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Comparison Between Mediastinum Thickness, Hormonal Levels, Nitric Oxide, and Testicular Hemodynamics in Baladi Bucks at Prepubertal and Postpubertal Stages

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Abstract

This study aimed to compare the testicular morphometry, mediastinum thickness, hormonal levels, hemodynamic, echogenicity, and heterogeneity in Baladi bucks at prepubertal and postpubertal stages. Five bucks (*Capra hircus*) were evaluated in two different stages of growth: prepubertal (age 4.5±0.6 months; 15.0±3.0 kg) and post-pubertal stages (age 13.0±1.3 months; 33.0±2.5 kg). Scrotal circumference, testicular dimensions, mediastinum thickness, echogenicity, heterogeneity, Doppler parameters, semen collection, testosterone, estradiol, follicle-stimulating hormone (FSH), luteinizing hormones (LH), and nitric oxide metabolites (NOMs) were measured. There was an ($P<0.05$) elevation of the testicular length, width, and scrotal circumference. Mediastinum thickness and colored areas toward and away from probe were increased ($P<0.05$) in post-pubertal age (2.18±0.01, 6556±32.58, and 7845±65.44) compared to pre-pubertal one (1.27±0.96, 4290±42.12, and 5144±54.24). The spectral graph was characterized by low resistance (RI), moderate pulsatility (PI) with high peak velocity, and low endpoints in the post-pubertal stage while the endpoint was equal to zero in young bucks. The post-pubertal age was associated with a marked decline ($P<0.05$) in echogenicity, heterogeneity, and RI, while estradiol, testosterone, and NOMs levels were increased ($P<0.05$). It could be concluded that the post-pubertal stage in Baladi bucks is associated with changes in testicular width, length, mediastinum thickness, RI, scrotal circumference, echogenicity, pixel heterogeneity, testicular colored area away and toward the probe, end-diastolic point, testosterone, nitric oxide, and estradiol levels, as all those variables are considered an accurate markers for the onset of sexual maturity in Baladi bucks.

KEYWORDS
Bucks, Semen; Puberty, Nitric oxide, Hormones, Doppler, Pixels

INTRODUCTION

Ultrasound practices are progressively vital in animal reproductive breeding strategies, offering both a means of diagnosis and a useful predictor of animal fertility (Pozor, 2005; Souza *et al*., 2014; Brito *et al*., 2015; Moxon *et al*., 2015; Abdelnaby *et al*., 2021a; 2021b). B-mode ultrasonographic tool allows the assessment of testicular dimensions, mediastinum thickness, and tissue echo-texture (Ahmadi *et al*., 2012), this echotexture is calculated by square elements called pixels (Giffin *et al*., 2014) with the aid of computer analysis (Ginther, 2007) in the form of numerical pixel values (NPVs) and standard deviation of numerical pixel values (sdNPVs) for calculation of testicular echogenicity (TE) and pixel heterogeneity (PH), respectively (Giffin *et al*., 2009). Those parameters could be used in the determination of male testicular maturation (Saaed and Zaid, 2018).

The achievement of puberty and sexual maturity is accompanied by an elevation in GnRH secretion that elevates both follicle-stimulating (FSH) and luteinizing hormone (LH) (de Souza *et al*., 2011), which is attended by an increase in testosterone levels from Leydig cells of the testes with the onset of spermatogenesis (Júnior *et al*., 2012), this hormone, plays an essential role in reproductive function and male sexual maturation (Ángel-García *et al*., 2015). Testosterone was correlated with testicular dimensions, scrotal circumference, and live sperm count (Kumbhar *et al*., 2019). in addition, testosterone and estradiol have a vasodilatation action on the testicular artery vascularization in humans (Tostes *et al*., 2003), adult male dogs (Zelli *et al*., 2013), and bucks (Samir *et al*., 2018), that associated with the marked decline in both Doppler indices as estradiol 17-ß (steroid hormone) causes a marked decline in the vascular resistance in the circulation. In addition, nitric oxide metabolites (NOMs) have been described to contribute to the vasodilation mechanism through the relaxation of cavernous tissue that helps in the erection of the penis (Gur *et al*., 2015). Pre and post-pubertal testicular vascularization have been poorly performed in Baladi bucks, as there was only one study present in rams (Camela *et al*., 2019). Testicular Doppler could give much information about organ functionality (Pozor and McDonnell, 2004). It was suggested that testicular Doppler parameters were important predictors of semen picture in dogs (Carillo *et al*., 2012). Little information is available for using this technique for testing the male reproductive performance of Bala-

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di bucks. Therefore, the main aim of this study was to compare for the first time (to the best of the authors' knowledge) testicular morphometry, mediastinum thickness, hormonal levels, testicular hemodynamic wave pattern, echogenicity, and heterogeneity in Baladi bucks at prepubertal and post pubertal ages to see if those parameters could be useful in sexual maturity assessment. Additionally, we measured Pearson correlations between testicular morphometry, hemodynamic, and semen pictures in Baladi buck.

MATERIALS AND METHODS

Ethical approval

The current study procedures had been accepted by the Institutional Veterinary Committee of animal use and care with approval sheet VET- CU- 24112020246.

Animals and management

The current study was conducted in Egypt at the Theriogenology Department at Giza square (30.0154° N, 31.2120°) with an average temperature of 30°. Baladi bucks (*Capra hircus*) were used in this study in two different stages of growth as follows: Prepubertal age (n. = 5; mean age of 4.5±0.6 months and weighing 15.0±3.0 kg); and Postpubertal mature age (n. = 5; mean age of 13.0±1.3 months and weighing 33.0±2.5 kg).

The age of puberty was achieved by the first production of fertile sperm through the appearance of motile spermatozoa in the collected sample with complete mating (de Souza *et al*., 2011) in subtropical climate (Alves *et al*., 2006) with an average of 8 months, while the sexual maturity was resolute when the semen presentation reached the excellence standards (de Souza *et al*., 2011) with full breeding capacity with an average 11-12 months. All bucks felt regular (once /week) clinical evaluation, visual check of the scrotum, and palpation of both testes to confirm their regularity, flexibility, and consistency (Kühn *et al*., 2016). All males were kept indoors with one-hour outdoor group pens and regularly vaccinated with free access to mineral salt and water all over the day. Males were fed a concentrated ration in the form of corn husked and soybean meal with 19% protein as each buck received 500 grams per day per animal.

Ultrasonography

Ultrasonographic evaluations were performed in the laying position. Each male was controlled with the two limbs separated. The scrotal hair was shaved then the gel was applied on the linear transducer without any pressure on the testicular surface. All examinations were conducted by only a single operator all over the period equipped with a B- and Doppler modes ultrasound scanner (EXAGO, made in France) prepared with a 5-7.5 MHz linear array probe (Maher *et al*.,2020a, 2020b) with the setting as follows: Doppler filter at 100 Hz, the angle of insonation was 45±5°, PRF is 4000KHz, and maximum velocity was 30 cm/sec (Farghali *et al*., 2022).Scrotal circumference was evaluated before ultrasound assessment and testicular dimensions (length, width, and depth) were measured in cm by electronic calipers in the ultrasound device as well as the testicular volume was calculated using the following ellipsoid equation = 4/3π abc; where length(a) × width(b) × height(c) (camela *et al*., 2019). In addition, mediastinum thickness was measured by caliper in mm that completely visualized as a white hyperechoic line in the testicular longitudinal view, finally, both echogenicity (TE) and heterogeneity (PH) were evaluated in a frozen B-mode image using computer further analysis software to measure mean numerical pixel value (NPVs) for TE and standard deviation of numerical pixel value (sd-NPVs) for PH (Giffin *et al*., 2009). The calculation was done (Brito *et al*., 2012) by gaining two 1 cm^2 spots selected approximately 1 cm above the mediastinum (Fig. 1).

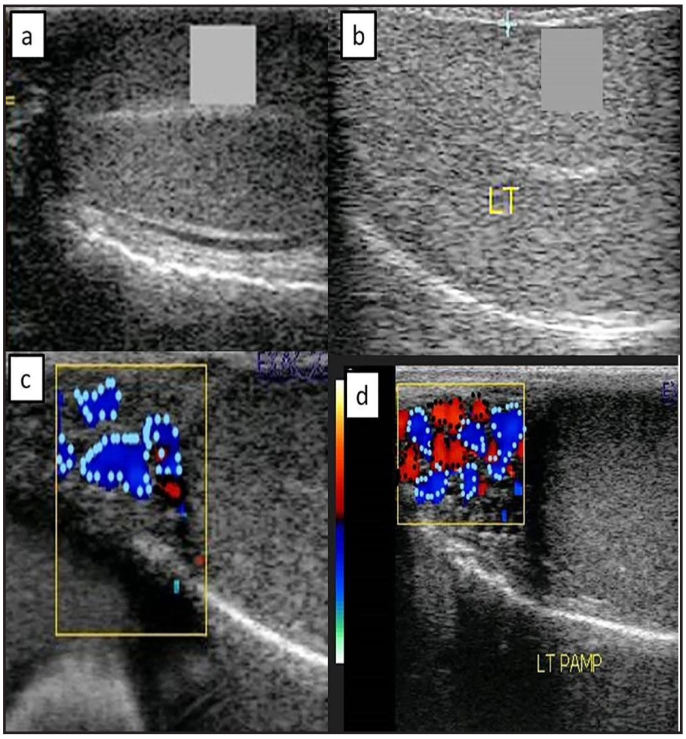

Fig. 1. Ultrasonograms of the buck testes in pre and post-pubertal ages, B-mode (a, b) showed a drawing two 1 cm^2 square designated about 1 cm above the mediastinum for computer-assisted analysis of testicular echogenicity and pixel heterogeneity by adobe Photoshop x64 software program as both parameters were reduced in post-pubertal age (b) as compared to prepubertal age (a), while in color Doppler mode (c, d)the colored area moves away (blue) or toward the probe (red) were calculated in order to measure the colored area/pixels by magnetic lasso tool, as both colored area /pixels were elevated in post-pubertal age (d) as compared with prepubertal age (c).

Testicular colored area

The pampiniform vascularization was assessed by measuring the number of colored area/pixels either away (blue) or toward (red) the probe, the frozen colored Doppler image was utilized in Adobe Photoshop cc x 64 software for determining the number of colored areas/pixels (Ribeiro *et al*., 2020).

Spectral graph evaluation

When the pulsed-wave Doppler mode icon is activated, the device setting is performed to open the gate with 1mm thickness in order to enter the testicular artery to assess its vascular perfusion (Ribeiro *et al*., 2020). The parameters were automatically calculated just after obtaining three successive spectral graphs in form of a cardiac cycle. The Doppler parameters that were measured were: resistive index (RI), pulsatility index (PI), peak velocity point (PV; cm/sec), and end velocity point (EV; cm/sec). All examination was done by the same operator to minimize handling errors.

Blood sampling and serum hormonal analysis

In the early morning on the same day of semen collection, blood samples were obtained from the jugular vein in a serum and plasma tube with 5ml then all samples were centrifuged at

3000 rpm for 10 min and then frozen at -18 ºC. Species-specific testosterone, estradiol, follicle-stimulating hormone (FSH), and luteinizing hormones (LH) were determined in plasma tubes by ELISA commercial (Sunlong Biotech Co) kit with test sensitivity was (0.012 ng/ml, 0.6 pg/ml, 0.1 ng/ml, and 0.037 ng/ml) with an intra and inter assays coefficients were ≤ 10 and ≤ 12 % for all hormones. For measuring those metabolites (NOMs) 100 µL of serum samples were mixed with a Griess reagent (equal volume) and incubated for 15 minutes at room temperature as previously measured (Abdelnaby *et al*., 2021a).

Semen collection and assessment

Semen samples were collected from Baladi bucks by the electroejaculation (EE) method with the application of short pulses of low voltage electrical current based on weekly basis. All males presented mild signs of pain from EE, such as vocalization (Abril-Sánchez *et al*., 2017). The semen sample volume (mL), sperm concentration (10^9/mL), consistency (1 to 4 watery, milky, thin creamy, and thick creamy in appearance were measured. Mass motility (MM; score), individual progressive motility (IPM, %), morphology (%), and live percentage were evaluated, the semen was placed in a graduated tube with plastic material, then the semen volume(mL), and aspect were calculated. Mass motility, individual progressive motility (IPM; %), and sperm concentration (10^9/mL) Sperm concentration was measured by a normal hemocytometer and then by CASA (CASA, AndroVision®-System,Fa. Minitube, Tiefenbach) using a camera connected to a microscope equipped with analysis computer software (Şavkaya *et al*., 2020).

Statistical analysis

Data were examined using SPSS version 20. All data were firstly tested for normality and homogeneity then all values were calculated using Student t-test between two stages of development and the Pearson type of correlation coefficient was set among testicular morphometry, hemodynamic, semen characteristics, and hormonal levels. Statistical significance was fixed at the 95% level (P < 0.05). All data are expressed as Mean±standard error of the mean (SEM).

RESULTS

Among bucks' variable data, no differences were observed between both sides of testes as well as both testicular arteries in the same observation site (P>0.05), so all data results were pooled and then analyzed on an animal basic. As depicted in (Table 1), the testicular dimensions became noticeable that puberty and sexual maturity in the bucks of this study was correlated and linked mainly with a significant (P<0.05) elevation of the testicular length, width and volume as well as the circumference of the scrotum, but the testicular depth was not significantly affected (P>0.05).

The thickness of the mediastinum line was increased in older

Table 1. Testicular dimensions, computer assisted analysis, hormonal levels, and testicular vascular perfusion parameters in Baladi bucks in pre and post-pubertal stages.

Item	Prepubertal stage (n=5)	Post pubertal stage (n=5)	P –value
Testicular dimensions			
Length(cm)	$3.05±0.14^a$	$4.82±0.24^b$	0.03
Width (cm)	$2.8±0.21^a$	$4.3±0.88^b$	0.03
Depth (cm)	3.5±0.04	3.3±0.05	0.17
Volume (cm^3)	$85.27±19.84^a$	$125±18.22^b$	0.03
MT (mm)	$0.18±0.96^a$	$1.08±0.01^b$	0.03
SC (cm)	$17.5±1.58^a$	$28.5±2.05^b$	0.02
Testicular computer analysis			
TE (NPVs)	$96.22±0.74^b$	$64.69±3.21^a$	0.01
PH (SdNPVs)	$28.36±0.68^b$	$22.17±0.74^a$	0.03
Colored area toward probe/pixels	$4290±42.12^a$	$6556±32.58^b$	0.04
Colored area away from probe/pixels	$5144±54.24^a$	$7845±65.44^b$	0.03
Hormonal levels			
Specific T(ng/mL)	$2.62±0.97^a$	$4.96±0.85^b$	0.03
Specific E2 (pg/mL)	$15.21±1.58^a$	$39.21±6.21^b$	0.01
Specific FSH (ng/mL)	29.66±5.12	26.85±2.33	0.13
Specific LH (ng/mL)	6.05±0.95	$4.97±0.33^b$	0.09
NOMs (µmol/L)	$36.66±3.62^a$	$75.67±6.25^b$	0.01
Testicular hemodynamic			
T.PSV (cm/sec)	15.98±1.02	17.08±0.95	0.16
T.EDV (cm/sec)	$0.16±0.01^a$	$2.11±0.09^b$	0.01
T. PI	1.12±0.02	0.94±0.02	0.09
T. RI	$0.86±0.02^a$	$0.54±0.01^b$	0.03

Data are expressed as Mean±SEM, Mean with different superscript are significantly different at P at least at 0.05. MT=mediastinum line thickness, SC=scrotal circumference, TE= testicular echogenicity, NPVs=numerical pixel value, PH=pixel heterogeneity, SdNPVs=standard deviation of numerical pixel value, T=testosterone, E2=Estradiol, FSH=follicle stimulating hormone, LH=luteinizing hormone, NOMs=nitric oxide metabolites. T.PSV= peak systolic velocity of testicular artery, T.EDV= end diastolic velocity of testicular artery, T. PI= pulsatility index of testicular artery, and T.RI= resistance index of testicular artery.

bucks (2.18±0.01) compared to young ones (1.27±0.96; Table1; Fig. 2), the line was visualized with 100% as a small white line with more echogenicity than testicular parenchyma in prepubertal bucks, but the line was increased in thickness become a large white line in post-pubertal age. As shown in (Fig. 3), both colored area/pixels toward probe (red color) and away from probe (blue color) were increased significantly (P<0.05) in post-pubertal age (6556±32.58 and 7845±65.44) when compared to pre-pubertal age (4290±42.12 and 5144±54.24).

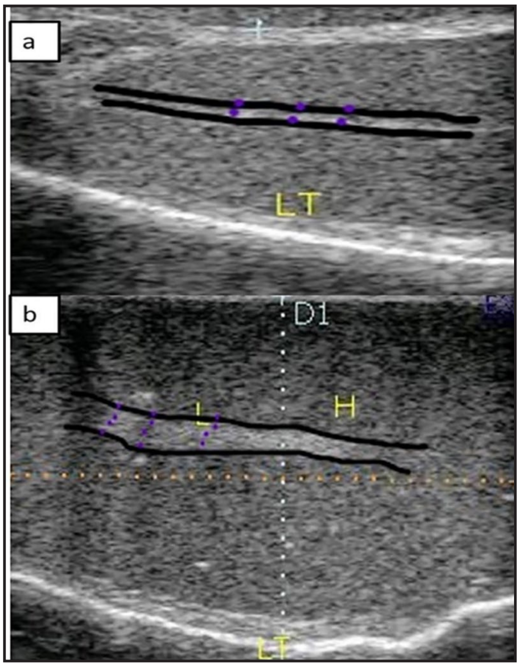

Fig. 2. B-mode ultrasonograms showed the mediastinum line thickness (MT) in bucks in prepubertal (5 months, a) and post-pubertal ages (12 months, b), by longitudinal view of the testes in order to obtain 100% visualization of the line.

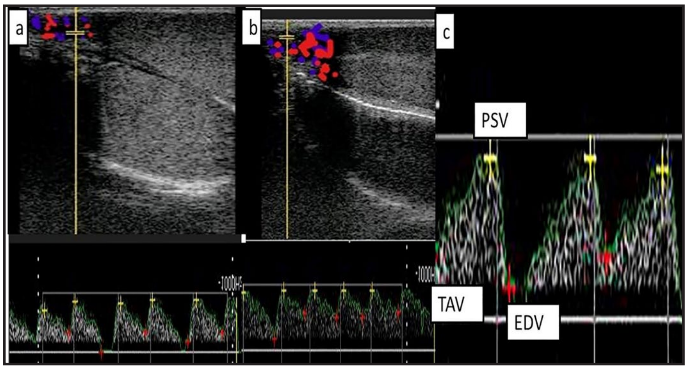

Fig. 3. Spectral Doppler mode showed a spectral line with opened gate (1mm in size) with a caliper placed in the testicular artery centrally in order to determine the Doppler parameters, note in immature young bucks the end-diastolic points equals zero with red dots (a) , while as the bucks reach sexual maturation the wave characterized by low resistance index with high end-diastolic points with red dots (b), the spectral graph showed the peak systolic point(PSV),the endpoint(EDV), and the average time taken to complete the cardiac cycle (TAV) in three successive waves were calculated in order to obtain the mean value (c).

The levels of testosterone were elevated (P<0.05; Table 1) in post-pubertal bucks in addition to testosterone was correlated positively with testicular dimensions (r=0.946; P<0.05) and blood flow colored area away probe (r=0.766; P<0.05), but no significant correlation was obtained with testicular echogenicity and pixel heterogeneity stages of testicular development in the growing male bucks.

Estradiol levels showed a significant elevation in post-pubertal bucks (39.2±6.21 pg/ml), while both FSH and LH were declined in sexually mature bucks (26.85±2.33 and 4.97±0.33ng/ml) when compared to young age (29.66±5.12 and 6.05±0.95), respectively. NOMs (nitric oxide metabolites) were elevated in the post-pubertal age (75.67±6.25 mmol/ml) compared to prepubertal age (36.66±3.62: Table 1). Both testicular echogenicity and pixel heterogeneity were significantly declined (P<0.05), in post-pubertal age (64.69±3.21 and 22.17±0.74) compared to prepubertal age (96.22±0.74 and 28.36±0.68).

In this current study, all bucks have showed a blood follow waveform monophasic pattern that characterized by low resistance , moderate pulsatility not exceed 2 with high peak systolic velocity and low end time points as shown in (Fig. 3), the end-diastolic point was equal zero in the young bucks when compared to older bucks reached maturity. No differences were noticeable between pre and post-pubertal stages in Doppler indices as shown in (Table 1) except the resistance index as this parameters was significantly declined (P≤0.05) in the mature bucks (0.54±0.01) compared to young bucks (0.86±0.02), while the testicular artery end-diastolic points was significantly elevated in sexually mature bucks(2.11±0.09) when compared to young bucks (0.12±0.01).

Semen samples could be collected by EE from 100% (5/5) of Baladi bucks at post-pubertal age and 20% (1/5) at a young age.

In the present investigation, semen characteristics were normal and the two semen characteristics that differ between both ages were mass motility and sperm concentration, which both were significantly elevated in older sexual mature Baladi bucks(mass motility was 5 and sperm cell concentration was 4.5×10^9/ml).

The semen normal; results were expressed as Mean±SEM as following: volume was 0.804±0.3 ml; mass motility was 5, individual progressive motility was 71.9±3.5%, sperm concentration was $3.61\pm0.57\text{x}10^9$/ml, normal morphological was 91.4±3.22%, and alive% was 81.25±2.31. The volume was negatively strongly correlated with testicular arteries Doppler indices in Baladi bucks (r=-0.96 for PI, P=0.03 and r=-0.64 for RI, P=0.04), also individual progressive motility was negatively related with both Doppler indices (r=-0.67 for PI, P=0.04 and r=-0.98 for RI, P=0.01), but no statistically significant correlation was obtained between buck testicular echotexture and semen parameters.

DISCUSSION

The similarity between both testes and both testicular artery results agree with previous studies in rams that reported the same manner of changes was observed on both sides (Chandolia *et al.*, 1997). In contrary, some studies reported that in ruminants both testes were different in size as the left one is larger with (Sisson and Grossmann, 1975) or without epididymis and pampiniform plexus (Karimi *et al.*, 2019). In this study Baladi bucks reach sexual maturity showed an elevation in the testicular width with scrotal circumference while the depth did not affect. Similarly, (Camela *et al.*, 2019) reported the marked increase in testicular length and width but with a decrease in the depth as well as the testicular volume did not change between pre and post-pubertal rams. This is a fascinating finding in testicular length and width dimensions development, but this could be due to the elongation and development of the shape in order to provide the greater testicular surface that plays a critical effect in thermoregulation mechanism especially in tropical climate (Bailey *et al.*, 1996).In addition, in some studies, there was a positive correlation between testicular dimensions recorded by ultrasound and that recorded at post mortem stage (Andrade *et al.*, 2014) as ultrasound could provide now an accurate gonadal measurement as previously reported (Gonzalez-Bulnes *et al.*, 2010).

The elevation of testosterone in post-pubertal bucks agrees with a human study (Herting and Sowell, 2017) reported that testosterone levels were 45 times higher in adults compared to boys at prepubertal age. The increase in testosterone levels could be related to the essential physiological events in order to achieve puberty and sexual maturity (Moura *et al*., 2002) that linked with activation of Leydig cells with germ cells proliferation, all those changes lead to development and maturation of hypothalamic-pituitary-testicles axis.

In this study, the testosterone levels correlated positively with testicular dimensions and blood flow colored area, but no correlation was showed between testosterone levels and echogenicity and heterogeneity, and between testosterone with testicular volume this could be explained by the differences in gonadotropin combination and testicular receptiveness to LH and FSH in bucks (Dias *et al*., 2017) and the growing rams (Camela *et al*., 2019) are more important factors in testosterone synthesis that number of Leydig cells.

Similarly, a strong positive significant correlation was observed between testicular size and testosterone levels in ram testes (Camela *et al*., 2019). During the breeding activity, testosterone levels was accompanied by estrogen levels as the testosterone to estradiol ratio is essential in erectile function, the process of spermatogenesis and libido index (Schulster *et al*., 2016). The increased estradiol levels in postpubertal bucks could be due to the vasodilator effect of estradiol on penile vasculature in the erection process (Shirai *et al*., 2004). Estradiol also inhibits the GnRH in the hypothalamus-pituitary axis and then reduces both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) at the level of the brain influence erectile function. Estradiol hinders the hypothalamus-pituitary gonadal axis and subsequently affects FSH and LH (Bagatell *et al*., 1994).

Some studies reported that there was a link between testosterone levels and testicular echo-texture in pre and post-pubertal ages in bull calves (Evans *et al*., 1996), but not in post-pubertal rams (Camela *et al*., 2019), the lack of correlation between testosterone and testicular echo-texture could be related to small numbers of Leydig cells in bucks (Sarma and Devi, 2017).

In this current study, NO was statistically elevated in post-pubertal age, it’s a logical finding as the elevation is associated with a marked increase in the testicular blood flow that reflected on colored area/pixels, a similar study concluded that during the erection process that occurs when animal reach sexual maturity, the amount of blood flow increased which positively associated with elevation of nitric oxide levels (Patel and Bennett, 2016). As NOMs is involved in the initiation and induction of penile erection through cavernous corpora muscle relaxation (Gur *et al*., 2015). As well as an endothelial form of NO is found mainly in the penile body (Taşolar *et al*., 2013).

Both testicular echogenicity and pixel heterogeneity were declined in post-pubertal age as compared to pre pubertal age ,those changes that were observed in both TE and PH could be due to many histological and morphological events throughout puberty in bucks, as in the early mitotic testicular growth the testicular echo-texture features related positively to percentage of seminiferous tubules with spermatocytes ,cell density and amount of germ cells in bucks(Sarma and Devi, 2017) and rams (Giffin *et al*., 2014), also testicular echogenicity that expressed by numerical pixel values (NPVs) linked positively with numbers of prespermatogonial or spermatogonial in bucks(Sarma and Devi, 2017)and rams (Giffin *et al*., 2014), therefore it’s reasonable to observe the marked decline in both TE and PH in sexually mature bucks due to presence of more mature germ cells in seminiferous tubules. In agreement with the obtained results; similar studies reported that the testicular echogenicity were slightly declined in the breeding season of mature animals compared to that of non-breeding season (Gouletsou *et al*., 2003), moreover a study reported that the testicular parenchymal echogenicity was elevated in prepubertal animals as compared with adult mature one (Abril-Sánchez *et al*., 2017). In this study, the thickness of the mediastinum line increased with higher echogenicity in post-pubertal age as compared to the prepubertal age, in agreement with our findings many studies reported both echogenicity and thickness were directly proportional with animal age (Gouletsou *et al*., 2003).

Doppler ultrasonography taken with color mode in order to reveal the colored area/ pixels toward and away from the probe ,as both were elevated in the postpubertal Baladi bucks that expressed by the decline in testicular vascular resistance index (RI) with a marked elevation in the end diastolic points (EDV) as compared to young ages, the spectral graph was represented by monophasic blood wave form pattern of lower RI and higher EDV , the lower in RI could be due to the elevation of blood supply to the testicular organ in sexual mature bucks that indirectly lead to a marked decline in vascular testicular RI as previously confirmed in stallions (Pozor and McDonnell, 2004), rams (Fadl *et al*., 2022), bucks (El-Sherbiny *et al*., 2022), buffalo bulls (Abdelnaby, 2022), and donkeys (Abdelnaby *et al*., 2021b). A decline in RI was observed in children at post pubertal age compared to young age (Paltiel *et al*., 1994), however; some studies reported that no significant difference between pre and post pubertal age for both Doppler indices in dogs (Zelli *et al*., 2013). The marked decline in EDV in young age agreed with (Rotaa *et al*., 2018) therefore; the visualization of end velocity point in bucks could be linked with sexual maturity.

The negative correlation that was observed between testicular volume and Doppler indices in Baladi bucks is almost logical as the testicular dimensions are considered a perfect indicator of the ability of animals to produce sperm (Gemeda and Workalemahu, 2017) as previously reported in rams (Salhab *et al*., 2001). Therefore with the advancement of age to the sexual maturity, the dimensions of testicular tissue were markedly increased with the reduction of both Doppler indices that reflected the improvement of the testicular blood supply, but in contrast to our result, a study reported that a negative correlation was observed between only testicular PI and semen volume in pre and post-pubertal rams (Camela *et al*., 2019).

In this study, there was no correlation between semen parameter and testicular computer-assisted analysis of testicular parenchyma, a study reported that only pixel heterogeneity of testicular tissue correlated negatively with the percentage of normal morphological sperm, but the testicular intensity is a poor predictor of semen quality in rams (Ahmadi *et al*., 2012). In bulls treating from scrotal insulation, authors reported that testicular intensity was positively correlated with the semen volume, but no correlation was observed with other semen parameters (Arteaga *et al*., 2005), otherwise, some studies reported that testicular echotexture was moderately correlated with semen ejaculate and concentration in bulls (Brito *et al*., 2003).

In summary, B – Color and spectral modes of ultrasonography in association with testicular image computer analysis, serum testosterone, estradiol, FSH, LH, and nitric oxide measurements offer additional evidence in comparison between young and sexual mature bucks with respect for semen quality and Doppler indices

CONCLUSION

The post-pubertal age in Baladi bucks is accompanied by a marked elevation in testicular width, length, mediastinum line thickness, scrotal circumference, testicular colored area away and toward the probe, testicular artery end-diastolic point, testosterone, nitric oxide, and estradiol levels with a marked decline in testicular echogenicity, pixel heterogeneity, and testicular artery resistance index (RI), and non-significant decline in FSH and LH levels. All those variables are considered an accurate marker for the onset of sexual maturity in Baladi bucks. The semen volume is negatively correlated with both RI and PI of the testicular artery in post-pubertal Baladi bucks.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interests related to this study.

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