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Original Research

Relationship Between Testicular Morphometry, Vascular Waveform Pattern, Semen Picture, and Specific Serum Testosterone Levels in Baladi bucks Approaching Puberty

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Abstract

This study aimed to determine testicular blood flow with semen imaging and describe accompanying variations in mediastinal thickness (MT), serum testosterone, and semen characteristics in Baladi bucks approaching puberty and sexual maturity. From 5–10 months of age, Baladi bucks (n = 5) underwent B-mode imaging, Doppler scanning, and blood sampling for testosterone assaying once a month. From 7 months, semen was collected and evaluated. The first semen was collected at a mean age of 7.2 months, while the first spermatozoa appeared at 8.3 months, and the mean age of sexual maturity was 9.4 months. The highest semen volume (1.02±0.09 mL), motility (mass score = 5 and individual = 78.00±1.58%), morphology (94.74±1.99%), alive% (85.21±1.32), and semen concentrations (5.24±0.32x10^9/mL) were noted in buck 4 (9.5 months; 25.0 kg) followed by others. Testicular width (TW) and MT were positively correlated ($r = 0.71$; $P = 0.03$) and increased from 8–10 months ($P < 0.05$). The spectral graph of young bucks revealed an absent end velocity point. Testicular flow expressed by colored area/pixels was positively correlated with age ($r = 0.855$), accompanied by increased testosterone levels. Semen characteristics and Doppler parameters were not significantly correlated, except Doppler indices negatively correlated with progressive motility percentage. In conclusion, only the testicular Doppler indices are useful for evaluating testicular function and selecting bucks for breeding since these were the only variables negatively correlated with progressive motility percentage. The spectral Doppler velocities are not useful in estimating the full picture of semen quality in sexually mature bucks. These values should be assessed cautiously since many alterations could lead to the elevation or decline of testicular Doppler parameters.

KEYWORDS
Buck, Puberty, Colored pixels, Semen, Testosterone.

INTRODUCTION

Developing a farming scheme involves good livestock management, especially from a reproductive aspect (Boukhliq *et al*., 2018). The male genital system must be sound to achieve successful breeding (Alves *et al*., 2006; Singla *et al*., 2017). For this reason, breeding soundness estimation (BSE) is decisive for reproductive success in goat production systems. BSE is based on the clinical examination of male genitalia and behavior, as well as the evaluation of semen quality and production (Tibary *et al*., 2018). The spermiogram is a vital indicator of male breeding capability and helps predict animal fertility (Mekasha *et al*., 2007). Puberty involves elevated gonadotropin-releasing hormone (GnRH) secretion, which increases follicle-stimulating (FSH) and luteinizing hormone (LH) levels (Wu *et al*., 1991; de Souza *et al*., 2011). This is a critical period when bucks can release sperm and reproduce successfully (Delgadillo *et al*., 2007). Since this is the onset of spermatogenesis, and elevated testosterone levels (Júnior *et al*., 2012), which play an essential role in reproductive function and male sexual maturation, accompany puberty (Ángel-García *et al*., 2015). To the best of the authors' knowledge, no studies have discussed testosterone levels with vascular characteristics during the sexual development of Baladi bucks. Testicular growth alterations occur during this period (Bo *et al*., 2020). The key factors affecting puberty and sexual maturity include testosterone levels, separation of the penis from the prepuce (Moulla *et al*., 2018), and sperm production. In sexually mature bucks, reproductive mating capacity and seminal characteristics are associated with maximum reproduction.

Ultrasound imaging technology is widely used to visualize the scrotal contents (Ragheb and Higgins, 2002; El-Sherbiny *et al*., 2022a) and evaluate the mediastinum testis with seminiferous tubules (SNT; Giffin *et al*., 2014; Abdelkhalek *et al*., 2022a). B-mode gray ultrasound has been effective in diagnosing testicular affections in goats (Cavalcante *et al*., 2008) based on tissue echogenicity (Ahmad *et al*., 1991). However, there is inadequate information on the utility of Doppler color and spectral modes in goat andrological and gynecological assessments (El-Sherbiny *et al*., 2022b ; Hashem *et al*., 2022). Combining both Doppler modes could provide more accurate data regarding tissue vascularity (Abdelnaby *et al*., 2021a; El-Sherbiny *et al*., 2022c). Among goat males, adults have a lower resistive index than a pre-pubertal age

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(Paltiel *et al*., 1994). A similar decline appeared in Doppler indices of pre- and post-pubertal age in equines (Rotaa *et al*., 2018). The color mode could be represented by two-color maps that related to the blood flow toward or away from the testes (Pozor and Mcdonnell, 2004), while the spectral mode offers more information about the artery of interest in form of Doppler velocities and indices (Batissaco *et al*., 2014; Abdelnaby *et al*., 2021b). These Doppler modes have been used in dogs (Günzel-Apel *et al*., 2001; Gumbsch *et al*., 2002) to diagnose andrological abnormalities, as well as to assess fertility in camels (Kutzler *et al*., 2011) and donkeys (Abdelnaby *et al*., 2021b). This study aimed to the accompanying variations in testicular size, mediastinal thickness, main testicular artery blood flow, serum testosterone, and semen characteristics in Baladi bucks approach puberty and sexual maturity.

MATERIALS AND METHODS

Ethical approval

This current study was approved by the veterinary institutional committee for animal use protocol (AUP) at the Faculty of Veterinary Medicine- Cairo University at Giza square, Egypt.

Animal housing and feeding

The study included 5 Baladi bucks born between late April and early May 2020, kept on a private farm from birth, and brought to the Theriogenology Department, Faculty of Veterinary Medicine, Cairo University (30.0154° N, 31.2120°). Each buck was fed a concentrated ration in the form of corn and soybean with 19% protein (500 g/day), with free access to mineral salt and water throughout the day. All males were kept indoors and regularly vaccinated. Clinical examination and testicular palpation were performed in all males (Kühn *et al*., 2016).

Experimental design

Between 5–10 months of age, bucks underwent monthly (on day 15 of each month±3.11 days) B-mode and Doppler testicular ultrasound scanning, as well as blood samples for hormone assay. Weight (13–15 kg) and body scores (3.5±0.01; range: 1–5) were recorded at 5 months. At 7 months, semen collection and evaluation were performed weekly until puberty and sexual maturity were observed, then semen collection was continued once weekly until the end of the study. The first semen collection began after complete penis detachment with release and exposure of the penis. The bucks were monitored weekly for penis detachment until complete separation occurred. Puberty was determined by the appearance of motile spermatozoa in the collected sample with complete mating (de Souza *et al*., 2011). Sexual maturity was determined when the semen picture reached the excellence criteria (de Souza *et al*., 2011), specifically, individual progressively motile spermatozoa value ≥50% with 20% total sperm abnormalities (Rowe, 2010; Ridler *et al*., 2012). Semen ejaculates were evaluated.

Ultrasonography

Examination by ultrasound was achieved once/month starting with a B-mode ultrasound scanner (EXAGO, made in France) linked to a 5 MHz linear array transducer then the Doppler icon was activated to measure the testicular vascular perfusion. Each animal was restrained with the two limbs separated. After shaving the scrotal hair, an adequate amount of gel was applied on the scrotum with very gentle pressing on the testicular surface.

Determination of testicular width and mediastinal thickness

With age advancement, it is very difficult to accurately determine testicular length because the whole testicle cannot be visualized on the ultrasound screen. Testicular width (TW) was determined using the transverse section of both testes (separately). MT was visualized on the longitudinal section after freezing the image. The thickness of the white mediastinum line was determined using an electronic caliper (Andrade *et al*., 2014).

Doppler assessment

Males were evaluated once a month starting from the 5th month of age. The main testicular artery, which emerges from the aorta and is located at the pampiniform plexus region, was identified by activation of the color Doppler mode with the presence of two main coloring spots (red and blue). The pulsed wave Doppler is then activated to obtain an entering gate by the specific window in the colored spots; the spot determining the standard spectral graph is considered an ideal Doppler wave. Three successive waves with accurate systolic and diastolic ends were measured. The device's automatic settings were as follows: blue and red colors on the coloring map, 70% brightness, 30 cm/sec maximum velocity, 30 dB gain, 45° angle, and 4000 Hz pulse repetition frequency (Abdelnaby *et al*., 2021 a, b). The same professional operator performed examinations to reduce handling errors and detect the same artery location throughout the study period. The Doppler parameters that appeared on the screen were as follows: resistive index (RI), pulsatility index (PI), peak velocity point (cm/sec), and end velocity point (cm/sec; Abdelnaby and Emam, 2022; El-Sherbiny *et al*., 2022d).

Bodyweight and scrotal circumference measurements

Bodyweight (kg) and scrotum circumference (cm) were measured once monthly before semen collection. Using a cloth measuring tape (Raji and Ajala, 2015; Abdelkhalek *et al*., 2022b), both testes were pushed downwards, and the circumference was measured three times to take the mean reading in centimeters.

Blood sampling and serum estosterone analysis

Before ultrasound examination, between 8:00 and 10:00 A.M. blood samples were collected from the jugular vein in a red serum tube (5 ml). This was allowed to clot for 25 min until after the ultrasound examination; the plasma which collected in a violet tube was centrifuged at 3000 rpm for 10 min then frozen at −18 ºC until further assessment. Species-specific testosterone was determined using an ELISA commercial (Sunlong Biotech Co) kit with a test sensitivity of 0.012 ng/ml and intra- and inter-assay coefficients of ≤10% and ≤12%, respectively.

Semen collection and semen characteristics evaluations

Semen collection

Semen was collected by electroejaculation (EE). The animal was restrained in the lateral position to reduce the risk of stress and injury, and the rectum was prepared before the insertion of a suitably sized and lubricated probe (Hargreaves and Hutson, 1997). This procedure involved probe insertion into the rectum while applying low-voltage short pulses of an electrical current directly to the pelvic nerves, which excite the ampullae, smooth muscles, and vas deferens. The animals showed mild signs related to distress or pain linked with electroejaculation, such as vocalization (Abril-Sánchez *et al*., 2017). All semen collections were done under the same circumstances.

Semen evaluation

After collection, the semen samples were placed in a plastic graduated tube. Then, the semen volume (ml), consistency, mass motility (score), individual progressive motility (IPM; %), alive sperm (%), normal/abnormal sperm morphology (%), and sperm concentration (10^9/ml) were evaluated as described by (Henry and Neves, 1998). Sperm concentration was measured by a normal hemocytometer and CASA as a confirmatory tool (CASA, AndroVision®-System,Fa. Minitube, Tiefenbach) using a camera connected to a microscope equipped with analysis computer software (Hahn *et al*., 2019; Şavkaya *et al*., 2020; Abdelkhalek *et al*., 2022a).

Image further assessment

Only colored images were analyzed by using Adobe Photoshop Cc X 64 software (Adobe Inc. USA) after exportation from the ultrasound device by the flash USB, the analysis was performed by using the lasso tool on the frozen image colored spots that connected with vascularization toward or far away from the probe by two-color (red and blue) respectively.

Statistical analysis

First, the data were assessed for normality using the Shapiro-Wilk test. TW, MT, and colored area/pixels away and toward the robe were measured using the mean value of both testes (right and left). These variables, along with the values of testicular blood parameters, testosterone, and semen characteristics, were analyzed via ANOVA. The Duncan multiple range tests were used to differentiate between significant means ($P < 0.05$). The age (months) of the first semen collection was calculated using the birth date of the Baladi bucks. The correlation coefficients among testicular Doppler parameters and semen images were also measured.in addition to correlation was done between SC and testosterone, SC and semen picture and finally between SC and testicular morphology (TW and MT) were also determined.

RESULTS

Ultrasonography

B-mode ultrasound measurements

B-mode ultrasound of both testes was used to measure the TW and MT. Notably, TW is considered the most accurate measurement of these dimensions with increasing animal age. In line with this, TW showed a marked alteration as depicted in Figure 1, specifically increased at 9 months compared to 5 months. MT also showed a small white line at 5 months (Figure 2a and 2b; pre-pubertal male buck) compared to a thick line at 9 months (Figure 2c and 2d). Both TW (mm) and MT (mm) were significantly affected ($P \leq 0.05$) by the buck's age (months). Both also showed a linear elevation, followed by a marked progressive significant increase between 8–10 months ($P < 0.05$; Figure 3). There were positive correlations between TW and MT ($r = 0.71$; $P = 0.03$) and between SC and MT, but these were not significant ($r = 0.377$).

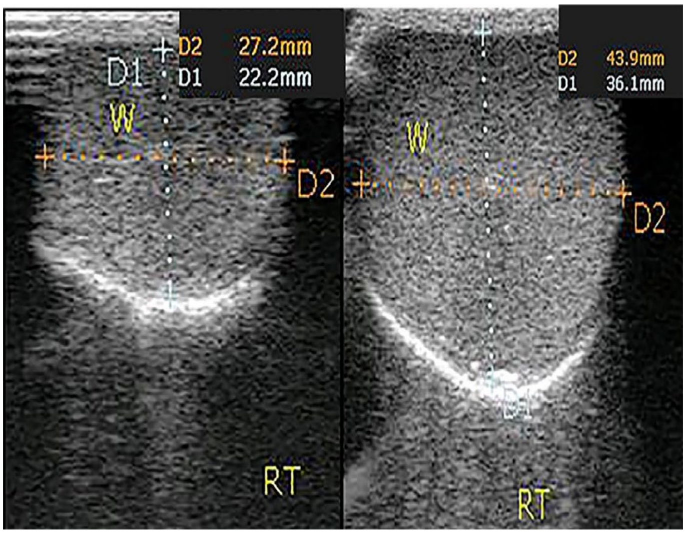

Figure 1. Grey B-mode ultrasonograms showed a right testicular width in a transverse section in Baladi buck at 5 (left) and 9 (right) months.

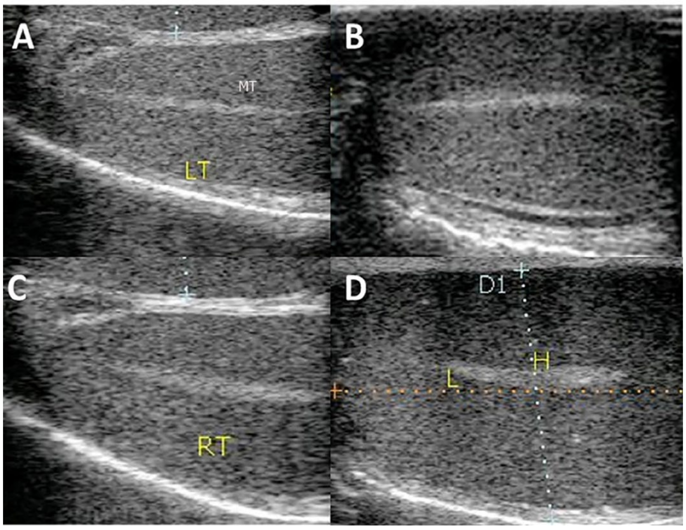

Figure 2. Grey B-mode ultrasonograms showed the echogenic mediastinum thickness in the testicular parenchyma in Baladi buck at 5 (A and B) and 9 (C and D) months. Note the small white line at 5 months (prepubertal male buck).

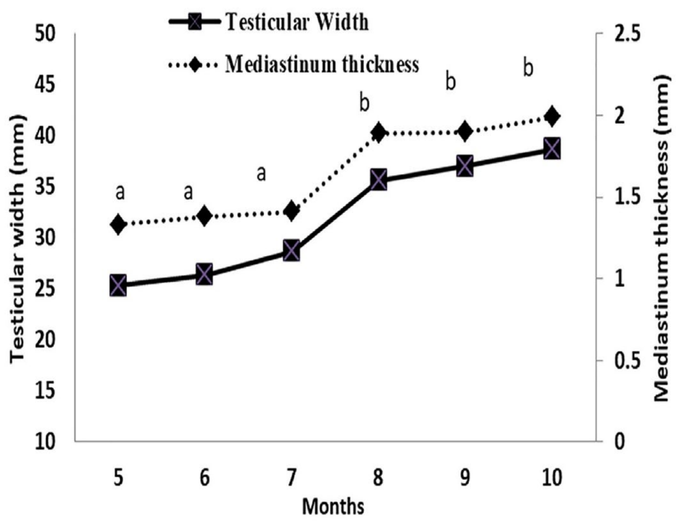

Figure 3. Mean±error of mean (SEM) of the testicular width (mm) and mediastinum thickness (MT; mm) measured in 5 Baladi bucks between 5 and 10 months of age. Different superscripts above show significant differences (at least at P<0.05).

Pulsed wave Doppler ultrasound measurements

Age significantly ($P < 0.05$) affected Doppler parameters, but not in the male Baladi buck. The main testicular artery PI declined linearly in the right (0.81±0.01) and left (0.79±0.01) sides at 10 months (Table 1). RI also declined significantly with age, with vales of 0.42±0.01 and 0.44±0.01 in the right and left side at 10 months, respectively. Buck age also had a significant effect in both Doppler velocity measurements. Both testicular peak velocity (cm/sec) and end velocity points (cm/sec) were significantly elevated (P < 0.05) until 8 months and then both showed a marked progressive significant increase from 8–10 months ((P < 0.05; Table 1). This data was obtained from the spectral waves, with the complete absence of the endpoint of the testicular blood flow in the young bucks (Figure 4).

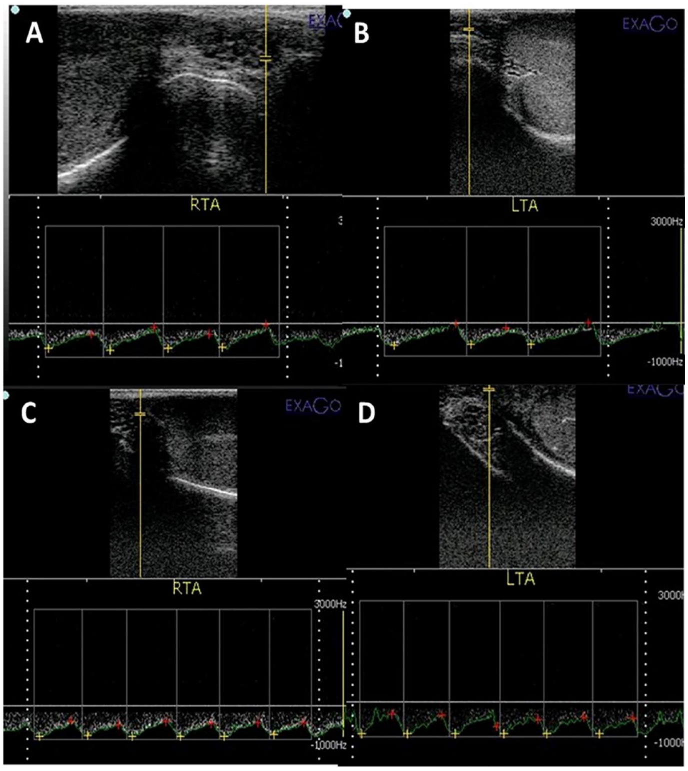

Figure 4. Main testicular artery vascularity assessment in both testes by pulsed Doppler in Baladi buck at 5 (A and B) and 9 (C and D) months. Note the absence of end diastolic velocity Doppler parameter at 5 months (prepubertal male buck).

Color Doppler ultrasound measurement

The amount of colored area either toward or away from the rectal transducer was evaluated via color mode icon activation. The plexus-colored area away from the probe toward the testis was higher in value than the colored pixels toward the probe away from the testis. The plexus-colored areas present both toward and away from the probe were significantly affected by age; both showed a marked abrupt, significant increase between 8–10 months (P < 0.05; Figure 5). Moreover, a lower coloration was observed at 5-month Baladi buck compared with coloration of the same buck at 9 month age (Figure 6). the changes by increase or decrease expressed by presence e of two main color map in the Doppler device (blue and red). There was a statistically positive correlation between total colored area with age advancement (r=0.855; P<0.01), but there was no coloration between coloration and semen picture.

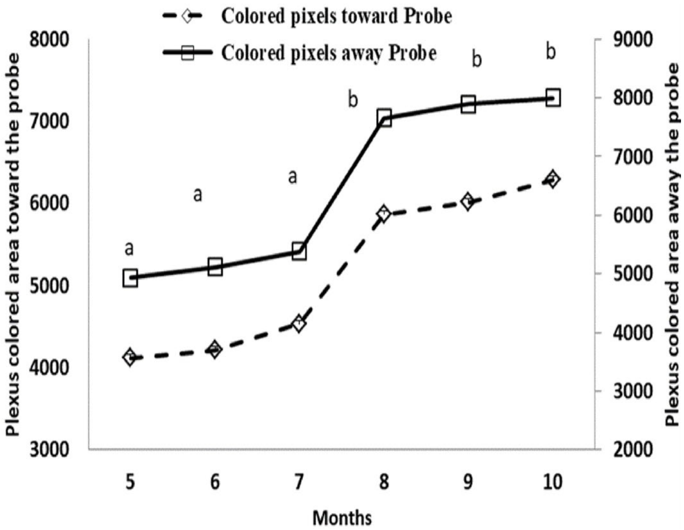

Figure 5. Mean±error of mean (SEM) of the plexus-colored area confirmed by the two colors either away or toward the probe measured by colored Doppler mode ultrasound (EXAGO) in 5 Baladi bucks between 5 and 10 months of age. Different superscripts above show significant differences of (at least at P<0.05).

Hormonal assessment

Serum testosterone level demonstrated a transitory but non-significant increase from 5–7 months (2.44±0.01 ng/ml to 2.74±0.08 ng/ml), and a significant elevation affected by age between 8–10 months (4.24±0.02 ng/ml to 4.93±0.65 ng/ml) (Table 1). There was a statistically positive correlation between scrotal circumference and serum testosterone levels (r = 0.986; P = 0.04), but no correlation was found between any of the Doppler parameters and testosterone level.

Semen evaluation

The first semen from the bucks was collected at a mean age

Table 1. Right and left main testicular artery vascularization in baladi bucks at the period between 5-10 months.

Age/Parameter	5 (October)	6 (November)	7 (December)	8 (January)	9 (February)	10 (March)
R. PI	1.51±0.02^c	1.12±0.02^b	1.01±0.02^b	0.85±0.01^a	0.84±0.01^a	0.81±0.01^a
R. RI	0.93±0.01^b	0.84±0.01^b	0.81±0.02^b	0.56±0.01^a	0.49±0.01^a	0.42±0.01^a
R. PV	13.95±1.02^a	15.06±0.85^b	15.03±1.25^b	17.64±1.81^c	18.87±0.55^c	19.01±0.01^c
R. EV	0.0±0.01^a	0.0±0.01^a	0.5±0.01^a	1.30±0.01^b	2.01±0.88^c	2.51±0.01^c
L. PI	1.49±0.01^b	1.13±0.01^b	1.11±0.01^b	0.89±0.01^a	0.81±0.02^a	0.79±0.01^a
L. RI	0.94±0.01^b	0.88±0.02^b	0.81±0.02^b	0.57±0.01^a	0.45±0.12^a	0.44±0.02^a
L.PV	13.85±2.58	15.11±1.39	15.01±1.69	17.66±0.95	18.66±2.33	19.32±2.01
L.EV	0.0±0.01^a	0.0±0.01^a	0.5±0.07^a	1.32±0.82^b	1.98±0.11^c	2.25±0.62^c
T (ng/mL)	2.44±0.01^a	2.63±0.55^a	2.74±0.08^a	4.84±0.02^b	4.81±0.25^b	4.93±0.65^b

Data are attained as mean±standard error of mean (SEM; n=5).
Means with different superscripts within row are significantly different (at least at p<0.05)
R=right side, L=left side, PI=pulsatility index, RI=resistance index, PV=peak velocity, EV=end velocity, and T=testosterone

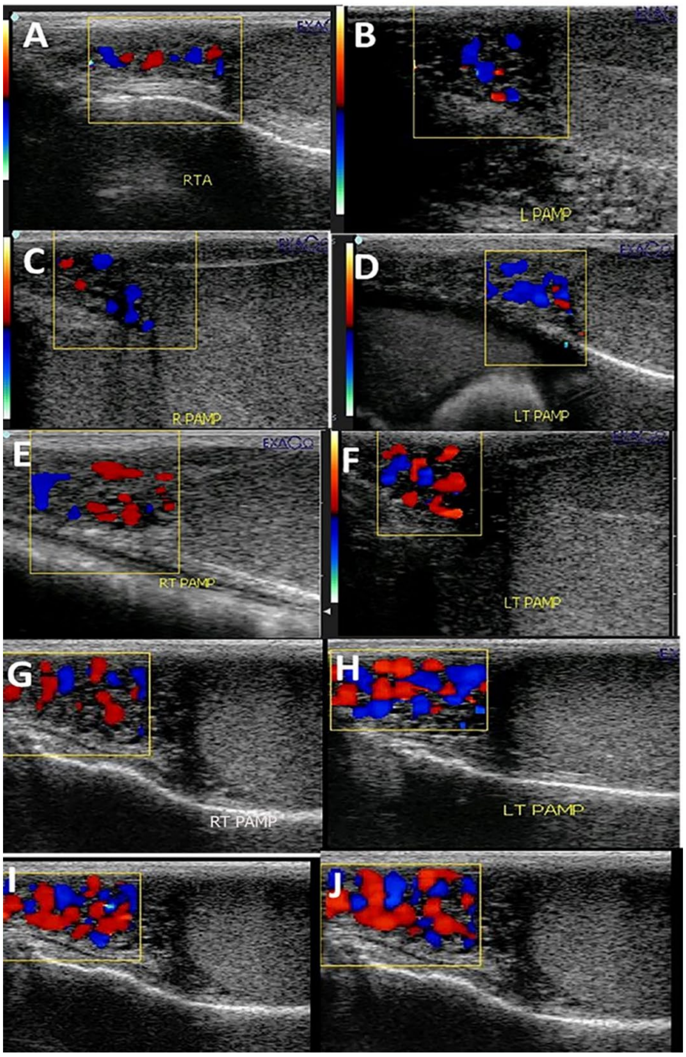

Figure 6. Color mode Doppler ultrasonograms revealed the changes in colored area away and toward the probe that expressed by two colored (blue and red) in Baladi bucks at 5 (A and B) ,6(C and D) ,7 (E and F),8 (G and H) and 9 (I and J) months. Note the lower coloration at 5 months (prepubertal male buck) compared with coloration at 9 months.

of 7.2 months, while the first spermatozoa appeared (puberty) at 8.3 months. Puberty was defined as the time when progressive motile sperm with at least 10% motility is seen in the ejaculate. Among the studied subjects, the mean age of sexual maturity was 9.4 months, and this age is associated with ≥50% progressive, motile sperm (Table 2). Upon reaching sexual maturity, their semen characteristics were calculated for age, body weight, and scrotal circumference. Among the 5 Baladi bucks, the highest age and body weight were noted in buck 5 (10 months, 25.5 kg) with a thin creamy semen consistency, followed by bucks 3 and 4 (9.5 months, 23.3–25 kg) and bucks 1 and 2 (9 months, 21.2–21.5 kg) with thin milky semen consistency (Table 3). The highest scrotal circumference was seen in buck 4, followed by bucks 5 (22.5 cm), 3, 2, then 1. The best semen volume was seen in buck 4 (0.61±0.09 ml), followed by bucks 3 (0.58±0.03), 1 (0.46±0.07), 2 (0.45±0.11), and 5 (0.43±0.05). The highest motility (mass and individual), morphology percentage, and semen concentrations were noted in buck 4, who also had the highest live percentage (85.21±1.32) compared to the others. Scrotal circumference had a significant positive correlation with semen characteristics (r = 0.657, P = 0.01). Semen characteristics were not significantly correlated with Doppler parameters, except for the Doppler indices (RI and PI), which were negatively correlated with progressive motility percentage (RI: r = −0.544, P = 0.01; PI: r = −0.877, P = 0.01). Lastly, there was a non-significant correlation between Doppler indices and abnormal sperm morphology percentage, but there was a negative correlation between progressive motility and abnormal sperm percentage (r = −0.840, P = 0.01).

DISCUSSION

In this study, both testicular width (TW) and mediastinum line thickness (MT) elevated with age advancement with a positive significant connection between both parameters (r=0.71), similar correlation coefficient value found in Sokoto bucks (r = 0.79), but in Afar bucks the correlation coefficient was r= 0.65 (Raji *et*

Table 2. First semen collection, first appearance of spermatozoa and after puberty at which individual progressive motile (IPM) sperm ≥50% in Baladi bucks (n=5) ages by month and date.

Buck	First semen collection (Month/Date)	First appearance of spermatozoa (Month/Date)	Sexual maturity (IPM sperm ≥50%) (Month/Date)
1	7(3rd December)	8(5th January)	9(3rd February)
2	7.5(15th December)	8(1st January)	9(1st February)
3	7.5(15th December)	8.5(16th January)	9.5(16th February)
4	7(3rd December)	8.5(18th January)	9.5(12th February)
5	7(5th December)	8.5(18th January)	10(1st March)
Mean	7.2	8.3	9.4

Table 3. Semen picture of mature baladi bucks (n=5) in relation to age (month), bodyweight (BW; Kg) and scrotal circumference (SC; cm).

Buck	Age	BW	SC (cm)	Consistency*	V (mL)	MM (score)	Morphology (%)			Sperm concentration (10^9/mL)
							IPM (%)	Live percentage	Normal sperm	
1	9	21.5	19.2	2	0.77±0.07	3.00±0.11	69.50±0.75	81.25±3.32	90.22±2.63	3.51±0.35
2	9	21.2	19.5	2	0.71±0.11	3.00±0.19	75.00±1.42	84.41±2.15	90.15±4.28	2.86±0.62
3	9.5	23.3	20	2	0.83±0.03	3.00±0.33	66.50±0.99	77.15±3.81	92.56±3.15	2.54±0.12
4	9.5	25	22.5	4	1.02±0.09	4.00±0.12	78.00±1.58	85.21±1.32	94.74±1.99	5.24±0.32
5	10	25.5	21.11	3	0.69±0.05	3.00±0.22	69.00±1.23	78.26±1.33	89.33±5.81	3.81±0.44
P –value					NS	NS	NS	NS	NS	NS

Data are expressed as mean±SEM.
* 1=watery, 2=milky, 3=thin creamy, and 4=thick creamy
BW=bodyweight, SC= scrotal circumference, MM=mass motility, IPM=individual progressive motility, V=volume

al., 2008). The positive connection between scrotal circumference (SC) and testicular dimensions recommend that all those parameters could provide an estimation in prediction of testicular functionality (Abba and Igbokwe, 2015; Abdelnaby *et al*., 2021b), similar study reported that SC with testicular length, width and weight can be used for selection of males for breeding purposes (Keith *et al*., 2009).

In the current study, the MT was visualized in all 5 bucks (100%). Similar studies have visualized the MT in 87% (Gouletsou *et al*., 2003) and 100% of test animals (Andrade *et al*., 2014), while another study in cattle and sheep found that the MT echogenicity increased with age (Moura *et al*., 2008). In line with this, the present study found a positive connection between testicular dimensions and testicular mediastinum white line thickness (Andrade *et al*., 2014). Determining testicular morphometry can be made easier via testicular vascularity detection (Abdelnaby *et al*., 2021b). Spectral wave Doppler imaging of the main testicular artery has been reported in many species (Abdelnaby *et al*., 2021b) because this artery is critical in thermoregulation (Brito *et al*., 2002). However, the Doppler velocities can have different measurements in the same animal (Gloria *et al*., 2018). Therefore, the most accurate measurements would be the PI and especially RI (Pinggera *et al*., 2008). In a previous study, Trautwein *et al*., 2019) recorded different values at different levels of the testicular artery at the supra location at the caput of the epididymis. To the best of the authors' knowledge, this is the first study that reported no correlation between Doppler parameters and semen parameters in Baladi bucks at puberty and sexual maturity.

Currently, there are no available testicular Doppler data in pre and postpubertal bucks. There are no reports on the changes occurring around this period either. There was lower plexus region coloration in the present study at 5 months (pre-pubertal) than at 9 months. Plexus coloration was positively correlated with age ($r = 0.855$, $p = 0.01$) but was not correlated with semen images. In contrast, a study reported a connection between the plexus-colored area/pixels and fertility; animals with high plexus coloration had higher value mass motility and sperm cell concentrations (Ribeiro *et al*., 2020). Testicular vascularity is a critical issue because the main testicular artery has a unique responsibility in thermoregulation and sperm production (Bergh and Damber, 1993). The strong correlation with age could be clarified since the testicular artery examined is the supra main branch close to the caput epididymis region. This artery originates from the aorta (Dogra *et al*., 2006) then extends to the plexus region to reach the testis. As animals age, the increases in testicular weight and size cause elevated blood flow to the testis to facilitate thermoregulation.

In the present study, the alteration in end velocity notch was measured with a neglected diastolic endpoint (end velocity = 0). There was a significant decrease in the main testicular RI at 5 months compared to 8–10 months (range: 1.30–2.55). Similarly, another study found that post-pubertal children had declined RI compared to pre-pubertal children, with the complete absence of an end-diastolic velocity pattern (Paltiel *et al*., 1994). Another study in pre-pubertal donkeys measured no diastolic endpoint as well (Rotaa *et al*., 2018). Therefore, the appearance of an end velocity point in bucks could indicate puberty and sexual maturity.

Another study also found no correlation between Doppler velocities and semen analysis parameters (Semiz *et al*., 2014) since the values of the Doppler parameters were not associated with the collected semen after sexual maturity. Only peak velocity could give more definite data for predicting abnormalities such as varicocele. Similarly, many studies reported a negative correlation between RI and progressively motile sperm (Pozor *et al*., 2014; de Souza *et al*., 2015; Gloria *et al*., 2018).

Many studies have focused on the relationship between both Doppler measurements and semen analysis parameters in the ejaculate (Zelli *et al*., 2013; Gloria *et al*., 2018). The difference between testicular hemodynamics and semen characteristics is critical since the semen analysis can be deceptive at the end of spermatogenesis (Omari *et al*., 2018). For this reason, many previous studies found no relationship between both parameters, especially during an assessment at the same days (Arteaga *et al*., 2005), but the examination became more accurate and significant when the pulsed wave Doppler ultrasound was performed later (Arteaga *et al*., 2005).

The present study reported a non-significant correlation between both Doppler indices and abnormal sperm morphology percentage. Similarly, another study reported that in terms of main testicular arterial function, there was a significant correlation between the Doppler indices and abnormal sperm morphology (Gloria *et al*., 2020) in dogs with abnormal spermatogenesis; however, there was no correlation in normal dogs. Another study found a positive correlation between RI and sperm abnormalities (Pozor *et al*., 2014). A negative relationship between both Doppler indices and total progressive motility has also been reported (Zelli *et al*., 2013), but England *et al*., (2017) found no relationship between the two indices and the total output of sperm. In rams, there was no correlation between Doppler parameters and sperm defect percentage (Batissaco *et al*., 2014),as the reduction in both Doppler indices referred to the elevation of the tissue vascular velocity and peak velocity(Abdelnaby, 2020;Abdelnaby *et al*., 2020; Abdelnaby *et al*., 2022).

Although complete exposure of the penis was noted in all 5 bucks, the full assessment of sexual behavior requires bucks to reach puberty and sexual maturity. Bucks have been reported to reach maturity at week 38 (de Souza *et al*., 2011), with an average scrotal circumference of 21.1±1.64 cm, which was in line with our findings. Additionally, our study reported no correlation between body weight and SC with increasing age, but on the other hand (Keith *et al*., 2009) found a positive connection between the two parameters.

In this study, the bucks reached puberty and sexual maturity at a mean age of 8.3 months and 9.4 months, respectively. The fresh semen characteristics of the Baladi bucks in this study were similar to those previously obtained in bucks. Buck 4 had the best semen quality, where there was a significant positive correlation with scrotal circumference. The absence of significant variations between repeated semen collection could be due to the semen characteristics, which were approximately the same during the examination period (Arrebola-Molina *et al*., 2020); similar findings were seen in a study of bulls (Gloria *et al*., 2018). Since our results considered only healthy Baladi bucks, we did not consider testicular affections such as orchitis and degeneration, which cause declines in semen quality (Ahmad and Niaz, 1998). Assessing the semen picture is an ideal tool for providing information about spermatogenesis (Leal *et al*., 2004). The positive correlation obtained in this study was consistent with another study in bulls which found a markedly positive correlation between SC and semen parameters ($r = 0.72$; $p < 0.05$) (Latif *et al*., 2009). Similarly (Islam *et al*., 2008) found that, in sexually mature blackbucks, the SC ranged from 14–16 cm, and semen volume and concentration ranged from 0.43–0.61 mL and 2.55–2.91x10^9 /mL, respectively, with very small variations among bucks. Likewise, the SC did not widely vary among the 5 bucks, as general semen production depends on many other factors like nutritional factors and endocrine hormonal balance (Goyal *et al*., 2007; Abu *et al*., 2016).

This study revealed a statistically significant negative correlation between progressive motile sperm and abnormal sperm percentages ($r = -0.840$; $p = 0.01$). Similarly, (Fernandes *et al*., 2009) found that a higher percentage of abnormalities in the sperm tail could result in a lower total and progressive motility percentage. When an animal reaches sexual maturity, the epididymal tissue phagocytoses any defects. Thus, higher progressive motility (≥70%) must accompany the onset of sexual maturity (Horn *et al*., 2002; de Souza *et al*., 2011).

The age of maturity in this study can be assumed as an image of the enhanced activity of Leydig cells, which is influenced by the hypothalamic-pituitary-testicular axis (de Souza *et al*., 2011). Moreover, the full development of the seminiferous tubules in bucks at around 26–28 weeks was associated with increased serum testosterones from puberty toward sexual maturity (Nishimura *et al*., 2000; Moura *et al*., 2002). The testosterone levels in the current study significantly elevated with age advancement (from 3.84±0.02 ng/ml to 3.93±0.65 ng/ml) between 8–10 months. Age was also correlated with body weight and scrotal circumference. Furthermore, a significant positive correlation was found between SC and serum testosterone levels, emphasizing the hormonal influence on testicular development after puberty. Studies reported an elevation of testosterone during full testicular development; this increase could indicate sexual maturity (Moura *et al*., 2002). A similar positive correlation between testosterone and SC was found in goats (Rachmawati and Ismaya, 2014) and bulls (Fadhli, 2016). Thus, this connection between SC with testosterone levels could be used as an indicator of puberty and sexual maturity (Brito *et al*., 2004; Reddy *et al*., 2014).

During puberty and outside of the breeding season, very low semen quality was observed in bucks (Ángel-García *et al*., 2015; Arangasamy *et al*., 2018), but increasing levels of testosterone was connected with very good semen quality in sexually mature bucks.

CONCLUSION

This study is the first to correlate main testicular blood flow with the semen picture in sexually mature Baladi bucks from 5–10 months old. Therefore, only the testicular Doppler indices (RI and PI) can be used to evaluate the testicular function and the selection of bucks for breeding since these negatively correlate with progressive motility percentage. Therefore, the spectral Doppler velocities are not useful in estimating the semen quality full picture in sexual mature bucks, as those Doppler velocities values should be assessed cautiously as many alterations could lead to elevation or declination of testicular Doppler parameters.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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