Doppler and B-mode ultrasonographic monitoring of accessory sex glands and testes in Barki rams during the breeding season

Ahmed Essam Elweza, Ahmed Mohamed Sharshar and Hamed Talaat Elbaz*

Abstract

The aim of this study was to perform ultrasonographic imaging of the testes and accessory sex glands in adult Barki rams during the breeding season. The impact of testosterone on the Doppler indices of accessory sex glands was also investigated. Scrotal contents, pelvic urethra and accessory sex glands of twelve mature Barki rams were scanned with multiple imaging of B-mode and colour Doppler ultrasonography. Serum concentrations of testosterone, FSH and LH were determined. The results revealed that the breeding season changed the echogenicity of testicular parenchyma, spermatic cord, epididymal tail, glans penis and echotexture of accessory sex glands. Serum testosterone was 7.27±0.37 ng/mL, FSH was 6.46±0.2 and LH was 5.6±0.28 m IU/mL. The pulsatility index (PI) for the supra-testicular artery (STA), marginal artery (MA) of the testes and epididymal tail was 1.01±0.07, 0.58±0.04 and 0.5±0.04. The resistive index (RI) for the same structures was 0.6±0.04, 0.33±0.04 and 0.3±0.03, respectively. Importantly, testosterone downregulated PI and RI of the ampulla, vesicular gland, prostate gland and bulbourethral gland. In conclusion, the breeding season changed the echogenicity of reproductive organs and accessory genital glands of rams, and testosterone regulated the hemodynamic parameters of the accessory sex glands.

Key words: *breeding season; colour Doppler; rams; sex glands; testes*

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Introduction

Sexual activity of rams may be affected by a range of factors, including seasonality, breeds, genetics, hormonal impact, post-weaning care, environmental temperature and nutritional state. Photoperiod is a major environmental factor that influences sheep reproductive capacity (Kridli et al., 2007). It greatly affects the onset of sexual activity, regardless of variations of its length between temperate and tropical zones (Hammoudi et al., 2010). Scrotal dimensions, testicular volume and weight are modified throughout the reproductive life of rams as a result of the complicated interaction between growth of neuroendocrine organs, testosterone, body development and sexual maturity (Maksimovic et al., 2016). The lowest values of reproductive characteristics (scrotal circumference, testosterone level, ejaculate volume and sperm motility) of Black Racka rams were found in winter, while the highest values were recorded in late summer and autumn (Sarlos et al., 2013). Moreover, recent records in rams revealed that breeding season greatly influences scrotal circumference, serum testosterone level and semen characteristics (Belkadi et al., 2017).

Ultrasonographic evaluation of the scrotal contents and accessory genital glands could be an important method for assessing the normality of morphological structures in mammals, including rams (Gouletsou et al., 2003). Gray-scale (B-mode) scrotal ultrasonographic examination was used to assess the testicular and epididymal echotexture, size and shape in rams (Ahmadi et al., 2012). Colour Doppler ultrasonography is a recent diagnostic tool applied in veterinary reproductive practice used as a stand-alone examination or in association with B-mode ultrasonography. Previous studies have recorded data on the haemodynamic parameters in the male genital system (Carvalho et al., 2008). Haemodynamic parameters evaluated in the testicular artery, particularly resistance index (RI) and pulsatility index (PI), could potentially be associated with semen quality (Zelli et al., 2013).

The accessory genital glands of ram are situated in the pelvic cavity and are commonly ignored during the breeding soundness examination (Gouletsou and Fthenakis, 2010). This is due to limitations in rectal palpation practice; however, their purpose is indirectly estimated via the calculation of semen volume (Camela et al., 2017). Transrectal ultrasonography has been applied to evaluate both major and minor alterations in the accessory genital glands (Chandolia et al., 1997; Clark and Althouse, 2003). Nevertheless, there are very few studies examining with the effects of breeding season on colour Doppler and B-mode ultrasonographic imaging of the accessory sex glands in rams, unlike for other animal species. A recent publication showed that in rams, achieving sexual maturity was related to the growth and changes in the echotexture of the accessory genital glands (Camela et al., 2017).

We recently demonstrated that sexual maturity in Barki rams was attributed to higher testosterone level, but that this was not associated with the Doppler indices during the non-breeding season (Elbaz et al., 2019). Therefore, the purpose of this study was to obtain normal ultrasonographic imaging of the testes, epididymis and accessory sex glands in sexually mature Barki rams during the breeding season. The impact of circulating testosterone levels on PI and RI values of accessory sex glands in breeding rams was also examined.

Materials and methods

This study was approved by the Ethics of the Animal Care and Welfare

Committee, University of Sadat City, Egypt.

Animals

The current study was conducted on twelve sexually mature Barki Egyptian rams aged between 2 and 2.5 years with an average body weight from 50 to 60 kg, raised at the educational farm (Faculty of Veterinary Medicine, University of Sadat City, Egypt) during the period from September to November, 2019. All rams were healthy, dewormed, vaccinated, and commonly housed in a free stall barn. They were fed a well-balanced ration consisted of pelleted concentrates with free access to water and green fodder (barseem clover). Rams were carefully selected after the breeding soundness examination, and all were verified to be free from any reproductive disorders.

B-mode and Colour Doppler ultrasonographic examination of the scrotum

No tranquilizing drugs were administrated to rams in order to avoid any effect on testicular vascular flow. Ultrasonographic monitoring of the testes and epididymis was performed in the standing position. The wool on both sides of the scrotum was thoroughly cleaned and shaved. Ultrasonographic examination of rams was performed using a linear and micro-convex probes (2.5-18 MHz) connected to the ultrasound scanner (Esaote MyLab™One VET, Italy). Gray scale (B-mode) ultrasonographic images were done first and the echotextures of the spermatic cord, testes and epididymal tail were recorded following the technique described by Gouletsou et al. (2003). The colour Doppler ultrasound probe was primarily situated at the scrotum neck to visualize the supra-testicular artery at the convoluted looping section. The transducer was then moved ventrally just over parenchyma of the testes to localize the marginal artery and the intra-testicular arteries. The colour gain was fixed and an electronic calliper (2–3 mm in length) was situated in the central area of the blood vessel with apertures to detect the spectral trace of vascular flow in addition to the spectral curve and haemodynamic indices: resistive index (RI) and pulsatility index (PI). The insonation angle was 60° and the high pass filter was set at 50 Hz. The Doppler gate was constantly reserved at 1 mm and at least three similar consecutive spectral curves were gained (Pozor and McDonnell, 2004; Gloria et al., 2018). All examinations were performed by the same person, all settings of the ultrasound machine were fixed and all assessments were performed at the same time (11:00 am) throughout the study. Spectral and Colour-Doppler imaging were performed every two weeks over 90 days.

B-mode and Colour Doppler ultrasound examination of the accessory sex glands and penis

Colour Doppler scanning of the accessory sex glands was performed after B-mode (grayscale) imaging. The transducer was adjusted in a self-manufactured connector to facilitate its manipulation per rectum. The ultrasound transducer was well lubricated with coupling gel, inserted into the rectum after faecal evacuation and then moved anteriorly to visualize the urinary bladder-pelvic urethral connection as a guide. The ampulla and vesicular glands were imaged near the urinary bladder. The pars disseminata of the prostate gland was visualized during imaging of the pelvic urethra. The bulbourethral glands were simply scanned during the removal of the probe from the anal opening and the echogenicity of accessory sex glands was recorded (Camela et al., 2017). The free portion of the penis was visualized after applying the ultrasound probe on the penile sheath.

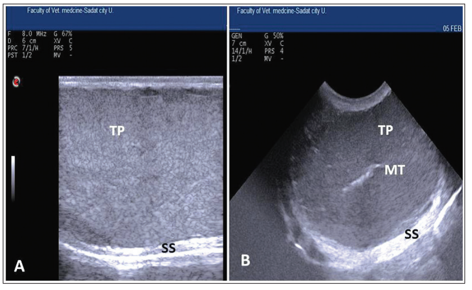

Figure 1. Ultrasonographic imaging (B-mode) of the testicular parenchyma (TP). (A) Longitudinal section of the testis showing moderate hypoechogenicity of the testicular parenchyma (TP) and highly echogenic white scrotal skin (SS). (B) Cross section of the testis showing the echogenic central mediastinum testis (MT)

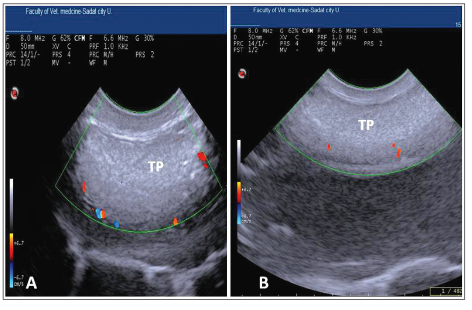

Figure 2. Colour Doppler ultrasonography of the testicular parenchyma (TP). (A) Colours of the marginal artery. (B) Colours of the the intra-testicular arteries

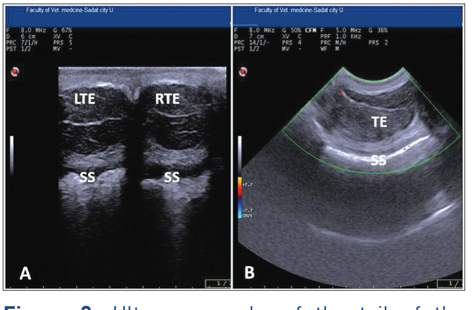

Figure 3. Ultrasonography of the tail of the epididymis. (A) B-mode showing the non-echogenic of the lumen of the right and left tail of epididymis (RTE, LTE) and echogenic white colouration of the scrotal skin (SS). (B) Colour Doppler image showing small, pale coloured dots at the proximal and distal part of the tail of the epididymis (TE)

Blood sampling

Blood samples (5 mL) were collected from the jugular vein of all rams regularly after ultrasonographic examination. Samples were placed in a slope direction of 45 ° at 4 °C for 12 hours to facilitate serum separation, clotted blood samples were centrifuged at 3000 rpm for 15 minutes and separated sera were stored at -20 °C in Eppendorf tubes until further analysis. Serum concentration of testosterone, FSH and LH were measured using ELISA kits and the microwell method (Calbiotech, Austin, CA, USA) and optical density (OD) absorbance was adjusted to 450±10 nm (Elbaz et al., 2019).

Statistical analysis

All data are presented as means ± standard error of the mean (SEM) of all measured values pulsatile index (PI) and resistance index (RI) of testes, epididymal tail and accessory sex glands. Statistical analysis was performed using GraphPad prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Values of $P<0.05$ were considered statistically significant. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to study the impact of testosterone on PI and RI of accessory sex glands.

Results

B-mode and colour Doppler ultrasonography of the scrotum and its contents

Ultrasonographic monitoring of the testes revealed that the testicular parenchyma was a homogenous, granular, grey structure and the mediastinum testis was a more echogenic white structure in the centre of testis (Fig. 1). Doppler ultrasound monitoring depicted the supra-testicular artery (STA) in the spermatic cord region in a tortuous pattern. During Doppler scanning of the testes, the marginal artery (MA) appeared with a linear pattern on the inner surface of the tunica albuginea and the intra-testicular artery appeared as light red patches or a dot near the centre of the testicular parenchyma (Fig. 2).

The tail of epididymis was globular, distended and appeared via B-mode ultrasonography as a black non-echogenic lumen with a hyperechoic line imaged in the centre. During Doppler colour scanning, the epididymal tail appeared as small, coloured dots at the proximal and distal parts (Fig. 3).

The spermatic cord appeared as an anechoic circular structure surrounded by hyperechoic regions and the colour Doppler of the pampiniform plexus appeared as large, different patches of varying orange and blue colouration during scanning. The waveforms of blood flow were monophasic and non-resistive (Fig. 4).

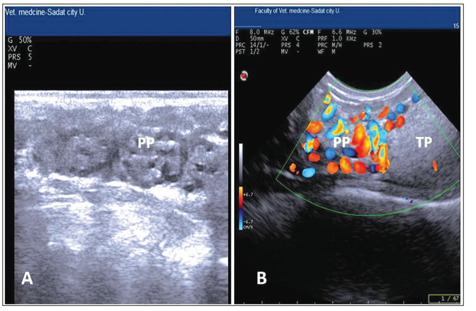

Figure 4. Ultrasonographic imaging of the spermatic cord. (A) B-mode of pampiniform plexus (PP) showing numerous anechoic black rounded or loop-like areas. (B) Colour Doppler showing blood flow within the convoluted part of the supra-testicular artery in the pampiniform plexus (PP), in comparison to the testicular parenchyma (TP)

B-mode and colour Doppler ultrasonography of accessory sex glands, pelvic urethra and penis

All accessory sex glands of rams were easily scanned and visible via trans-rectal ultrasonography. Ampullae were visualized dorsally to the urinary bladder as non-echogenic linear lumens with echogenic glandular lining (Fig. 5). Vesicular glands appeared posterior to the urinary bladder as large pyriform, moderately echogenic structures, surrounded by a circumscribed echogenic line. The glands were seen as a small red dot in the ventral aspect (Fig. 6). *Pars disseminata* of the prostate gland appeared as a slightly hypoechoic to moderately echogenic along the lumen of the pelvic urethra. The glands appeared as small red and blue dots (Fig. 7). Bulbourethral glands were scanned at the exit of the linear probe from the rectum and appeared as hypoechogenic to moderately echogenic with a low hypoechogenic area surrounding the glands with an echogenic dorsal rim.

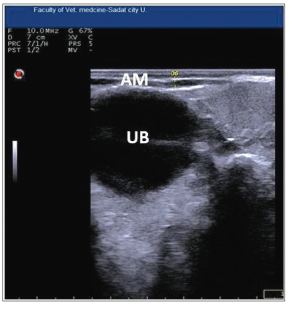

Figure 5. Ultrasonographic imaging of the ampulla (AM). Note the small, black, non-echogenic lumen with echogenic glandular lining above the non-echogenic urinary bladder (UB)

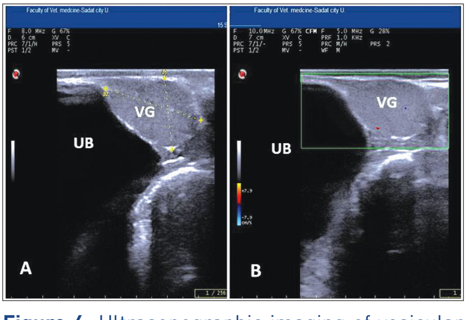

Figure 6. Ultrasonographic imaging of vesicular gland (VG). (A) B-mode showing large pyriform of moderate echogenicity with circumscribed echogenic line posterior to the black, non-echogenic urinary bladder (UB). (B) Colour Doppler showing a smaller red dot in the ventral aspect

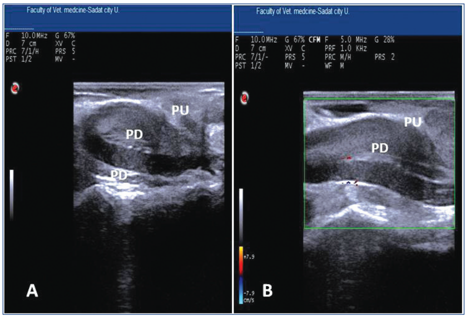

Figure 7. Ultrasonographic imaging of the *pars disseminata* of the prostate gland (PD). (A) B-mode showing the echogenic part of the *pars disseminata* (PD) surrounding the lumen of the pelvic urethra (PU). (B) Colour Doppler showing a small red dot near the lumen

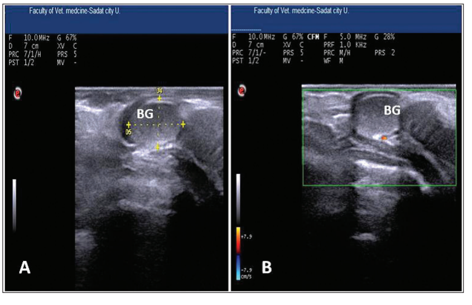

Figure 8. Ultrasonographic imaging of the bulbourethral glands (BG). (A) B-mode showing hypoechogenic to moderate echogenicity with a low hypoechogenic area surrounding the glands. (B) Colour Doppler showing a single orange dot in the ventral aspect

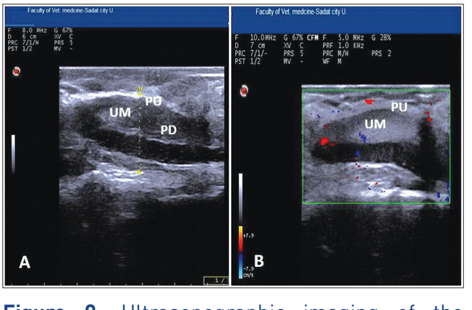

Figure 9. Ultrasonographic imaging of the pelvic urethra (PU). (A) B-mode showing the black, non-echogenic lumen surrounded by the hypoechogenic urethral muscle (UM). (B) Colour Doppler showing small red to blue dots distributed along the urethra

The glands had a single orange dot in the ventral aspect (Fig. 8). The pelvic urethra appeared as a anechogenic lumen surrounded by hypoechogenic urethral muscle. It showed tiny red to blue dots distributed along the urethra (Fig. 9). The free part of penis (*glans penis*) appeared hypoechogenic to moderately echogenic with non-echogenic *corpus cavernosum urethrae* in the ventral part. It had small dots of varying colouration in the lower part (Fig. 10).

Doppler indices of the testes, epididymal tail and accessory sex glands

The pulsatility indices (PI) of the supra-testicular artery (STA), marginal artery (MA) of testes and epididymal tail were 1.01±0.07, 0.58±0.04 and 0.5±0.04, respectively. The resistive indices (RI) of the above structures were 0.6±0.04, 0.33±0.04 and 0.3±0.03, respectively (Table 1). The PI and RI values of the ampulla, vesicular gland, prostate gland and bulbourethral glands are shown in Table 2.

Serum hormonal concentrations

The serum testosterone in breeding rams was 7.27±0.37 ng/mL. Furthermore, levels of FSH and LH were 6.46±0.2 and 5.6±0.28 m IU/mL, respectively.

Effect of serum testosterone on the PI and RI of accessory sex glands

Level of circulating testosterone clearly downregulated the values of PI and RI of the ampulla, vesicular gland, prostate gland and bulbourethral glands (Figs. 11 and 12).

Discussion

Breeding season may affect testicular echogenicity and Doppler indices in parallel with changes in circulating androgen and oestrogen in small ruminants (Samir et al., 2018). In the present study, the testicular parenchyma was a homogenous, granular, grey structure, and the mediastinum testis was a more echogenic structure in the centre of the testis. Similar results have been reported in the literature (Ahmad et al., 1991; Gouletsou et al., 2003; Andrade et al. 2014; Elbaz et al., 2019). The echogenicity of the testes was slightly reduced during the breeding season, and the current results support records in rams by Gouletsou, (2017) and Elbaz and Abdel Razek, (2019). We hypothesized that changes of testicular echogenicity during the breeding season might be induced by the improvement of testicular function of breeding rams. Hence, activity of the testes and seminiferous tubules in rams were higher during autumn and winter compared to summer (Barkawi et al., 2006; Al-Ghetaa 2012). Moreover, sperm total defects were positively correlated to testicular blood indices (Batissaco et al., 2013) and assessment of the haemodynamic of the testicular artery was used to diagnose testicular disorders (Pozor, 2007).

Doppler ultrasound monitoring of the testes revealed that the marginal artery (MA) appeared with scattered red and blue patches or dots on the inner surface of the tunica albuginea, and the intra-testicular artery appeared as pale red patches or dots near the centre of the testicular parenchyma. Our results were in accordance with previously published data in rams (Samir et al., 2015; Camela et al., 2017; Elbaz et al., 2019; Hedia et al., 2019). Moreover, the mean value of resistive index (RI) and pulsatility index (PI) of the MA were 0.33±0.04 and 0.58±0.04, respectively. The obtained values were in accordance with previously values reported by Elbaz et al. (2019) and Hedia et al. (2019). However, Hedia et al. (2019) recorded that during summer, the values of RI and PI of testicular blood flow were increased with reduced testicular volume in rams. This difference might be attributed to seasonal,

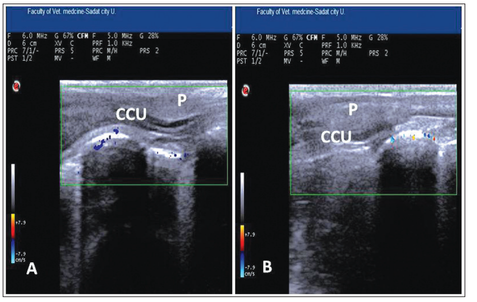

Figure 10. Ultrasonographic imaging of the penis (P). (A) and (B) Colour Doppler imaging of the penile part showing pale dots of varying colour in the lower part and the black, non-echogenic *corpus cavernosum urethrae* (CCU)

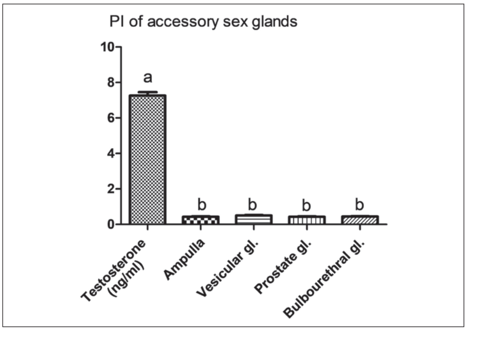

Figure 11. Effect of testosterone on the pulsatility index (PI) of the accessory sex glands of breeding rams (*n*=12). Bars with different superscript letters differ significantly at *P*<0.05. Data are presented as mean ± SEM

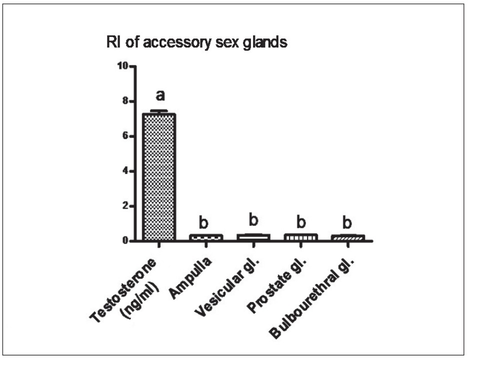

Figure 12. Effect of testosterone on the resistive index (RI) of the accessory sex glands of breeding rams (*n*=12). Bars with different superscript letters differ significantly at *P*<0.05. Data are presented as mean ± SEM

Table 1. Doppler parameters (PI and RI) of testes and the epididymis tail of Barki rams (*n*=12). (STA) supra-testicular artery, (MA) marginal artery, (RI) resistive index, (PI) pulsatility index. Data are presented as mean ± SEM

Testes				Tail of epididymis	
STA		MA			
PI	RI	PI	RI	PI	RI
1.01±0.07	0.6±0.04	0.58±0.04	0.33±0.04	0.5±0.04	0.3±0.03

Table 2. Doppler parameters, pulsatility index (PI) and resistive index (RI) of the accessory sex glands (ampulla, vesicular, prostate, bulbourethral) of Barki rams (*n*=12). Data are presented as mean ± SEM

Ampulla		Vesicular gland		Prostate gland		Bulbourethral gland	
PI	RI	PI	RI	PI	RI	PI	RI
0.42±0.04	0.33±0.02	0.5±0.03	0.35±0.03	0.43±0.04	0.36±0.03	0.45±0.03	0.32±0.02

vascular resistance and/or testosterone level variations.

Testicular volume is a principle indicator of sperm production as the seminiferous tubules represent the major constituent of testicular tissue (Sarlos et al., 2013). Moreover, some factors such as nutrition, temperature and relative humidity could affect seasonality and photoperiod in sheep (Rosa and Bryant, 2003). The present data showed that the tail of the epididymis was visible as a globular and distended structure, with a black, non-echogenic lumen and a hyperechoic line imaged in the centre, as described previously (Ahmad et al., 1991; Gouletsou et al., 2003). We hypothesized that large epididymal tail might be beneficial for increasing storage capacity. The increased sperm cell concentration in rams in autumn as compared to summer supports this hypothesis (Hedia et al., 2019).

The spermatic cord appeared as a black, non-echoic circular area surrounded by whiter hyperechoic regions, and the colour Doppler of the pampiniform plexus was visualized as large, differing patches with varying orange and blue colouration during scanning. These results were similar to those previously recorded in rams (Camela et al., 2017; Elbaz et al., 2019; Hedia et al., 2019). The waveforms of blood flow were monophasic and non-resistive, in accordance with previous results obtained by Elbaz et al. (2019) and Hedia et al. (2019). However, the Doppler blood flow of the stallion had a resistive and biphasic character (Pozor and McDonnell, 2004). These differences might be attributed to species, testis position or blood vessels.

Doppler testicular blood flow indices and testosterone are imperative to predict the future ejaculate volume and active progressive motility of spermatozoa in rams (Camela et al., 2017). Unfortunately, there are few relevant reports concerning the examination of accessory genital glands in rams by B-mode and colour Doppler ultrasonography (Gouletsou et al., 2003; Camela et al., 2017; Elbaz et al., 2019; Hedia et al., 2019). Here, we reported that circulating testosterone downregulated the blood indices of accessory sex glands of breeding rams. Thus, we assume that high testosterone levels induced the low values of Doppler indices, for more vascular supply to stimulate the activity of the accessory sex glands during the breeding season. Therefore, the present results might be essential for monitoring the reproductive performance of Barki rams.

Our results showed that the ampullae appeared as a black, non-echogenic linear lumen with echogenic glandular lining, similar to the results of Camela et al. (2017). Vesicular glands appeared as large, pyriform and moderately echogenic with a circumscribed echogenic line, in agreement Camela et al. (2017) and Elbaz et al. (2019). The lower echogenicity of the vesicular gland is caused by plasma-storing microcystic structures within the glandular parenchyma, which provides most of the seminal plasma (Westfalewicz et al., 2017). The glands had a small red dot in the ventral aspect seen by colour Doppler scanning, similar to reports by Camela et al. (2017). The pars disseminata of the prostate appeared as slightly hypoechoic to moderately echogenic along the lumen of the pelvic urethra, supporting the results of Camela et al. (2017) and Elbaz et al. (2019). The glands had small red and blue dots seen by colour Doppler scanning. The bulbourethral glands appeared as hypo-echogenic to moderately echogenic, with a low hypo-echogenic area surrounding the glands and an echogenic dorsal rim, in agreement with the results obtained by Camela et al. (2017) and Elbaz et al. (2019). The gland had a single orange dot in the ventral aspect and the pelvic urethra had small red to blue dots distributed along the urethra by colour Doppler ultrasonography, as also reported by Camela et al. (2017). The glans penis appeared as hypoechogenic to moderately echogenic, with a black non-echogenic *corpus cavernosum urethrae* in the ventral part. It had small dots of varying colour in the lower part, in accordance with Gouletsou et al. (2003).

The current data showed that serum testosterone of breeding Barki rams was 7.27±0.37 ng/mL, with FSH and LH values of 6.46±0.2 and 5.6±0.28 m IU/mL, respectively. The increase of testosterone and FSH levels during the breeding season stimulated oestradiol 17β secretion from the Sertoli cells, which in turn resulted in a remarkable decrease in both RI and PI values (Hedia et al., 2019). Testosterone concentrations in fat-tailed rams in Egypt were altered by air temperature and photoperiod length (Hedia et al., 2019). Reversely, Elbaz and Abdel Razek, (2019) showed that the testosterone level was 1.73±0.43 ng/mL, and FSH and LH were 4.63±0.38 and 2.05±0.14 m IU/mL, respectively, during the non-breeding season. Moreover, testosterone concentration was affected by season, temperature, relative humidity and nutrition in rams (Rosa and Bryant 2003; Hedia et al., 2019).

Conclusions

The breeding season altered the echogenicity of the external reproductive organs and accessory genital glands in breeding rams. Serum testosterone concentrations also regulated the haemodynamic parameters of the accessory sex glands in rams during the breeding season. Further studies are needed to clarify the impact of testosterone on secretions of accessory sex glands and therefore the reproductive performance in rams.

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Praćenje akcesornih spolnih žlijezda i testisa u ovnova pasmine barki pomoću Dopplera i ultrazvuka u B modu tijekom rasplodne sezone

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Ovo je istraživanje provedeno radi uspostavljanja normalnog ultrazvučnog (UZV) snimanja testisa i akcesornih spolnih žlijezda u odraslih ovnova pasmine barki tijekom rasplodne sezone. Osim toga, istraživan je i utjecaj testosterona na akcesorne spolne žlijezde temeljem uporabe Dopplera. Višestrukim snimanjem u B-modu i ultrazvučnim kolor Dopplerom pretraživan je sadržaj skrotuma, zdječna uretra te akcesorne spolne žlijezde u 12 odraslih ovnova pasmine barki. Određivane su serumske koncentracije testosterona, FSH i LH. Dobiveni su rezultati pokazali da u rasplodnoj sezoni dolazi do promjena u ehogenosti parenhima testisa, sjemenskog užeta, repa epididimisa, glansa penisa i ehoteksture akcesornih spolnih žlijezda. Razina serumskog testosterona iznosila je 7,27±0,37 ng/mL, FSH 6,46±0,2, a LH 5,6±0,28 m IU/mL. Pulzirajući indeksi (PI) supra arterije testisa (SAT), periferne arterije (PA) testisa i repa epididimisa bili su 1,01±0,07, 0,58±0,04, odnosno 0,5±0,04. Istovremeno, indeksi rezistentnosti (RI) gornjih kriterija su bili 0,6±0,04, 0,33±0,04, odnosno 0,3±0,03. Važno je spomenuti da je testosteron snižavao PI i RI ampule (a), vezikularne žlijezde, prostate i bulbouretralne žlijezde. Zaključno, rasplodna je sezona utjecala na promjene ehogenosti reproduktivnih organa i akcesornih spolnih žlijezda u ovnova. Nadalje, testosteron je regulirao hemodinamične pokazatelje akcesornih spolnih žlijezda tijekom rasplodne sezone u ovnova.

Ključne riječi: *rasplodna sezona, kolor doppler, ovnovi, spolne žlijezde, testisi*