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Ultrasonographic Examination and Measurements of Testes, Epididymis and Accessory Glands at Different Growth Stages of Chios Rams

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ABSTRACT

Background: Testes, epididymis and accessory glands measurements should be mandatory as an imminent for male selection. This study aimed at defining the ultrasonographic characteristics and measurements of testes, epididymis and accessory glands at different growth stages in Chios rams.

Methods: Sixteen rams were assigned by age class into: group A (pre-pubertal, n=3), group B (pubertal, n=5) and group C (mature, n=8). Scrotal circumference (SC) was measured using a tape. Scrotal contents were examined using a B-mode ultrasound. Testicular breadth (TB), thicknesses of tunica albuginea (TA) and mediastinum (MS) and the largest dimension of epididymal tail (EpT) were estimated. Trans-rectal ultrasound was carried out to estimate the echogenicity and diameter of bulbourethral gland (BUG), height of pars dessiminata of prostate (PDP) and maximum breadth of seminal gland (SG).

Result: SC increased curvilinear with age. Testicular echogenicity increased with age. Thickness of TA did not differ with age. MS could not detect in pre-pubertal rams, reached maximum thickness with puberty and became relatively thinner by maturity. EpT evidently enlarged with puberty. BUG, PDP and SG exhibited moderate echogenicity. They reached their greatest size with sexual maturity. SC estimated by tape and TB estimated by US correlated with most of the ultrasonographic measurements of the epididymis and accessory glands.

Key words: Accessory glands, Epididymis, Scrotal circumference, Testes, Ultrasonography.

INTRODUCTION

In evaluating rams for use in breeding, physical examination, measurements of genital tract and semen analysis are imperative (Hassani *et al*., 2014; Ridler *et al*., 2012; Al-kawmani *et al*., 2018; 2020; Belkhiri *et al*., 2019). An important measurement taken during the breeding sound examination of rams is the scrotal circumference (SC). This measurement is strongly related to the semen production capacity (Barkley, 2020). Moreover, female progeny from rams with larger SC reach puberty earlier than progeny from rams with smaller SC (Gouletsou and Fthenakis, 2010).

Diagnostic ultrasonography has been extensively used for inspection of the reproductive tract of female, but more scantily in male. Ultrasonography (US) can provide useful information in the assessment of male reproductive health. There existed a potential practical application for US combined with other techniques to monitor the changes in testicular histomorphology, histophysiology and hemodynamic (Omer *et al*., 2012; Ahmadi *et al*., 2013; Giffin *et al*., 2014; Bartlewski et al., 2017; Ntemka *et al*., 2018). Testicular length and width estimated by US in pre-pubertal age and scrotal circumference estimated by caliper in post-pubertal age have been proved as indicator for selection of bucks (Kumbhar *et al*., 2019).

Trans-rectal ultrasound (TRUS) of the internal genitalia may be used as an aid in the diagnosis of reproductive tract health. TRUS could reveal prostatic masses and seminal gland echo-texture abnormalities suggestive of inflammation (Pozor and McDonnell, 2002; Schnobrich *et al*., 2016). Relatively, little information is existing on male TRUS in veterinary practice.

This study aimed at defining the ultrasonographic characteristics of testes, epididymis and accessory glands at different growth stages of clinically normal Chios rams raised in the subtropics.

MATERIALS AND METHODS

Animals

Sixteen healthy Chios rams with no history of genital tract pathology were included in this study. Their ages and weights

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ranged from 3 to 24 months and from 16 to 50 kg, respectively. Animals were assigned by age class into: group A (pre-pubertal, < 6 months, average 4.33 ± 0.67 months, n=3), group B (pubertal, 6-12 months, average 10 ± 0.2 months, n=5), and group C (mature, > 12 months, average 20.25 ± 1.1 months, n=8) (Belibasaki and Kouimtzis, 2000; Ribeiro *et al*., 2017). Rams were maintained in Al-Azhar agriculture farm, Al-Azhar University, Assiut-Egypt under general husbandry condition. This experiment took place during the months of September and October (Updated, 2020).

Clinical and ultrasonographic examinations

Clinical examination of genitalia was performed from the rear, with the ram in the standing position and restrained by two assistants. Scrotal circumference was measured using a tape at the widest point with constant tension (Ley *et al*., 1990).

US of the scrotal contents was carried out using a 6/8 MHz linear array transducer connected to B-mode ultrasound scanner (Pie Medical, 100 LC, Maastricht, Holland). Before examination, one testis was 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. To enhance contact and image quality, an ample ultrasound coupling gel was used. The transducer gently applied to the caudal scrotal surface. One testis was pushed dorsally and the other forced ventrally, with the transducer placed vertically on the latter testis, about intermediate from the caput and cauda epididymides. Once the first testis is examined, the relative positions of the two testes are reversed, and the second testis examined. Thereafter, both testes were gently forced ventrally, and the transducer is placed transversely and moved consecutively along the length of the scrotum, allowing simultaneous examination of both testes. The entire scrotum and testes, including epididymis, testicular tunics and spermatic cords were examined. Testicular breadth (TB, a medial to lateral line at the width of the testis), thicknesses of tunica albuginea (TA) and mediastinum (MS) and the largest dimension of epididymal tail (EpT) were estimated from frozen images.

TRUS was performed after removing the feces from the rectum and applying ultrasound transmission gel. Examination was done using the same US device. The diameter of bulbourethral gland (BUG), the height of pars dessiminata of prostate (PDP) and maximum breadth of seminal gland (SG), of both sides were recorded from frozen images. US was carried out by a single operator.

Statistical analysis

Data were expressed in means ± SE. Analysis of variance (ANOVA) was used for comparison among age groups, with LSD as the post-ANOVA test. Relationships were determined by correlation coefficients. Significance was set at P < 0.05. Statistical analysis was carried out using SPSS program, version 21.

RESULTS AND DISCUSSION

There were no statistically significant differences in measurements between the left and right sides of paired structures (testes, epididymis, and accessory glands), so their measurements were pooled for one value for each animal.

SC increased curvilinear with age, with a rapid increase in group B, but at a slower rate in group C (P=0.001). There was a positive correlation between SC and age (r=0.836, P=0.01) (Table 1, Fig 1). Testicular tissue was commonly homogenous and moderately echogenic (Fig 2). The echogenicity increased with age (*i.e*. the parenchyma appeared brighter). TB estimated by US increased rashly in groups B, but at a relatively leisurelier rate in group C (Table 2). Changes in testicular echogenicity seems to occur simultaneous with pubertal changes in testicular histology (Brito *et al*. 2012). There was a strong linear correlation between SC and TB (r = 0.95, P = 0.0001) (Fig 3). So, TB can be used as an alternative measure of SC in breeding sound examination of rams (Schurich *et al*., 2009; Lotti and Maggi, 2015; Bartlewski *et al*., 2017).

TA appeared as a bright, hyper-echogenic line separating the testis and scrotum (Fig 2 B). Thickness of TA did not differ with age (Table 2). There was no significant correlation between SC and TA, or between TB and TA. MS appeared as a hyper-echogenic line or spot when the positioning of the transducer is parallel or perpendicular, respectively, relative to the long axis of the testis. It could not observe in group A, rapidly developed in group B, and became relatively thinner in group C (Fig 2 A and B, Table 2). No correlation was found between SC and MS, or between TB and MS. MS thickness changed with age, where it could not observe in pre-pubertal rams. Gouletsou *et al*. (2003) detected MS in 87% of rams and 77% of clinically healthy testes.

EpT was found just beyond the ventral border of the testes. It was easily detected; less echogenic than the testis, clearly delineated from it, and with a heterogeneous structure (Fig 4 A). EpT diameter was larger in group B than in group A (P = 0.004), but there was no significant difference between groups B and C. This appears to be related to the onset of

Table 1: Scrotal circumference (means ± SE) estimated by tape at different ages of Chios rams.

Measurement	Age			
	Group A (pre-pubertal, 3-5 month, n=3)	Group B (post-pubertal, 6-12 months, n=5)	Group C (mature, >12 – 24 months, n=8)	P value
Scrotal circumference (SC)	12.33 ± 1.46[a]	20.4 ± 1.13[b]	24.75 ± 0.89[c]	0.001

Table 2: Measurements (means ± SE) estimated by ultrasonography for testes, epididymis and accessory glands at different ages of Chios rams.

Measurements	Age			
	Group A (pre-pubertal, 3-5 month, n=3)	Group B (post-pubertal, 6-12 months, n=5)	Group C (mature, >12 – 24 months, n=8)	P value
Testicular breadth (TB)	1.47 ± 0.19[a]	3.006 ± 0.15[b]	3.68 ± 0.12[c]	0.001
Tunica Albuginea (TA)	0.23 ± 0.05[a]	0.21 ± 0.05[a]	0.19 ± 0.05[a]	0.5
Mediastinum testis (MS)	0.00 ± 0.00[a]	0.42 ± 0.07[b]	0.19 ± 0.07[c]	0.004
Epididymal tail (EpT)	0.97 ± 0.23[a]	1.65 ± 0.18[b]	1.88 ± 0.14[b]	0.02
Bulbourethral gland (BUG)	0.91 ± 0.23[a]	0.9 ± 0.19[a]	1.24 ± 0.12[b]	0.03
Prostate (pars disseminate, PS)	0.6 ± 0.08[a]	0.55 ± 0.06[a]	0.83 ± 0.04[b]	0.006
Seminal gland (SG)	0.75 ± 0.22[a]	0.91 ± 0.16[a]	1.42 ± 0.17[b]	0.03

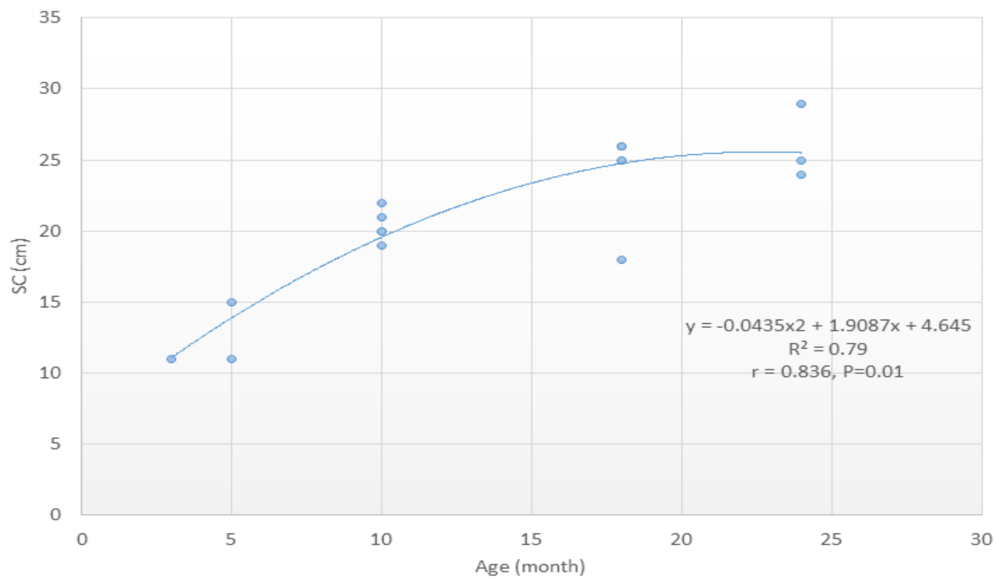

Fig 1: Correlations between scrotal circumference and ram's age.

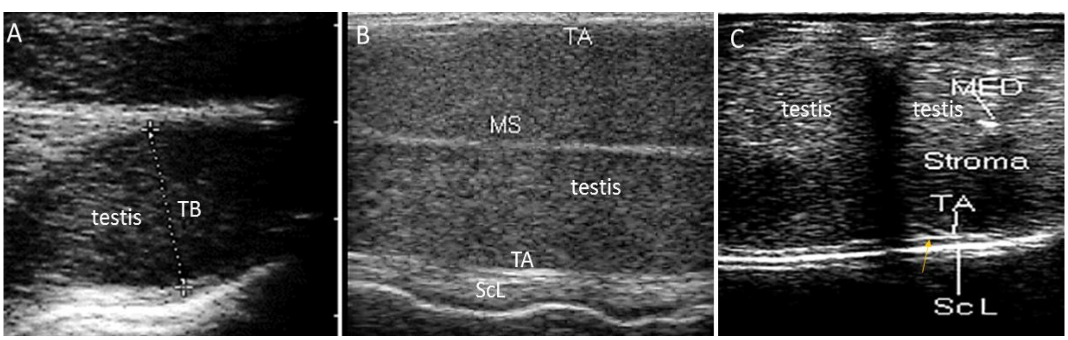

Fig 2: Ultrasonography of the testes in Chios rams: (A) longitudinal plane for a testis of a pre-pubertal lamb showing low echogenicity with no mediastinum testis (MS), testicular breadth (TB) is a medial to lateral line at the width of the testis; (B) longitudinal plane for a testis of mature ram showing echogenic echotexture, MS appears as a bright line extends along the center of the testis, tunica albuginea (TA) looks as a bright line separating testis from the scrotal layer (ScL). (C) Transverse plane in the testes of pubertal ram with moderate echotexture, MS appeared as a bright spot in center of the testis, the fine black line between TA and ScL represents the fluid of tunica vaginalis (arrow).

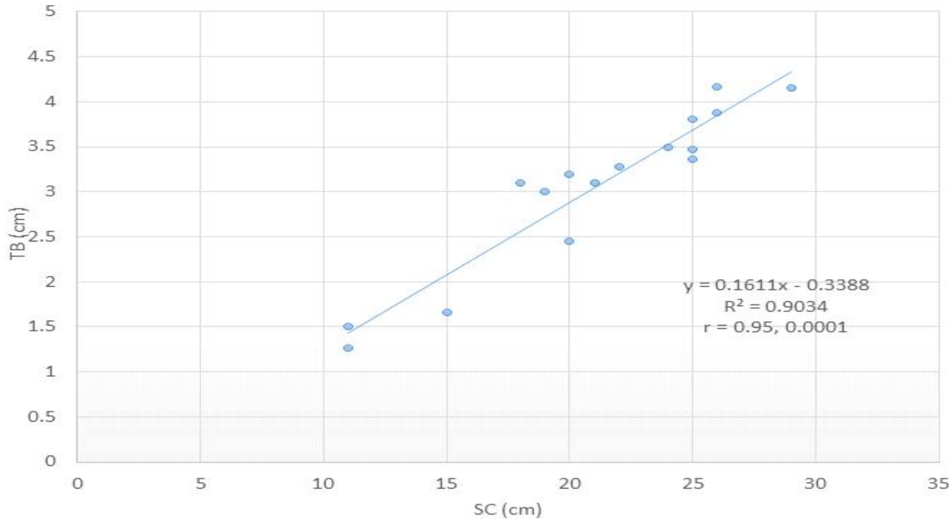

Fig 3: Correlation between scrotal circumference (SC) estimated by tape and testicular breadth (TB) estimated by ultrasonography.

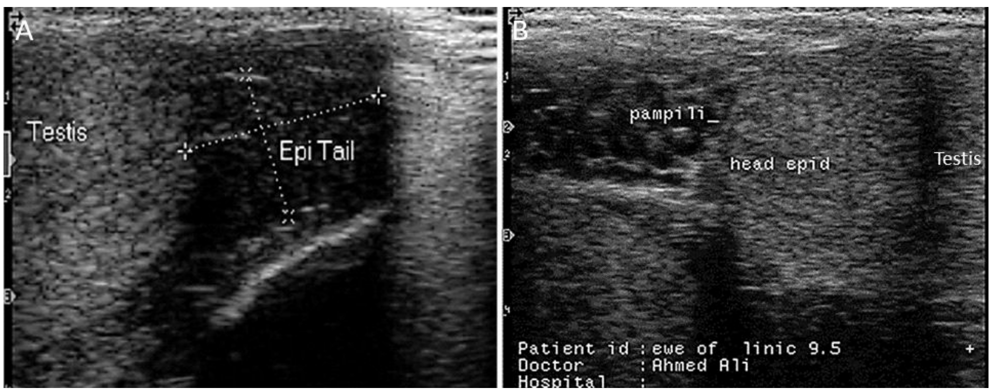

Fig 4: Ultrasonography of the epididymis in Chios rams: (A) Epididymal tail in a mature ram, it appears less echogenic than the testis, visibly demarcated from it, with a heterogeneous assembly; (B) Epididymal head in a mature ram, it is relatively near to the testicular echotexture, with a clear demarcation between them, the pampiniform plexus was evidently scanned just dorsal to the epidyidymal head as a network structure comprising frequent black rounded vesicles representing the spermatic vessels.

semen production. Puberty averaged 6.3 months in Chios ram (Belibasaki and Kouimtzis, 2000). Based on the results of the current study, the greater the SC and TB, the larger the EpT. This is due to the correlation of testicle dimensions with the rate of sperm production. There were positive correlations between SC and EpT (r = 0.737, P = 0.0001) and between TB and EpT (r = 0.626, P = 0.0009). The head of the epididymis was located on the proximal and cranial aspect of the testis, comparatively close to the testicular echogenicity, with a clear distinction between them (Fig 4 B). It was regularly incompletely imaged. Both the body of the epididymis and ductus deferens were along the caudal–medial border of the testis; they were small, tubular structures that were hard to image. The pampiniform plexus was clearly imaged just dorsal to the epidyidymal head as a network structure containing numerous hypo-echogenic rounded vesicles representing the spermatic vessels (Fig 4 B) (Gervasi and Visconti, 2017; Leahy *et al*., 2019),

BUG was observed immediately when entering the rectal probe, at the most caudal part of the pelvic urethra. It appeared as a well demarcated round, moderately echogenic structure (Fig 5 A). Its diameter was greater in group C than in groups A and B (P=0.03). Likewise, achievement of sexual maturity in Dorper rams was linked with enlargement of BUG (Camela *et al*., 2017).There was a positive correlation between SC and BUG (r = 0.663, P = 0.02). No correlation was found between TB and BUG. BUG are responsible for producing a pre-ejaculate fluid called Cowper's fluid, which is secreted during sexual excitement, neutralizing the acidity of the urethra in preparation for the passage of sperm cells (Chughtai et al., 2005).

PDP was detected just cranial to the BUG, and above and along the pelvic urethra. It had a homogeneous, moderately echogenic echotexture. The pelvic urethra (PU) looked less echogenic than the PDP (Fig 5 A). The height

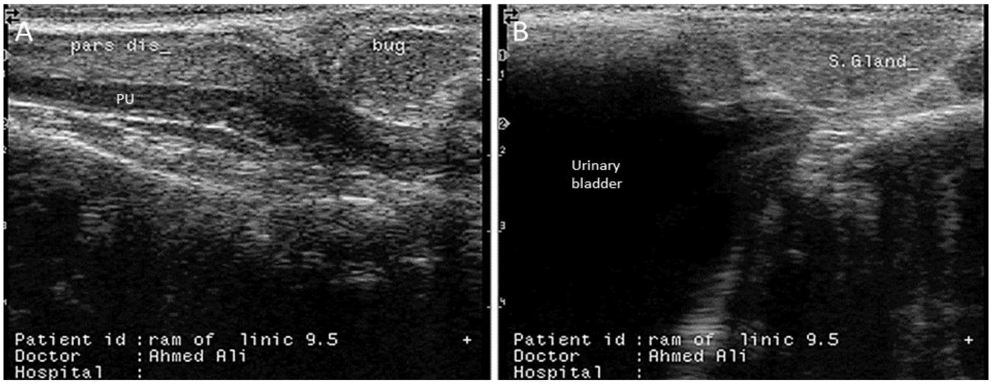

FiG 5: Accessory sex gland in a mature Chios rams: (A) Bulbourethral gland (bug) was detected instantly by introducing the rectal probe, at the most caudal part of the pelvic urethra, it is well demarcated round and moderately echogenic structure; (B) Pars disseminate of the prostate (pars dis) is found just cranial to the Bulbourethral gland , and above and along the pelvic urethra (PU), it has a homogeneous, moderately echogenic echotexture, PU seen less echogenic than the prostate; (B) Seminal gland (S. Gland) is seen close to the urinary bladder at the cranial end of the pelvic urethra, it appeared as a homogenous, moderately echogenic structure with a well-demarcated irregular and echogenic outline.

of PDP did not differ between groups A and B, but rashly increased in group C (P = 0.006). A positive correlation was found between SC and PDP (r = 0.632, P = 0.02), and a tendency for positive correlation between TB and PDP (r = 0.541, P = 0.06). PDP produce fluid that forms part of semen.This prostatic fluid is slightly alkaline. The alkalinity of semen helps neutralize the acidity of the vaginal tract, prolonging the lifespan of sperm (Barrett, 2019).

SG was imaged close to the urinary bladder at the cranial end of the pelvic urethra. It appeared as a homogenous, moderately echogenic structure with a well-demarcated irregular and echogenic outline (Fig 5 B). The maximum breadth of the SG did not differ between groups A and B, but speedily increased in group C (P = 0.03). There were positive correlations between SC and SG (r = 0.734, P = 0.004) and between TB and SG (r = 0.652, P = 0.02). SG secretes fluid that partly composes the semen. The seminal fluid consists of nutrients including fructose and citric acid, prostaglandins, and fibrinogen (Hall, 2016). The growth and maturation of accessory glands are chiefly controlled by testosterone hormone, which secreted from the testes (Risbridger and Taylor, 2006). Both testicular and serum testosterones were higher in mature than pre-pubertal Awassi rams (Saaed and Zaid, 2019).

CONCLUSION

The present data provide expedient clinical guiding principle for US morphology and size measures of testis, epididymis and accessory sex glands of Chios rams. The normal reference data defined in this study will be useful in the clinical evaluation and selection of Chios rams. Scrotal US remains a first-line imaging technique for the assessment of male reproductive health due mainly to its adaptability and lower cost. US permits to make refined management decisions constructed on elucidation of anatomical images as they relate to the physiological status of the reproductive system. TRUS assists studying the accessory glands right in the living animals as a non-invasive technique. TB can be used as an alternative measure of SC in breeding sound examination of rams.

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