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## **GONADAL AND EPIDIDYMAL SPERM COUNTS OF GROWING OSSIMI RAMS IN UPPER EGYPT**

(With 3 Tables)

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**مخزون الحيامن في خصية وبربخ كباش الأوسيمي النامية**

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تم في هذا البحث دراسة مخزون الحيامن بالخصية والبربخ لعدد ٤٨ ذكر أوسيمي تراوحت أعمارهم من ٦ الى أكثر من ٢٤ شهرا ومذبوحة بمزرعة مجاورة ومجزر أسيوط. وبينت الدراسة وجود حيامن لأول مرة في خصية ذكربن من إجمالي ٥ ذكور عند عمر ٧- ٨ شهور وخلال الفترة من ٨-١٢ شهرا كان هناك زيادة واضحة في عدد الحيامن لكل جرام من نسيج الخصية ثم تضاعف معدل الزيادة حتى عمر ١٥ شهرا. تبين أن الزيادة التي لوحظت بعد هذا العمر كان لزيادة وزن الخصية عنه. في انتاجية نسيجها لوحدة الجرام الواحد. كانت هناك علاقة وطيدة في زيادة مخزون الخصية وأجزاء البربخ الثلاثة مع وزن هذه الاعضاء أثناء فترة النمو من الشهر التاسع حتى أكثر من ٢٤ شهرا. احتوى ذيل البربخ نسبة ٦٤,١ % ، ٨٠,٧ % من المخزون الكلي للحيامن في البربخ عند عمرى ٩ ، ١٨ شهرا على التوالي. بينت احصائيات البحث وجود علاقة وطيدة معنوية بين وزن الخصية والبربخ بتقدم العمر وكذلك بين أوزان الخصيه والبربخ ومحتوياتهما الاجمالية من الحيامن في الحيوانات المتقدمة في العمر خاصة بعد ١٨ شهرا من العمر.

### **SUMMARY**

Ossimi is the most abundant breed of sheep in Egypt. As compared with other sheep, the age of puberty and reproductive capacity of this local breed received little attention. For both purposes, gonadal and epididymal sperm counts were studied in 48 healthy slaughtered growing Ossimi ram lambs. Animals were classified into 8 equal age groups (6, 7, 8, 12, 15, 18, 24 and >24 month). Testis and epididymis (caput, corpus and cauda) of one side were dissected and weighed separately. The whole testis and each region of epididymis were cut seperately into small pieces then homogenized with known volumes of saline using an electro mixer. To 5 ml of each homogenate, 3 drops

sodium hydroxide (2%) and 2 drops eosin solution (3%) were added and gently mixed. Stained sperm heads, without elongated spermatids, were counted twice with a cytometer. At 6 months, no sperm could be detected from any organ while at 7 months 2 cases had sperm in their testes only. In both cases testicular weight, sperm per g tissue and total sperm per gonad averaged 48.5 g,  $23.6 \times 10^6$  and  $1.14 \times 10^9$  sperm respectively. At 8 months, sperm were detected from all testes and epididymes. At this age, testis weighed  $51.3 \pm 1.8$  g, while sperm count per g tissue and per organ were  $26.1 \pm 4.2 \times 10^6$  and  $1.34 \pm 0.23 \times 10^9$  respectively. Moreover, total sperm count in epididymis was  $1.64 \pm 0.19 \times 10^9$ . After this age, testes and their total sperm counts showed significant increases ( $P < 0.01$ ) until 24 months. However, number of sperm per g tissue was maximum at earlier age (15 months) and reached  $90.2 \pm 7.9 \times 10^9$ . Epididymis (weight and total sperm) developed slowly from 7 to 12 months then rapidly until 24 months. Each region of epididymis grew independently. Ratios of caput and corpus weights to whole epididymis were 41.3% and 20% respectively at 8 months. These ratios decreased to 39.7% and 13.6% at 18 months, respectively. Ratios recorded for cauda increased from 38.7% to 46.7% at the same ages, respectively. There were similar trends for sperm counts. At 24 months, cauda contained  $45.15 \pm 7.9 \times 10^9$  sperm representing 80.7% of total epididymal sperm. Significant correlations ( $P < 0.01$ ) were present between testis-epididymis weights and their sperm counts. It is evident that Ossimi ram lambs reached puberty at 7-8 months of age while maximum reproductive capacity attained at 2 years. Data obtained might be of value in rams selected for breeding especially with regard to evaluate the size of testes and cauda epididymides.

**Key words:** *Ossimi rams, testis, epididymis, sperm*

## INTRODUCTION

The number of sheep in Egypt reached 5.35 million (FAO, 2006). Moreover, Ossimi breed which originated in Ossim vellage, Giza Governorate, is the most popular among other breeds all over the country. They are subtropical fat tailed sheep, medium size and raised mainly under intensive cropping conditions to produce edible meat and coarse-carpet wool with colored fibers (Mason, 1996). Breeding seasons are not present and usually 3 mating seasons per 2 years were managed (Almahdy *et al.*, 2000).

Puberty in ram lambs ranged from 84-456 days depending on breed and body weight as cited by Haynes and Shanbacher (1983). The age of puberty in Ossimi and its crossbreeding with Chios french sheep was reported by Hassan *et al.* (1992) to be  $296.8 \pm 35.4$  and  $318.5 \pm 39.7$  days respectively depending upon the first appearance of sperm in the ejaculate. In Saidi ram lambs in Upper Egypt, Salem *et al.* (2005) determined the age of puberty, from the first detection of sperm in smear taken from the penile sheath, to be  $363.8 \pm 13.3$  days at body weight of  $50.3 \pm 3.0$  kg.

Knowledge about gonadal and extragonadal sperm reserves is of vital importance to assess the reproductive capacity of the male and as well the age of puberty. The available literature showed that Almquist and Amann (1961) were the first authors who determined the gonadal and extragonadal sperm reserves in cattle by direct counts. Osman (1972) could modified the technique in buffalo bulls by adding 1% sodium hydroxide to the homogenate for clarification before counting and reported that gonadal sperm reserves in this species are lower than those in cattle. The sperm production rates were investigated in different breeds of sheep with variable results even within the same breed due to the variable applied techniques (Bielanski and Wierzbowski, 1961, Voglmayr *et al.*, 1966, Abdo *et al.*, 1978 and Queiroz and Cardoso, 1987).

Van Rooyen (2001) recommended that testes size is generally considered as an indirect measure of reproduction in ram. Moreover, Schoenian (2005) reported that most ram lambs reach puberty between 5 and 7 months of age at 50-60 % body weight. In Tanyang ram lambs, Cui *et al.* (2003) suggested that 4-5 months of age is a critical period for pubertal changes. Manson (2004) cited that sperm are stored in the epididymes particularly in its lower region, the tail, which can be felt by palpation as a large lump under each testis and a large firm tail indicates that the ram has good sperm reserves. In two adult Ossimi rams, Osman (1998) found that the total number of sperm in two exhaustion trials (11 and 13 ejaculates per trial respectively) represented 33.8 % and 36.7 % of the total sperm reserved in the cauda epididymides of both rams respectively after sexual rest and slaughter.

The aim of the present work is to study the quantification of gonadal and epididymal sperm reserves during the new pubertal period with regard to age and body weight in Ossimi rams to give an idea about their age of puberty and reproductive potential of their genitalia with the advance of age.

## MATERIALS and METHODS

From a local farm and a slaughterhouse, the testes and epididymes of 48 growing Ossimi rams, with determined age and live body weight, were taken to the laboratory. All animals were healthy and their carcasses were fit for consumption. The testes and epididymes of each animal were taken to the laboratory. Each gonad was free from surrounding structures and dissected carefully from the connected epididymis, vas deferens and spermatic cord so as to leave the tunica albuginea intact. The corresponding epididymis was separated from the surrounding tissue and cut into 3 regions (head, body and tail). The testis and each region of the epididymis were weighed separately (g).

The whole testis was cut into small pieces then homogenized with 500 ml saline using an electromixer for 3-4 minutes according to Osman (1972). To 5 ml of each homogenate 3 drops of sodium hydroxide (2%) and 2 drops of eosin (2%) were added and gently mixed. The addition of sodium hydroxide could clarify greatly the homogenate leaving the ovoid sperm heads and some remnant testicular tissues and faint elongated spermatids. The eosin-stained sperm heads could be easily differentiated and counted twice with a Thoma-rulling cytometer. The dilution rate was calculated for each trial from the ratio between the weight of organ and volume of saline.

Each region of the epididymis was similarly treated but 200 ml saline was used for tissue homogenization (Osman and El Azab, 1969). The dilution rates averaged about 1:10 for the testis and 1: 100 for each of the epididymal regions.

The results were expressed in Mean  $\pm$  SEM and correlations between two factors were statistically analysed according to Snedecor and Cochran (1980) and Pc-Stat (2003).

## RESULTS

The obtained results are presented in Tables 1-3. As there were no marked differences between the characters of right and left testes-epididymes, the results of only one side were taken into consideration. The live body weight showed a progressive increase with the advance of age till the end of study with significant correlation ( $r= 0.9634$ ) between them ( $P < 0.01$ ). However, maximum daily gain in body weight observed up to one year (143.3, 230 and 270 g between 6-7, 7-8 and 8-12 months respectively). After that age, the daily gain ranged between 28-51 g.

On the other hand, the testes increased dramatically after 8 month of age till 24 month only. The ratio of testes weight (both sides) to live body weight increased sharply from 0.27 % at 6 month of age to 0.69 % at 2 years of age. Among different age groups ( 6, 7, 8, 12, 15, 18, 24 and >24 months of age) the recorded ratios were 0.28, 0.28, 0.26, 0.30, 0.42, 0.54, 0.70 and 0.58 % respectively. Significant correlation between testes weight and body weight was present ( $r=0.9267$ ) among age groups ( $P<0.01$ ).

It is noteworthy to mention that at 6 month of age no sperm could be detected from either the testes or epididymes of all individuals. At 7 month of age 2 out of 6 animals have sperm in their testes only. At 8 month of age, however, sperm could be detected from all testes and epididymes (Table 1) with some increase in live body weight and testes weight. Between 8 and 12 month of age, marked increase in the number of sperm per gram testicular tissue followed by double folds increase upto 15 month of age when no further increase could be observed. The steady increase in the total sperm count per gonad, after 12 month of age, coincides mainly with the pondreal increase observed in the testis weight which reached maximum value at two years.

The increase in weight and sperm count of the 3 epididymal regions was variable with the advance of age (Table 2). From 8 to 24 month of age the rates of increase in caput, corpus and cauda epididymides weights were 3.3, 2.4 and 4.16 folds respectively. The corresponding values with regard to sperm per gram tissue and total sperm count per caput, corpus and cauda epididymides were 5.3, 8.5 and 10.3 (Sperm per gram tissue) and 17, 20.75 and 43 (total sperm count per region) folds respectively. It is evident from these results that each region of the epididymes grew independently from each other. In addition, the absolutely vast rates of increase were noticed with the cauda epididymides. The accumulation of sperm and epididymal secretion in this region, appeared associated with such high rate of development which observed for the cauda epididymides in aged rams. It is of interest to mention that most of the incised large cauda epididymides in older Ossimi rams showed milky secretion which contained high concentration of motile sperm under the microscope after dilution with warm saline.

Table 3, showed the proportions in weights and total sperm reserves between the caput, corpus and cauda epididymides with the advance of age. The ratios of caput weight did not show any valuable changes with age but the ratios of corpus weight decreased from 20.4 %

to 13.9 %. On the other hand, the ratios of cauda epididymides weight increased from 40.7 % (at 6 month of age) to 46.5 % (at 24 month of age). With regard to the total sperm counts, the ratios decreased markedly in the caput (from 26.2 % to 13.4 %) and corpus epididymides (from 9.7 % to 6.0 %). The reverse was true with the cauda epididymides where marked increases were noticed in the ratios of sperm counts with the advance of age (from 64.1 % at 8 month to 80.7 % at 24 month of age) as related to the total sperm counts in the whole epididymes.

The obtained data revealed the presence of significant correlations ( $P < 0.01$ ) between live body weight and epididymal ( $r=0.9088$ ) weights among age groups. Moreover, significant correlations ( $P < 0.01$ ) were present between weights of testes and epididymes ( $r=0.9942$ ) as well as between each of them and its total sperm count ( $r=0.9939$  and  $r=0.9807$  respectively). The whole epididymes (weight) represents from 14 % to 16 % as related to the testes in all groups without significant difference with the advance of age.

**Table 1:** Testicular weight and sperm reserves as related to age and live body weight in slaughtered growing Ossimi ram.

Age Groups (Month)	Body Weight (Kg)	Testis Weight (g)	Sperm /g ( $\times 10^6$ )	Sperm/Organ ( $\times 10^9$ )
6	28.2 $\pm$ 4.3	38.5 $\pm$ 2.3	-	-
7	32.5 $\pm$ 4.6	46.3 $\pm$ 2.8	23.6 (n=2)	1.14
8	39.4 $\pm$ 5.2	51.3 $\pm$ 1.8	26.1 $\pm$ 4.2	1.34 $\pm$ 0.23
12	47.5 $\pm$ 4.8	72.4 $\pm$ 2.1	40.8 $\pm$ 2.8	2.56 $\pm$ 0.53
15	50.1 $\pm$ 7.1	106.0 $\pm$ 2.2	90.2 $\pm$ 7.9	9.56 $\pm$ 0.53
18	54.7 $\pm$ 6.9	150.3 $\pm$ 4.3	90.0 $\pm$ 7.9	13.53 $\pm$ 1.46
24	58.1 $\pm$ 7.6	201.2 $\pm$ 7.8	93.3 $\pm$ 6.5	18.77 $\pm$ 2.01
> 24	65.5 $\pm$ 6.6	190.2 $\pm$ 6.6	92.4 $\pm$ 5.9	17.57 $\pm$ 2.11

N = 6 In each group

Mean  $\pm$  SEM

**Table 2:** Epididymal regions weight and sperm reserves as related to age in slaughtered growing Ossimi rams

Age Groups (Month)	Caput Epididymidis			Corpus Epididymidis			Cauda Epididymidis			Total Sperm per ** Epididymis
	Weight (g)	Sperm * per g	Sperm ** per region	Weight (g)	Sperm * per g	Sperm ** Per region	Weight (g)	Sperm * per g	Sperm ** per region	
6	2.1 ± 0.09	-	-	1.1 ± 0.08	-	-	2.2 ± 0.08	-	-	-
7	2.7 ± 0.07	-	-	1.3 ± 0.07	-	-	2.6 ± 0.09	-	-	-
8	3.3 ± 0.08	0.13 ± 0.02	0.43 ± 0.08	1.6 ± 0.06	0.10 ± 0.1	0.16 ± 0.02	3.1 ± 0.32	0.34 ± 0.05	1.05 ± 0.36	1.64 ± 0.9
12	4.2 ± 0.3	0.18 ± 0.02	0.76 ± 0.12	1.7 ± 0.17	0.13 ± 0.03	0.22 ± 0.05	4.3 ± 0.19	0.48 ± 0.11	2.06 ± 0.3	2.94 ± 0.33
15	7.0 ± 0.19	0.5 ± 0.03	3.5 ± 0.32	2.2 ± 0.14	0.45 ± 0.03	0.99 ± 0.05	7.9 ± 0.67	1.6 ± 0.23	12.6 ± 1.25	17.1 ± 6.21
18	9.1 ± 0.57	0.58 ± 0.03	5.28 ± 0.6	3.1 ± 0.03	0.62 ± 0.11	1.92 ± 0.51	10.7 ± 1.03	2.61 ± 0.32	27.82 ± 4.45	34.47 ± 8.6
24	10.9 ± 0.8	0.69 ± 0.13	7.52 ± 1.2	3.9 ± 0.24	0.85 ± 0.14	3.32 ± 0.51	12.9 ± 0.4	3.5 ± 0.38	45.15 ± 7.9	55.9 ± 11.04
> 24	10.5 ± 0.68	0.64 ± 0.11	6.72 ± 0.82	3.7 ± 0.31	0.81 ± 0.12	3.0 ± 0.38	12.3 ± 0.52	3.28 ± 0.29	40.34 ± 6.81	50.06 ± 9.56

Mean ± SEM

N = 6 In each group

\* = x10 6

\*\* = x10 9

**Table 3:** Proportions of weight and sperm reserves in the epididymal regions of slaughtered growing Ossimi rams

Age Groups (Months)	Caput Epididymidis		Corpus Epididymidis		Cauda pididymidis	
	Weight %	Sperm %	Weight %	Sperm %	Weight %	Sperm %
6	38.9	-	20.4	-	40.7	-
7	40.9	-	19.7	-	39.4	-
8	41.3	26.2	20.0	9.7	38.7	64.1
12	41.2	25.0	16.7	7.2	42.1	67.8
15	40.9	20.5	12.9	5.8	46.2	73.7
18	39.7	13.7	13.6	5.6	46.7	80.7
24	39.4	13.5	14.1	5.8	46.5	80.7
> 24	39.4	13.4	13.9	6.0	46.5	80.6

## DISCUSSION

Reviewing the literature of Watson *et al.* (1956), Skinner (1971), Dyrmondsson and Lees (1972) and Osman (1980), it is evident that Ossimi breed is smaller in size than other breeds (Merino, Dopper and Clun Forest). El Alamy *et al.* (1982) reported that Ossimi ram weighed 73.5 kg at 2 years of age, an average which is heigher than 58.1 kg reported here. On the other hand, Youssef and Saleem (1962) reported an average value of 25.0 kg for the body weight of twin males at 6 month of age which is slightly less than that recorded in the present work ( $28.2 \pm 4.3$  kg). Individual variations and types of breeding may be responsible for such difference.

The average daily gain in body weight reported in the present work from 6-12 months of age (average 214.7 g) is larger than 129 g, at 8 months, as reported by Gueseous *et al.* (1998). The sharp decrease in daily gain after one year (43.3 g per day) denoted that Ossimi ram lambs achieved their mature size economically during their first year of life. It seems possible that the different levels of nutrition and managements of the studied ram lambs might be responsible for such differences in body



weight gain and ram development in comparison with the results of their authors.

The marked increase in the relative weight of testes to live body weight in Ossimi rams with age denoted higher rate of testicular development especially after 7-8 months (age of puberty). Moreover, the rams possess the highest ratio between testes and live body weight in relation to other animals, 0.63 % adult Ossimi rams versus 0.07% dairy bulls (Boyd and Van Demark, 1957) and 0.045 % buffalo (Osman and Zaki, 1971). Manson (2004) cited that rams are 25 times as large, relative total weight, as human testes.

The age of puberty in Ossimi rams seems to start at 7 months of age where testicular sperm could be first detected in the tissue homogenates of 2 out of 6 ram lambs. Such age appeared delayed than 4-6 month reported by different authors in other foreign breeds of sheep (Rossouw and Grabbelaar (1975), Yarney and Sanford (1989), Madani *et al.* (1989) and Cui *et al.* (2003). On the other hand, Hassan *et al.* (1992) determined the age of Ossimi ram lambs at first ejaculation to be 9.9 months which is delayed than that reported here (7-8 months). The absence of sperm in the epididymes of both Ossimi rams at 7 month of age denoted the presence of a transitional phase of about 4 weeks from the onset spermatogenesis till the appearance of sperm in the epididymes.

The number of sperm per gram testicular tissue in Ossimi rams did not show any increase after 15 month of age where the increase in total sperm counts were due to increase in testicular weight. Mcmillan and Hafs (1968) cited that Holstein bulls attain their mature rate of spermatogenesis per gram testicular parenchyma by 1 year of age. Moreover, buffalo bulls attain such maximum rate by 2 ½ years of age (Osman 1972). Variations between species are expected with such criteria.

In comparison to other animals the rate of sperm production per gram of testicular homogenates is much higher in adult rams, 93.3 million in Ossimi rams versus 54.8 million dairy bulls (Almquist and Amann, 1961), 57.0 million buffalo bulls (Osman, 1972) and 47.2 million Egyptian camel (Osman and El Azab, 1974). However, Queiroz and Cardoso (1987) reported 111.4 million sperm per gram testis in Brazilian Hary rams which is higher than that found in Egyptian Ossimi rams.

The marked increase in total sperm counts in the testes and epididymes of Ossimi rams from 12 to 24 month of age, coincides

greatly with the findings reported by Abdou *et al.* (1978) in Awasi rams. Osman (1998) reported triple fold increase in the size of Ossimi ram lambs testes-epididymes between 2 and 18 month of age and individuals which possessed larger testes- epididymes at early ages attained maximum gain in size during their growth. In the present study maximum reproductive capacity of Ossimi rams with regard to gonadal and epididymal size and their sperm counts attained at 24 months of age. In Magyar Tarka Bulls Osman (1970) reported that maximum development in the size of gonads reached at 8 years of age in animals more than 2 years old.

The storage capacity of the epididymes appeared to developed at a higher rapid rate than the capacity of the testes to produce or store sperm. From 12 to 24 month of age, the total sperm count increased from 2.56 to 18.77 and from 2.94 to 55.9 billions in the testis and epididymis respectively. Moreover, the cauda is the distinguished region of the epididymides to store sperm with the advance of age. Such heigher growth rate of the epididymes may occurred to accommodate the strong activity of the testes to produce sperm in rams. However, the presence of 80.7 % of the total epididymal sperm in the cauda might reflect its peculiar role in the storage capacity. Osman (1998) reported that the total sperm numbers in two trials for exhaustion in Ossimi rams (13 and 11 consecutive semen collections per each trial) represent 33.8 % and 36.7 % from the total sperm reserves in the couda epididemes respectively after rest and slaughter.

The data with regard to the presnce of significant correlations between live weight and testes weight as well as between the testes and epididymes and their sperm counts in Ossimi rams appeared in harmony with other investigators (Amann,1970, Osman, 1980, Madani *et al.*, 1989 and Plant (2004).

As a amajor conclusion from the present work, Ossimi ram reached puberty at 7-8 months of age while maximum reproductive capacity achieved at 2 years. Data obtained may be of value in rams selected for breeding especially with regard to the size of testes and cauda epididymedes.

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