ranged from 0 to 18.0 ng/ml. As manufacturers' description, the kit had a sensitivity value of 0.05 ng/ml with inter- and intra-assay run precision coefficient of variations of 3.7 and 5.0%, respectively.

Statistical analysis

Data were statistically analyzed using General Linear Model (GLM) and correlation coefficients procedures (SAS, 1996). Differences among means were detected by Duncan's Multiple Range Test (Duncan, 1955). The following model was used for analysis of variance:

$Y_{ijk} = \mu + A_i + B_j + (A*B)_{ij} + e_{ijk.}$ Where:

μ = overall mean,

Ai = Effect of age,

Bj = Effect of scrotum circumference

A*Bij= Interaction between age and scrotum circumference, and e ijk = the experimental errors.

Effect of interaction between age and scrotum circumference was not significant, so the interaction was excluded from the results. Percentage values (e.g. live, dead and abnormal spermatozoa) were transferred to arc-sign and measured as means.

RESULTS AND DISCUSSION

Effect of age:

Sexual behaviour, testicular measurements and testosterone concentration

By the end of the experiment, rams of group B showed higher (P< 0.001) body weight relative to group A. Differences in body weight between A and B groups were almost the same in the beginning and the end of the experiment (~19 kg). Number of mounting without ejaculate, testicular measurements and testosterone concentration after ejaculation were affected by age except reaction time and testosterone concentration pre-copulation behaviour (Table 1).

These results are in agreement with those of Price *et al.* (1991) and Kridli *et al.* (2007) indicating that younger rams have lower mating efficiency than mature ones. Also, there was a relationship between scrotal circumference and number of mating and between age and testosterone concentration. These results are explained in the light of Snowder *et al.* (2002) and Simitzis *et al.* (2005) findings; they reported that sexual behaviour of the rams is influenced by age.

Table 1. Effect of age (LSM ± SE) of Ossimi rams on sexual behaviour, testicular measurements and testosterone concentration of group A (1-1.5 years old) and group B (2-2.5 years old)

Items	Group A (n= 18)	Group B (n= 22)	% of change relative group A	P value
Final body weight (kg)	38. 8 ± 1.6	57.6 ± 1.7	48	0.001
Reaction time (s)	27.2 ± 11.0	23.5 ± 7.1	(- 13)	0.77
Mounting with no ejaculation*	12.7 ± 1.9	19.9 ± 2.4	57	0.02
Scrotal circumference (cm)	24.0 ± 0.7	27.4 ± 0.7	14	0.01
Testicular diameter (cm))	6.2 ± 0.3	7.0 ± 0.2	13	0.02
Testicular length (cm)	9.2 ± 0.3	10.5 ± 0.3	14	0.01
Testosterone concentration (ng/ml)**	0.6 ± 0.1	1.4 ± 0.1	133	0.001
Testosterone concentration (ng/ml)***	0.4 ± 0.1	0.7 ± 0.1	75	0.12

* Frequencies of mounts without ejaculation before sexual exhaustion (loss of libido)
** Testosterone concentration after pre-copulation behaviour and ejaculation
*** Testosterone concentration pre-copulation behaviour and ejaculation

Table 2. Effect of age (LSM ± SE) of Ossimi rams on semen characteristics of group A (1-1.5 years old) and group B (2-2.5 years old)

Items	Group A (n= 18)	Group B (n= 22)	% of change relative group A	P Value
Ejaculate volume (ml)	0.8 ± 0.1	1.1 ± 0.1	37	0.01
Semen pH	7.0 ± 0.1	6.8 ± 0.0	(- 3)	0.01
Mass motility	2.7 ± 0.4	4.2 ± 0.3	55	0.01
Sperm concentration/ml ($X10^9$)	2.0 ± 0.5	2.9 ± 0.3	45	0.12
Total sperm / ejaculate ($X10^9$)	1.9 ± 0.5	3.5 ± 0.5	84	0.04
Live (%)	66.3 ± 4.5	73.9 ± 3.1	11	0.16
Dead (%)	33.7 ± 4.5	26.1 ± 3.2	(- 22)	0.17
Total abnormal (%)	30.1 ± 2.4	19.7 ± 2.6	(- 34)	0.01

Semen characteristics

Age of rams showed a significant effect on ejaculate volume, semen pH, mass motility, total sperm per ejaculate (x 10^9) and total abnormal (%), spermatozoa concentration / ml (x 10^9) and live (%). However non-significant effect was found in the rest of semen traits (Table 2). These results may be due to rams in group B (that of higher body weight) had higher testes measurements and higher testosterone concentration (Table 1), which may reflects better function of testes in semen production. This suggestion is supported by the findings of Toe *et al.* (2000) and Mahmoud (2002).

The authors reported a positive correlation between testes measurements and semen quality. Moreover, increasing testosterone concentration in rams of group B (Table 1) may stimulate testicular function (Dufour *et al.*, 1984, Kilgour *et al.*, 1985 and Matos and Thomas, 1991) and sexual activity (Bearden and Fuquay, 1997) and semen quality (Kishk, 2008). Effect of testosterone on semen quality attributed to its effect on Sertoli cells and the process of spermatogenesis (Hafez, 1993).

Effect of scrotal circumference

Sexual behavior, testicular measurements and testosterone concentration

Scrotal circumference of rams showed significant effect on number of mounting without ejaculation (libido), testicular measurements and testosterone concentration after ejaculation. This effect was not significant on reaction time and testosterone concentration pre-copulation behavior (Table 3). Higher testosterone concentration in the group having scrotal circumference > 25 cm may be the main factor affecting testes measurement, and better libido compared to rams having scrotal circumference ≤ 25 cm.

These results are in agreement with those of Charles *et al.* (2002) and Stellflug *et al.* (2004) indicating the important role of testosterone hormone for regulating sexual behaviour (libido). Increase LH and testosterone levels in peripheral blood leads to string sexual behavior (Sanford *et al.*, 1982 and 1984). Concentrations of these hormones were found to be greater in sexually active than in sexually inactive rams when exposed to estrus ewes (Perkins *et al.*, 1992, Alexander *et al.*, 1999 and Stellflug *et al.*, 2004).

Semen characteristics

Scrotum circumference of rams showed significant effect on all studied traits of semen (Table 4**).** These results most probably attributed to the rams had scrotal circumference > 25 cm showed higher testicular measurements and higher testosterone concentration (Table 3) which means better function for testes in semen production.

These results agree with those of Bearden and Fuquay (1997) and Kishk (2008) indicating high positive correlation between testosterone level and semen quality. Consequently scrotum circumference is a good index of sperm production in the ram (Toe *et al.*, 2000 and Oberst *et al.*, 2011).

Table 3. Effect of scrotal circumference, SC (LSM ± SE) of Ossimi rams on sexual behaviour, testicular measurements and testosterone concentration

Items	S.C.≤ 25 cm (n= 16)	S.C.>25 cm (n= 24)	% of change relative group S.C.≤ 25 cm	P Value
Body weight (kg)	44.1±3.1	52.5±2.2	19	0.02
Reaction time (s)	36.7±12.3	17.4±6.1	(- 52)	0.12
Mounting with no ejaculation*	10.2±2.1	21.0±1.9	105	0.000
Scrotal circumference (cm)	22.7±0.6	27.9±0.5	23	0.000
Testicular diameter (cm)	5.9±0.3	7.1±0.2	20	0.000
Testicular length (cm)	8.5±0.3	10.8±0.2	27	0.000
Testosterone concentration(ng/ml)**	0.9±0.2	1.6±0.1	78	0.001
Testosterone concentration(ng/ml)**	0.6±0.2	0.9±0.1	50	0.09

* Frequencies of mounts without ejaculation before sexual exhaustion (loss of libido)
** Testosterone concentration after pre-copulation behaviour and ejaculation
** Testosterone concentration pre-copulation behaviour and ejaculation

Table 4. Effect of scrotum circumference (LSM ± SE) of Ossimi rams on semen characteristics

Items	S.C.≤ 25 cm (n= 16)	S.C. > 25 cm (n= 24)	% of change relative group S.C.≤ 25 cm	P Value
Ejaculate Volume (ml)	0.7±0.1	1.2±0.1	71	0.000
Semen pH	7.1±0.4	6.8±0.0	(- 4)	0.000
Mass motility	2.7±0.4	4.1±0.2	52	0.00
Sperm concentration/ml ($X10^9$)	1.1±0.1	3.6±0.3	277	0.000
Total sperm / ejaculate ($X\ 10^9$)	0.8±0.1	4.4±0.41	450	0.000
Live (%)	57.5±3.1	79.7±2.1	38	0.000
Dead (%)	42.5±3.1	20.3±2.5	(- 52)	0.000
Total abnormal (%)	34.4±2.2	16.8±1.2	(- 51)	0.000

Correlation coefficient

There are positive correlations between number of mounting, scrotal circumference and testosterone concentration. On the other hand, there are negative correlation was found between reaction time and each of number of mounting, scrotal circumference and testosterone concentration (Table 5). These results are in agreement with those of Kishk (2008) who found that there was negative correlation ($P<0.001$) between reaction time and testosterone concentration in rams and Matos and Thomas (1991) who reported that testicular size was

correlated with plasma testosterone concentration. Price *et al.* (1991) and Kridli *et al.* (2007) indicated that there were found positive correlation between scrotal circumference and number of mating.

CONCLUSION

It could be concluded that the sexual activity and semen characteristics in the ram improved by age progress and increasing the testicular measurements. Selecting rams of mating could be applied based on the scrotal circumferences, which could be good indicator to testicular function and sexual activity.

Table 5. Correlation coefficients among sexual behaviour, scrotal circumference (cm) and testosterone concentration (ngLml) of Ossimi rams

	Mounting with no ejaculation***	Scrotal circumference	Testosterone concentration
Reaction time	- 0.34*	- 0.34*	- 0.22
Mounting with no ejaculation* **		0.72**	0.56**
Scrotal circumference			0.77**

***** P< 0.05 ** P<0.001

*** Frequencies of mounts without ejaculation before sexual exhaustion (loss of libido)

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تأثير العمر ومحيط الصفن علي السلوك الجنسي و تركيز التستستيرون وصفات السائل المنوي ومقاييس الخصية في كباش الأغنام الأوسيمى

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أجريت هذه الدراسة على أربعون من ذكور الأغنام الأوسيمى عالية الرغبة الجنسية بغرض دراسة تأثير كا من مقاييس الخصية، السلوك الجنسي، تركيز هرمون التستستيرون وصفات السائل المنوى تحت تأثير محيط كيس الصفن والعمر. وقسمت ذكور الأغنام الى مجموعتين كل مجموعة لها نفس العمر ووزن الجسم. المجموعة الأولى (مجموعة أ) من 1 – 1.5سنة والمجموعة الثانية (مجموعة ب) من 2 – 2.5 سنة. وقسمت كل مجموعة الى مجموعتين على حسب محيط كيس الصفن ≥ 25 سم و > 25 سم وقبل إجراء التجربة تم تسجيل وزن جسم الحيوان، السلوك الجنسى ومقاييس الخصية. وأثناء التجربة تم تجميع السائل المنوى بالمهبل الصناعي لمعرفة صفاته. وتم أخذ عينتين دم من كل ذكر قبل إختلاط الذكر بالأنثى وبعد جمع السائل المنوى لتقدير هرمون التستستيرون.

أظهرت النتائج المتحصل عليها أن العمر لة تأثير معنوى على كل من وزن الجسم، مقاييس الخصية، صفات السائل المنوى، تركيز هرمون التستستيرون وعدد مرات الوثب على الأنثي بدون جمع للسائل المنوى حتى الإجهاد. ولم يظهر تأثير للعمر على الوقت الذي يستغرقه الحيوان للوثب على الأنثى عند الإختلاط بها والسلوك ما قبل الجمع.

ووجد أن الحيوانات التى يكون فيها محيط كيس الصفن > 25 سم أظهرت تقدم ملحوظومعنوى فى السلوك الجنسى (الرغبة الجنسية)، صفات السائل المنوى وتركيز هرمون التستستيرون مقارنة بالذكور التى لها محيط كيس الصفن ≥ 25 سم.

ووجد علاقة موجبة بين تركيز هرمون التستستيرون، عدد مرات الوثب على الأنثي ومحيط كيس الصفن، كما وجد أيضآ علاقة سالبة بين الوقت الذي يستغرقه الحيوان للوثب على الأنثى عند الإختلاط بها وبين كل من عدد مرات الوثب على الأنثي، محيط كيس الصفن وتركيز هرمون التستستيرون.

ويصفة عامة عند إختيار الذكور للتربية لابد أن نأخذ فى الإعتبار السلوك الجنسى للذكر مع كبر محيط كيس الصفن حيث يكون > 25 سم. ووجد أن التغير فى السلوك الجنسى وصفات السائل المنوى مرتبط بالعمر وبالتغير فى محيط كيس الصفن الذى يتبعه تغير فى تركيز هرمون التستستيرون.