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STUDYING THE DEVELOPMENT OF THE CHIOS RAM GENITALIA FROM 6 TO 24 MONTHS AGE USING ULTRASONOGRAPHY

(With 3 Tables and 8 Figures)

By

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**تطور نمو الجهاز التناسلي لكباش الكيوس من عمر ٦:٢٤ شهر باستخدام
الموجات فوق الصوتية**

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خططت هذه الدراسة لمتابعة التغيرات التي تحدث نتيجة لنمو الجهاز التناسلي لكباش الكيوس باستخدام جهاز الموجات فوق الصوتية وأجهزة قياس الخصية (الشريط والمساك). استعمل في هذه الدراسة عدد عشرون كبشا ثم تقسيمها إلى أربعة مجموعات : المجموعة أ (متوسط العمر ٦ أشهر ومتوسط الوزن ٣ و١٩±٢ و٨ كجم) ومجموعة ب (متوسط العمر ١٢ شهر والوزن ٢ و٢٨±٤ و٨ كجم) والمجموعة ج (متوسط العمر ١٨ شهر والوزن ٣٤±٣ و٨ كجم) ومجموعة د (متوسط العمر ٢٤ شهر والوزن ٤٢±٥ و٧ كجم) تم في كل مجموعة قياس متوسط محيط كيس الصفن (SC) وعرض الخصية (TB) وقطر الغدة البصلية المبالية (BUG) ارتفاع جزء البروستاتة حول المبالى الحوضي (PDP) وكذلك الغدة المنوية (SG) وتم حساب العلاقة بين نموهم . وقد وجد ازدياد ملحوظ في قياسات محيط الصفن في الفترة من ٦-٨ شهر وكان الازدياد بطيئا في الفترة من ٢٢ الى ٢٤ شهر وبالمثل كانت الزيادة في عرض الخصية. وكان قياس ارتفاع جزء البروستاتة ثابتا طوال ١٢ شهر من العمر ثم لوحظ ازدياده في الفترة من ١٢-٨ شهر. كما كانت الزيادة في حجم الغدة المنوية ملحوظة في الفترة من ١٢-٢٤ شهر. وقد وجدت علاقة ايجابية ملحوظة بين نمو محيط الصفن وعرض الخصية وكل من الغدة البصلية المبالية وارتفاع البروستاتة وكذلك الغدة المنوية ومن البحث نخلص إلى أن الأعضاء التناسلية لكباش الكيوس تنمو في أنماط مختلفة ما بين ٦-٢٤ شهر وقد ساعدت الدراسة بالموجات فوق الصوتية في دراسة نمو الأجزاء التناسلية الموجودة في الحوض وذلك في الحيوانات الحية دون الحاجة إلى ذبحها كما كان يحدث من قبل.

SUMMARY

Testes and accessory glands measurements are taken as a potential for male selection. This study was designed to follow up the developmental changes of Chios rams genitalia using ultrasonography, tape and caliper. Twenty rams were allocated into equal four age groups: A (6 months, BW: 19.3±2.8 kg), B (12 months, BW: 28.2±4.8 kg), C (18 months, BW: 34±2.8), and D (24 months, BW: 42.7±7.5 kg). In each group, the mean values of scrotal circumference (SC), testicular breadth (TB), and diameters of bulbourethral gland (BUG), pars disseminate of prostate (PDP), and seminal glands (SG) were measured and correlated. SC increased rapidly between 6 and 18 months and non-significantly between 18 and 24 months. Similarly, TB increased rashly between 6 and 18 months and slowly between 18 and 24 months. BUG increased slowly between 6 and 18 months and non-significantly between 18 and 24 months. PDP remained constant until 12 months, then increased significantly between 12 and 18 months, to remain constant again between 18 and 24 months. SG increased non-significantly between 6 and 12 months, then increased rapidly between 12 and 24 months. Positive correlations were found between SC, TB and each of BUG, PDP and SG. It could be concluded that male genitalia of Chios rams developed in different patterns between 6 and 24 months age. Ultrasonography assists studying the accessory glands directly in the living animals, which was unattainable before.

Key words: Chios ram, genitalia, development, ultrasonography

INTRODUCTION

Fertility evaluation of rams, which includes an examination of the genitalia and an assessment of libido, should be carried out in flocks before the beginning of the breeding season. It may also take place during selection of ram-lamb replacement, before the private purchase of a ram, or when investigating reproductive problems in a flock (Edgar 1963, Boundy 1992 and Bruere and West 1993, Gouletsou *et al.*, 2003). In evaluating rams for use in natural mating, three parameters have been shown to be the most reliable and repeatable. A complete breeding sound examination is normally conducted by veterinarian and includes: physical examination, measurements of scrotal size and semen evaluations.

Methods for assessment of the health of ram external genitalia were previously limited to palpation, measurements of the scrotal circumference and testicular dimensions as well as evaluation of semen. Other methods like thermography, tonometry and biopsy have also been employed as diagnostic aids but have not gained widespread acceptance in veterinary medicine (Pechman and Eilts 1987). At the same time, studying the development of the reproductive tract has been carried out previously by removing the tract after slaughter or indirectly by analyzing semen composition for presence and secretion of accessory glands. Recently, the same basic principles and technique of transrectal ultrasonography used for female can also be applied to the ram for examination of the pelvic genitalia of the ram. Ultrasound has an increased the number of diagnostic tools applied in veterinary practice, with both large and small animals. Being non invasive, it has considerable advantages over other methods. It allows the clinician to obtain instant information about a wide range of body systems and in some cases the dynamic function of organs can be assessed. In addition, ultrasonography has led to new insights into basic anatomy and physiology processes (Goddard, 1995).

The present study was designed to study the developmental changes of Chios rams genitalia from the age of 3 months to 2 years old using ultrasonography, tape and caliper.

MATERIALS and METHODS

Clinical Examination:

Twenty Chios rams maintained under general husbandry conditions were included in the present study. General health examination was formerly performed for these rams. Clinical examination of genitalia was carried out and followed as previously described by (Fthenakis *et al* 2001), with the ram in the standing position and restrained by two assistants, one of them lifted its tail. The scrotum was observed for the presence of lesions and asymmetry of the two halves. Exerting pressure on through squeezing it gently between fingers tested consistency of testicles and pain reaction. Free movement of the testes into the scrotum and lack of other masses therein were confirmed. The spermatic cord was palpated as far as possible and similarly the epididymal head and tail.

The rams were allocated in four age and weight groups (n=5 each): A (6 months, BW: 19.3±2.8 kg), B (12 months, BW: 28.2±4.8 kg), C (18 months, BW: 34±2.8), and D (24 months, BW: 42.7±7.5 kg).

The scrotal length (SL) as estimated from the origin at the inguinal region until free border of scrotum and scrotal circumference (SC: at the most largest point) were recorded using measuring tape. The three dimensions, length, width and depth of testes were measured using the caliper after deduction of scrotal layer thickness. Also the largest dimension of epididymal tail was recorded using the caliper.

Ultrasonographic Examination:

Ultrasonographic imaging of the testis and the epididymis was carried out using a 6/8 MHz linear array transducer connected to B-mode ultrasound scanner (Pie Medical, 100 LC, Holland). Before examination, the testes were pulled down into the scrotum and retained by grasping the spermatic cord at the neck of the scrotum with one hand of the examiner while the other hand was used to remove the transducer across the organ. This was done over the caudal surface while the animal was restrained in standing position. The probe was placed, after applying water-soluble jelly, on the caudal surface of the scrotum along its longitudinal axis and was moved from left to right to monitor the scrotal tunics, the testicular parenchyma and mediastinum. The probe was also applied transversely to show a transverse image of the testes. The tail of the epididymis was visualized from a diagonal plane near the distal end of testis. The thickness of tunics, testicular width (TW), thickness of mediastinum and the largest dimension of epididymal tail were estimated from frozen images by the scanner.

The pelvic genitalia were scanned after removing the feces from the rectum and applying ultrasound transmission jelly. The accessory glands and pelvic urethra were examined using 6 MHz linear probe. The maximum breadth of seminal gland (SG), the height of pars disseminata of prostate (PDP) and the diameter of the bulbourethral gland (BUG) were recorded from frozen images.

All data were entered into an excel spreadsheets. Means and standard deviation were presented for the recorded values. The correlations between age and the recorded results were calculated.

RESULTS

Table (1) presents the developmental changes in SC, SL and testicular dimensions for Chios rams from 6 to 24 months age. The SC

increased rapidly between 6 and 18 months and non-significantly between 18 and 24 months and length increased significantly from 6-12 month. Similarly, TB increased rashly between 6 and 18 months and slowly between 18 and 24 months

The ultrasonographic longitudinal images of ram lamb testis from group A appeared as a hypoechogenic structure with the absence of the characteristic hyper echogenic mediastinum from the middle of image (Fig 1). In mature ram the same image (Fig 2) showed a centrally located mediastinum testis represented by a hyperechogenic line surrounded by homogenous echogenic structure. The tunica albugenia and scrotum layers were evident as clear hyperechogenic lines at the border of testis image, which separated by a thin non-echogenic layer of fluid seemingly between two layers of tunica vaginalis. The mediastinum testis appeared as round spot in transverse ultrasonographic plane (Fig 3). The tail of the epididymis was clearly visible, where it appeared less echoic than testicular parenchyma and with a heterogenic structure (Fig 4). The pampiniform plexus could be seen as a dome shaped structure on the upper pole of the testis, which appeared as numerous convoluted sonlucent tubular structures (Fig 5). Developmental changes of tunics, breadth and mediastinum of testes were estimated from ultrasonographic images and reported in Table (2). The data showed that the testis breadth increased significantly until 12 months while the tunica albugenia decreased steadily with the advancement of age. The mediastinum showed non-significant slight increase in its growth pattern. The epididymal tail showed significant growth from age 6 to 12 month and from 18 to 24 month.

The circumscribed homogenous hyperechogenic appearance of BUG is demonstrated in Fig 6. Table (3) reported that, BUG increased non-significantly between 6 and 18 months and significantly from 18 to 24 months. PDP, which appear as homogenous hyperechogenic area and pelvic urethra demonstrated in fig 7. It was found, that PDP remained constant until 12 months, then increased significantly between 12 and 18 months, to keep constant once more between 18 and 24 months (Table 3). The ultrasonographic image of SG in fig 8 appear as moderately echogenic areas separated by hyperechogenic CT. The gland increased non-significantly between 6 and 12 months, then increased significantly between 12 and 24 months (Table 3). Positive correlations were found between SC and TB and each of BUG, PDP and SG.

Table 1: Developmental changes of scrotum and testes of Chios rams as measured by caliper and tape in relation to age and body weight (M±SD).

| Age (M) | Body Weight(Kg) | SC(cm) | SL(cm) | TL(cm) | TD(cm) | TB(cm) |
|---------|-------------------------|--------------------------|-------------------------|------------------------|------------------------|------------------------|
| 6 | 19.33±2.89 ^a | 12.33±2.31 ^a | 6.33±0.58 ^a | 2.75±1.06 ^a | 1.50±0.00 ^a | 1.45±0.49 ^a |
| 12 | 28.20±4.87 ^b | 20.40±1.14 ^b | 14.20±1.10 ^b | 5.00±0.93 ^b | 2.50±0.35 ^b | 2.90±0.35 ^b |
| 18 | 34.00±2.83 ^c | 24.00±3.39 ^{bc} | 15.00±1.73 ^b | 7.80±0.84 ^c | 4.20±1.15 ^c | 5.60±0.93 ^c |
| 24 | 42.67±7.50 ^d | 26.00±2.65 ^c | 15.67±2.89 ^b | 9.50±0.87 ^d | 4.27±0.25 ^c | 5.90±0.50 ^c |

Means in the same column with similar letter do not differ significantly at $p>0.05$
 SC: scrotal circumference, SL: scrotal length, TL: testis length, TD: testis depth,
 TB: testis breadth

Table 2: Developmental changes of testes and epididymal tail of Chios rams as measured from ultrasonographic images (M±SD).

| Age(M) | Tb(cm) | Ta.(cm) | Med. (cm) | Epi. tail(cm) |
|--------|------------------------|-------------------------|-----------|-------------------------|
| 6 | 1.47±0.20 ^a | 0.23±0.04 ^a | 0.20±0.02 | 1.10±0.14 ^a |
| 12 | 3.01±0.33 ^b | 0.21±0.08 ^a | 0.20±0.19 | 1.64±0.11 ^b |
| 18 | 3.68±0.41 ^b | 0.18±0.09 ^{ab} | 0.21±0.04 | 1.77±0.67 ^{bc} |
| 24 | 3.67±0.42 ^b | 0.15±0.04 ^b | 0.23±0.07 | 2.05±0.13 ^c |

Means in the same column with similar letter do not differ significantly at $p>0.05$
 Tb: testis breadth, Ta: Tunica albugenia, Med.: Mediastinum,
 Epi. Tail: Epididymal tail

Table 3: Developmental changes in pelvic genitalia of Chios rams as measured from ultrasonographic images (M±SD).

| Age (M) | BUG(cm) | PDP (cm) | SG(cm) |
|---------|------------------------|------------------------|------------------------|
| 6 | 0.91±0.56 ^a | 0.60±0.04 ^a | 0.75±0.50 ^a |
| 12 | 0.90±0.13 ^a | 0.55±0.05 ^a | 0.91±0.13 ^a |
| 18 | 1.08±0.27 ^a | 0.85±0.19 ^b | 1.20±0.23 ^b |
| 24 | 1.47±0.25 ^b | 0.79±0.03 ^b | 1.71±0.19 ^c |

Means in the same column with similar letter do not differ significantly at $p>0.05$
 BUG: Bulbourethral Gland, PDP: Pars Disseminata,
 SG: Seminal Gland

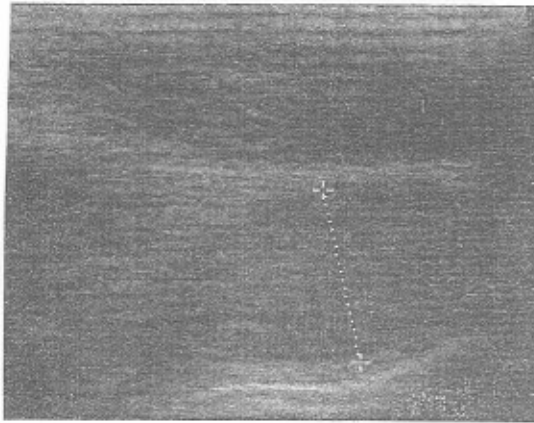


Fig. 1: longitudinal image of lamb ram (prepuberty)

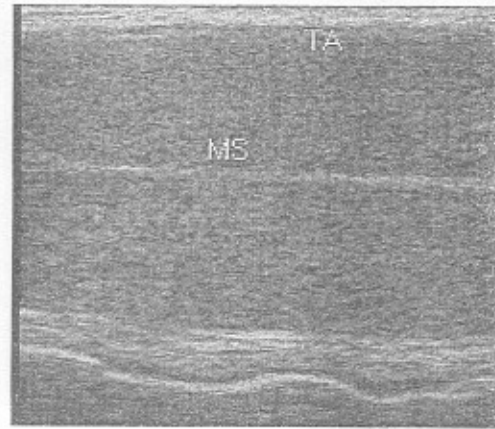


Fig. 2: Longitudinal image of testis (post pubertal) : Mediastinum (MED) Tunica Alb (TA)

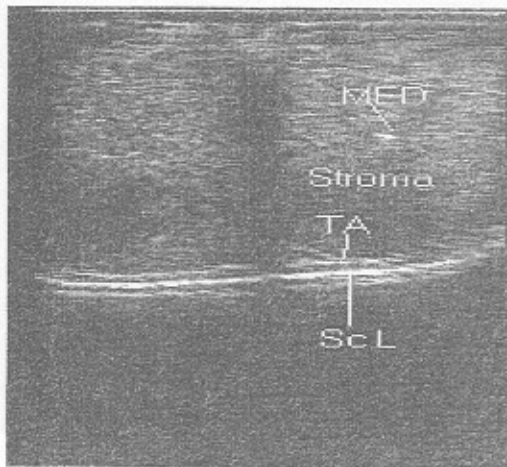


Fig. 3: Transverse image of testes (post pubertal): mediastinum (MED), tunica albugenia (TA), scrotal layer (Sc L)

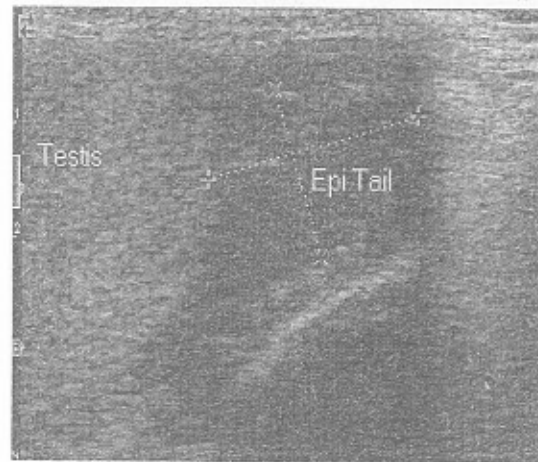


Fig. 4: Lower pole of Testis and Epidydmal Tail (post pubertal)

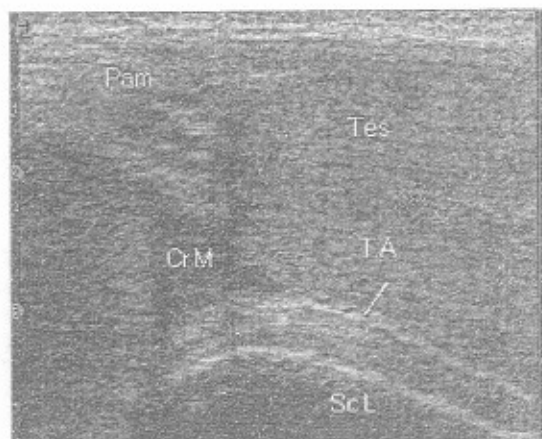


Fig. 5: Upper pole of testis(post pubertal): Pampiniform (Pam), Cremasteric: Cr M, Tunica Alb (TA), scrotal layer (ScL), testis (tes).



Fig. 6: Bulbourethral Gland (BUG) and caudal part of Pelvic Urethra (PUr) (post pubertal).

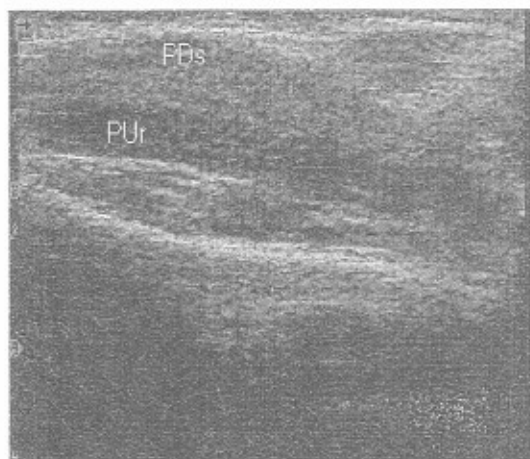


Fig. 7: Pelvic urethra (PUr) and Disseminate part of prostate (PDP) (post pubertal).

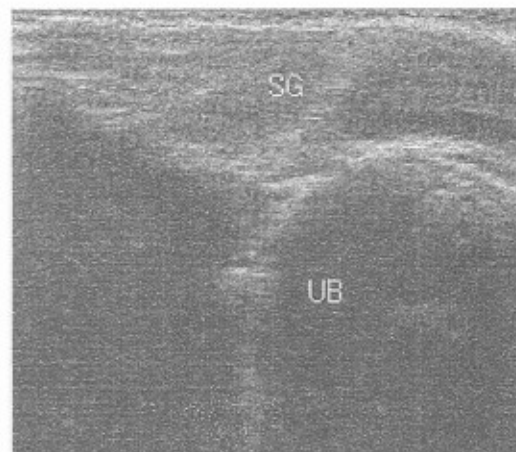


Fig. 8: Seminal Gland (SG) and Urinary Bladder (UB) (post pubertal).

DISCUSSION

The development of testes in Chios rams follows a uniform pattern among age groups and body weights at which the particular changes were observed along the study. SC measurements are taken in the bull and the ram due to their positive correlation with testicular weight and testicular volume (Ott, R.S. and Menon, 1980, Coulter and Keller 1982, Gipson *et al.* 1985, Makerchain *et al.* 1985 Ott 1987, Smith *et al.* 1989, Foster *et al.*, 1989 and Palasz *et al.*, 1994). The rate of SC and testicular growth are slow in the first 6 months, after that it grows rapidly between 6-18 months then subsequently slow. Similar results were cited by Lapwood (1980). This study revealed that the body weight has significant correlation with testicular growth as well as the age of animals. These results are in accordance with that of Salhab *et al.*, 2001 who recorded that, testicular measurements increased progressively and were correlated with body weights although in this aspect Lapwood (1980) mentioned that the role of weight is much stronger than the age. Significant increase in epididymal tail size was recorded only after 18 months, this is estimated to be the age at which testicular maximum sperm production rate is obtained for this breed. Colas *et al.*, (1986) reported that, scrotal circumference in rams is not routinely used to assess testicular weight. Instead testicular diameter measurements have been used. While, Wildeus and Entwistle (1982) stated that, the measurement of scrotal circumference in bulls has a good correlation with testicular weight which is, in turn, directly related to sperm production.

As in the females trans-rectal and trans cutaneous methods of real time ultrasonography have been employed to examine the accessory sex glands as well as testes and epididymides of rams. The use of real time ultrasonography in breeding management programs has the potential to increase the ability to manage males and females in efficient manner. It also allows to make educated management decisions based on interpretation of anatomical images as they are related to the physiological status of the reproductive system. In this aspect, the ultrasound scanning was used to follow up the growth of ram genitalia where the estimated parameters supported the measurements by convenient methods. Scanning and interpretation of the ultrasonographic finding did not present special difficulties; as the testis are easy to access, do not have any other organs near them. In the same time, images of pelvic genitalia were extremely accurate where pelvic urethra and related accessory glands are nearly fixed and have predicted places

for its detection and examination with the linear probe. No detailed studies were available for the ultrasonographic appearance of the pelvic urethra and accessory genitalia of rams although few studies were conducted on examination of testes and epididymes (Ahmed *et al.*, 1991 and Gouletsou *et al.*, 2003).

High correlations existed between the rates of growth of the testes and those of the epididymal tail, seminal glands and bulbourethral glands. Significant growth rate of the accessory glands were pronounced from 12 months of age. This was parallel to the peak level of testosterone as recorded by Drymundeson (1973).

Finally, it could be concluded that male genitalia of Chios rams developed in different patterns between 6 and 24 months age. Ultrasonography assists studying the accessory glands directly in the living animals, which was unattainable before without animal slaughtering.

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