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Relationship between Main Testicular Hemodynamics and Computer-assisted Analysis in Prepubertal Age for Breeding Selection in Baladi Bucks

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ABSTRACT

This study aimed to determine the relationship between testicular hemodynamics and assisted analysis in pre-pubertal bucks at age of 4-6.5 months. Baladi bucks (n=5) weighting (13-15 kg) and aging (4 months) were examined once a week from week 16 till week 26 of age. Buck's body weight, scrotal circumference, testicular volume, mediastinum thickness, Doppler, and image analyses were evaluated. Both scrotal circumference and testicular volume were increased (P<0.05) especially in buck 4 followed by buck 2. Both testicular echogenicity (TE) and pixel heterogeneity (PH) showed an increase (P<0.05) in buck 4 then buck 2. Plexus colored area showed a pattern of an abruptly increase either toward or away from the probe (P<0.05) from week 23 of age without marked differences were shown between all buck testes. Both pulsatility (PI) and resistance indices (RI) showed a marked significant (P<0.05) decrease in buck 4 followed by buck 2 from week 23. In addition, testicular peak systolic velocity (PSV) showed a marked linear increase at week 23. There was a positive correlation between TE and PH and both indices. In conclusion, the assessment of testicular hemodynamics and assisted analysis has added a power to the pre-pubertal bucks' reproductive performance as both tools could help in breeding selection.

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Introduction

In Egypt, goats are considered an important meat source; in addition, goats are one of the most economically essential livestock (Galal *et al*., 2005). Therefore, the choice of males with great reproductive performance capacity is very crucial in small ruminant herds (Saaed and Zaid, 2018; Camela *et al*., 2019). In this feature, breeding soundness estimation is vital for reproductive achievement based on clinical examination of male genitalia as well as determination of semen quality (Kennedy *et al*., 2002).

B-mode ultrasound enables the estimation of the shape, size, and echotexture of testicles (Ahmadi *et al*., 2012) and imaging of the entire scrotum with its content. An ultrasonogram is considered a pattern of square intensity components known as pixels (Camela *et al*., 2019). Pixel intensity is known as testicular echogenicity (TE) that determined by computer analysis of the frozen image which is suggestive for the testicular tissue histomorphology (Ahmadi *et al*., 2013; Pozor *et al*., 2017). Therefore, TE had a clearer relationship with upcoming semen quality than with the current semen (Andres *et al*., 2005). In addition to TE has been utilized for testicular development determination in males (Moxon *et al*., 2015). Testicular parenchyma is usually defined as a tissue of pixel heterogeneity (PH) which reflects the presence of scattered area with hyper- and hypo-echogenicity (Giffin *et al*., 2009). These quantifiable calculations have been primarily conducted in farm animals (Brito *et al*., 2012), stallions (Pozor *et al*., 2017), and stud male dogs (Moxon *et al*., 2015) testes.

Testicular Doppler ultrasound could offer adequate information on vascular reliability as well as blood supply in the male genital system (Pozor and McDonnell, 2004; Carvalho and Chammas, 2008; Abdelnaby *et al*., 2021a). It was suggested that testicular vascular perfusion indices, for example, vascular testicular resistance (RI) and pulsatility indices (PI), in addition to testicular peak velocity point (PSV) were important interpreters of future semen quality (Carillo *et al*., 2012; Zelli *et al*., 2013) as well as penile blood flow (Abdelnaby *et al*., 2021b).

For Baladi bucks, this critical period from week 16 until a week 26 were not previously evaluated. Additionally, computer digital analyzing technique supports ultrasonographic data to be a measurable tool. Little information is available for using this technique for testing the male reproductive performance of bucks in Egypt. Therefore, the objective of this study was

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to evaluate the changes in testicular echo-texture, and hemodynamics at prepubertal age (4-6.5 months) for breeding selection in Baladi bucks, and to determine the correlations between testicular plexus, scrotal circumference, and mediastinum thickness, as well as between testicular hemodynamics parameters and Baladi bucks' age.

Materials and methods

Ethical agreement

This study was in accordance with Institutional Animal Committee (VET-Iacuc) of the Faculty of Veterinary Medicine, Cairo University with approval sheet VET- CU- 24112020246.

Animals and housing

This investigation was started in the Faculty of Veterinary Medicine, Cairo University, Egypt. Five healthy pre-pubertal Baladi bucks (*Capra hircus*), weighting 13-15 kg, body score 3.5±0.01 (range: 1-5) and 16 weeks old, were subjected to this current study. Bucks were examined once weekly from week 16 till week 26 of age. Animals were housed under natural light and temperature with artificial lighting at night (10 h at night). Animals were kept in the Theriogenology Department at Giza square (30.0154° N, 31.2120°) with an average temperature of 30° C. The bucks were kept indoors. All bucks were undergone routine clinical examinations, scrotal visualization, and testicular palpation for estimation of the testicular symmetry (Kühn *et al*., 2016). Concentrated feed from soybean and corn with 19% crude protein and the feed mixture was supplied in the amount of 500 g / animal. In addition to animals were unrestricted access to water, mineral salt licks, and unrestricted corn silage.

Body weight and scrotal circumference measurements

Bucks' body weight (kg) and the circumference of the scrotum (cm) were determined once weekly in the prepubertal bucks. The scrotal circumference was determined in centimeters at the greatest diameter of the scrotum by using a cloth measuring tape (Raji and Ajala, 2015). The scrotal measurements were repeated three times and the average was recorded. Scrotal contents were palpated to observe freely moving testes.

Ultrasonography

Ultrasonographic examination was performed once/week with a B-mode ultrasound scanner (EXAGO, Echo Control Medical, France) connected to a 5-7.5 MHz linear array transducer (Ginther, 2007; Abouelela *et al*., 2021). Each male was restrained with the two limbs separated. The buck testes were shaved then the gel was applied on the probe, the operator pressed gently on the surface of the testes.

Testicular dimensions and mediastinum thickness measurement

Testicular measurements for the bucks involved viewing the transverse (TS) and the longitudinal sections (LS) for both testes. The testicular parameters measured once weekly were the width of both testes (W) was taken on the TS separately for both testes. The length of the testis (L) was taken on the LS using the electronic caliper. In the lateral view, rete testes appeared as straight line (mediastinum) in the center; the thickness of mediastinum (MT/cm) was also measured and appeared as a hyper-echoic line in both testes. The testicular volume was calculated using the known ellipsoid equation (4/3π abc) where length (a) × width (b) × height (c) (Camela *et al*., 2019). In addition to testicular echogenicity (TE) and pixel heterogeneity (PH) were also evaluated in the grey B-mode frozen image in a selected area depicted by a box cursor using a computer analysis method (Moxon *et al*., 2015).

Colour and pulsed Doppler modes application

All bucks were examined once /week without any sedation. The testicular artery is located near the cranial pole of the testis at the region of the pampiniform plexus. After identifying the main testicular artery by the specific spectral pattern graph, the three-five best continuous successive waves with complete systolic and diastolic of a cardiac cycle are measured to evaluate the following Doppler indices expressed by resistance index (RI) as well as pulsatility index (PI). In addition to peak systolic velocity (PSV; cm/sec) that was measured automatically (Ginther, 2007). Once the colored Doppler mode is activated, the Doppler screen automatically gives two colors; the first one is the red color which related to the direction of blood flow toward the probe away from the specific organ, while the second color is the blue color which related to the direction of blood flow far away from the probe toward the specific organ (Ginther, 2007).

For the pulsed wave Doppler mode, the main testicular artery is recognized by the spectral graph to obtain a cardiac cycle with peak systolic and end-diastolic velocities with the Doppler filter at 100 Hz and the angle of insonation was 40±5° (Strina *et al*., 2016).

Pampiniform plexus hemodynamic vascular features

In bucks, the measurement of pampiniform plexus vascularization in form of colored plexus/ pixels and colored plexus percentage was assessed as the colored plexus/ pixels was determined as endorsed to the blood perfusion in plexus which previously validated to assess the intensity of colored pixels in the frozen colored image captured by the color Doppler mode (Ribeiro *et al*., 2020). Pampiniform colored plexus percentage was measured as previously done by Ribeiro *et al*. (2020) throughout the assessment scoring system that ranged from 1-5.

Image analysis

Grey brightness mode/colored Doppler images were exported from the EXAGO device in order to make further analysis of the image using the computer-assisted program as Adobe Photoshop Cc X 64 software (Adobe Inc. USA), which delivered the vascularization, pampiniform plexus coloration either toward the probe (red color) or away from the probe (blue color) and testicular echogenicity (TE) with pixel heterogeneity (PH) by using additional magnetic lasso tool with the histogram in adobe Photoshop.

Testicular echogenicity (TE) and pixel heterogeneity (PH) were measured by computer-assisted analysis using Adobe Photoshop cc by gaining two-three 1 cm^2 spots selected approximately 1 cm above the mediastinum (Brito *et al*., 2012). Then, the averages of testicular measurements and pixel intensity value were recorded. Means for NP from the six spots (three from each testis) were calculated to be used in the statistical analysis (Ahmadi *et al*., 2012). Testicular pixel intensity symbolized within the selected areas depends on the gray color shade (Camela *et al*., 2019).

Statistical analysis

Data were subjected to analysis of variance, correlation, and regression analysis using the Statistical for Social Sciences package (SPSS version: 20.0). One-way ANOVA was used to obtain means ±standard errors of parameters to determine the effect of weeks (16-26) on body weight, scrotal circumference, testicular volume numerical pixels values, and Doppler parameters. The correlation coefficients among blood flow parameters and testicular morphology were calculated. Regression of scrotal circumference, body weight, total testicular volume, and the total thickness of the mediastinum line inside testes was also determined. The Duncan multiple range tests were used to differentiate among significant means.

Results

Data of visually symmetrical right and left testes of the examined bucks were included in the study, no significant difference was observed between both buck testes. Both testes had free movability inside the scrotum. Both spermatic cords were easily palpated as well as the epididymis (Table 1).

Among 5 bucks; buck 4 has a higher testicular volume and scrotal circumference values compared to others (Figure1). Body weight increased from 14.21±0.22 Kg at week 16 to 18.12±0.96 Kg, at week 26 with a marked increase at week 23 (17.02±0.51 Kg) but it does not share any differences between bucks. Also, scrotal circumference and testicular volume significantly ($P<0.05$) increased linearly till week 23 (16.22±1.22 and 17.85±0.01) then abruptly elevated after that especially in buck 4 followed by bucks 2 and 5. The testicular volume of both testes was nearly the same measures as shown in Table 1. In this study, the mediastinum line appeared totally as a hyper-echoic line. The thickness measurement was done from week 16 until week 26 as shown in Table 2, the thickness of mediastinum is weakly increased in all bucks ($P<0.05$) and ranged from 0.09±0.01 at week 16 to 0.19±0.01 at week 26, while both testicular echogenicity (TE) and pixel heterogeneity (PH) were the same in both testes and showed a pattern of moderate increase till week 22 then both parameters showed a marked significant increase in both testes ($P< 0.05$) from week 23 (61.13±2.36 and 61.20±2.22 for TE) and (18.36±2.85 and18.66±1.96 for PH). In addition, both total TE and PH showed a marked abrupt elevation from week 23 which was observed in buck 4 then buck 2 as depicted in Fig. 2. As shown in Fig. 3, scrotal circumference (cm; Fig. 3a), body weight (kg; Fig. 3b), total testicular volume (cm^3; Fig. 3c) and total thickness of mediastinum line inside testes (cm; Fig 3d) increased proportionally with buck age with a linear equation and regression equations presented on each measure ($y=0.4065x+13.395$ with $R2=0.9286$), ($y=0.5858x+10.83$ with $R2=0.892$). ($y=1.1915x+6.866$ with $R2=0.9693$) and ($y=0.0113x+0.0742$ with $R2=0.9598$), receptively.

Weeks significantly affected the pampiniform plexus colored area/pixels and plexus colored area % in both testes as shown in Table 3, colored plexus showed a pattern of an abruptly significant increase in the right and left testes either toward probe or away from the probe in all bucks ($P<0.05$) from week 23 (4942.11±21.15 and 5026.82±25.23 toward the

Table 1. Data summary (mean ±SEM) of scrotal circumference, body weight and testicular volume of both testes in 5 male Baladi goats from week 16 until week 26.

Age (weeks)	Scrotal circumference (cm)	Body weight (kg)	Testicular volume(cm^3)	
			Right	Left
16	12.23±0.39^a	14.21±0.22^a	8.05±0.65^a	8.02±0.33^a
17	12.32±0.02^a	14.45±0.39^{ab}	9.01±0.74^b	9.02±1.02^b
18	12.54±0.25^a	14.65±0.01^{ab}	10.24±0.36^b	10.23±2.96^b
19	12.85±2.45^a	14.85±0.99^{ab}	11.32±0.44^c	11.33±2.14^c
20	13.32±0.96^b	15.11±0.06^b	13.65±0.05^d	13.64±0.96^d
21	13.52±0.22^b	15.33±0.33^b	13.85±0.85^d	13.88±3.15^d
22	13.69±0.32^b	15.52±0.75^b	14.36±1.65^e	14.35±1.25^e
23	16.22±0.22^c	17.02±0.51^c	17.85±0.01^f	17.85±0.01^f
24	16.55±0.85^c	17.27±0.21^c	18.02±0.87^g	18.09±0.32^g
25	17.02±0.04^d	17.55±0.68^c	18.86±1.25^h	18.88±0.82^h
26	17.33±1.65^d	18.12±0.96^d	18.96±1.05^h	18.99±0.96^h
P-value	0.03	0.002	0.001	0.001

Means with different superscripts within column are significantly different at $P<0.05$

Table 2. Data summary (mean ±SEM) of thickness of mediastinum line, testicular echogenicity (TE) and pixel heterogeneity (PH) of both testes in 5 male Baladi goats from week 16 until week 26.

Age (weeks)	Mediastinum thickness (cm)		Testicular echogenicity (Npv)		Pixel heterogeneity (Sdnpv)	
	Right	Left	Right	Left	Right	Left
16	0.09±0.01^a	0.09±0.01^a	39.42±9.31^a	38.65±3.66^a	13.02±0.96^a	13.05±0.66^a
17	0.11±0.01^a	0.11±0.01^a	40.63±6.21^a	39.95±5.93^a	13.21±0.02^a	13.22±0.52^a
18	0.11±0.01^a	0.11±0.01^a	42.82±4.05^{ab}	42.96±7.20^{ab}	13.52±0.85^a	13.54±0.31^a
19	0.11±0.01^a	0.11±0.01^a	42.92±3.58^{ab}	42.99±8.02^{ab}	13.66±0.42^a	13.69±0.02^a
20	0.14±0.01^b	0.14±0.01^b	43.02±3.22^{ab}	43.25±3.65^{ab}	14.21±0.13^{ab}	14.11±0.96^{ab}
21	0.14±0.01^b	0.14±0.01^b	46.01±4.69^{bc}	46.06±7.31^{bc}	14.36±0.25^{ab}	14.26±0.85^{ab}
22	0.14±0.01^b	0.14±0.01^b	52.02±6.92^{bc}	52.31±6.12^{bc}	14.32±3.21^{ab}	14.33±1.32^{ab}
23	0.18±0.01^c	0.18±0.01^c	61.13±2.36^c	61.20±2.22^c	18.36±2.85^c	18.66±1.96^c
24	0.18±0.01^c	0.19±0.01^c	66.74±7.32^c	66.72±4.02^c	18.96±3.22^c	18.99±2.05^c
25	0.19±0.01^c	0.19±0.01^c	71.62±6.82^d	71.69±8.26^d	22.65±0.52^d	22.78±2.82^d
26	0.19±0.01^c	0.19±0.01^c	72.21±7.93^d	72.31±7.56^d	23.45±1.32^d	23.14±0.04^d
P-value	0.03	0.03	0.001	0.001	0.001	0.001

Npv=numerical pixel value; Sdnpv= standard deviation of numerical pixel value. Means with different superscripts within column are significantly different at $P<0.05$

probe), (5222.51±11.25 and 5552.84±12.22 away from the probe) of age when compared to weeks before. In addition, plexus colored area% showed a marked significant (P<0.05) elevation from week 23 (38.76±4.33 and 38.25±6.35) in both testes. No marked differences were showed between both buck testes.

The hemodynamic structures by Doppler examination did not show any difference between the right and left testes as shown in Table 4. Both testicular pulsatility (PI) and resistance indices (RI) decreased linearly till week 22 (1.44±0.01 and 0.72±0.01) then showed a marked significant (P<0.05) decline from week 23 (1.12±0.01 and 0.53±0.01).

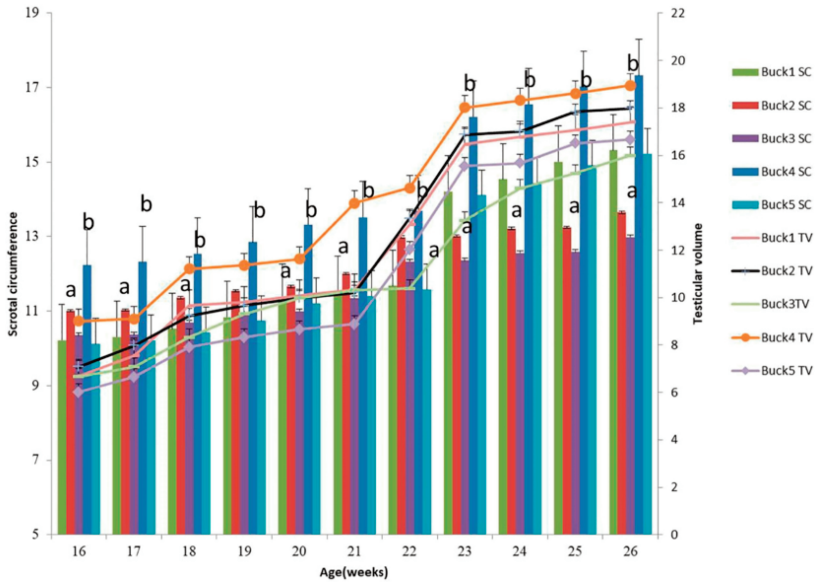

Fig. 1. Bucks scrotal circumference (cm) and testicular volume (cm^3) expressed by (mean± SEM) in 5 male Baladi goats from week 16 until week 26. Different superscripts above show differences between bucks of P < 0.05.

Table 3. Pampiniform plexus hemodynamic vascular features (mean ±SEM) of both testes in 5 male Baladi goats from week 16 until week 26.

Age (weeks)	Plexus colored area/ pixels				Plexus colored area%	
	Right to Probe	Left to Probe	Right away Probe	Left away Probe	Right	Left
16	3854.36±14.82^a	3908.28±22.36^a	4006.32±71.25^a	4032.32±41.66^a	28.15±1.48^a	28.16±1.48^a
17	3905.21±32.21^a	3911.25±15.27^a	4099.01±62.52^a	4069.02±41.02^a	30.12±1.96^b	30.69±1.05^b
18	3927.82±21.72^a	3929.31±55.27^a	4192.32±22.33^a	4165.44±32.12^a	31.07±1.87^b	30.99±1.74^b
19	4031.33±14.01^b	4062.83±25.32^b	4312.97±42.87^b	4355.82±65314^b	31.25±6.02^b	31.12±2.65^b
20	4087.87±32.72^b	4098.62±15.12^b	4362.96±55.82^b	4396.55±67.42^b	31.22±4.25^b	31.25±3.82^b
21	4298.32±12.12^c	4245.32±35.17^c	4402.21±34.25^c	4411.65±52.66^c	32.36±7.28^c	32.00±2.68^c
22	4299.42±19.27^c	4241.74±32.75^c	4432.02±54.95^c	4463.65±36.77^c	32.49±3.05^c	32.46±7.32^c
23	4942.11±21.15^d	5026.82±25.23^d	5222.51±11.25^d	5552.04±12.22^d	38.76±4.33^d	38.25±6.35^d
24	5319.62±19.56^e	5313.65±16.57^e	5596.01±88.34^e	5599.32±45.63^e	39.02±3.22^d	39.95±9.25^d
25	5325.47±23.52^e	5337.15±21.62^c	5599.39±11.81^e	5599.88±14.82^e	42.02±4.47^e	42.05±6.35^e
26	5328.41±31.02^e	5339.24±25.27^c	5614.21±14.85^e	5634.74±33.25^e	42.12±5.22^e	42.11±2.25^e
P-value	0.01	0.01	0.01	0.01	0.01	0.01

Means with different superscripts within column are significantly different at P<0.05

Table 4. Main testicular artery Doppler indices and Doppler velocity of both testes in 5 male Baladi goats from week 16 until week 26.

Age (weeks)	Main testicular PI		Main testicular RI		Main testicular PSV cm/sec	
	Right	Left	Right	Left	Right	Left
16	1.66±0.01^d	1.68±0.01^d	0.80±0.02^e	0.79±0.01^e	13.05±0.06^a	13.02±0.31^a
17	1.56±0.01^d	1.57±0.01^d	0.80±0.01^e	0.79±0.01^e	13.09±0.06^a	13.08±0.12^a
18	1.51±0.01^{cd}	1.49±0.01^{cd}	0.78±0.02^d	0.76±0.01^d	13.24±0.03^b	13.24±0.02^b
19	1.51±0.01^{cd}	1.49±0.01^{cd}	0.78±0.01^d	0.76±0.01^d	13.33±0.05^b	13.35±0.05^b
20	1.49±0.01^{cd}	1.47±0.01^c	0.75±0.01^c	0.73±0.01^c	13.41±0.04^b	13.40±0.12^b
21	1.46±0.01^c	1.47±0.01^c	0.75±0.01^c	0.73±0.01^c	13.75±0.06^c	13.80±0.18^c
22	1.44±0.01^c	1.44±0.01^c	0.72±0.01^c	0.72±0.01^c	13.85±0.11^c	13.85±0.11^c
23	1.12±0.01^b	1.12±0.01^b	0.53±0.01^b	0.53±0.01^b	16.32±0.12^d	16.32±0.12^d
24	1.11±0.01^b	1.11±0.01^b	0.53±0.01^b	0.51±0.01^b	16.89±0.03^d	16.88±0.01^d
25	1.09±0.01^b	1.11±0.01^b	0.51±0.01^a	0.48±0.01^a	16.98±0.13^d	16.89±0.06^d
26	1.06±0.01^a	1.03±0.01^a	0.51±0.01^a	0.48±0.01^a	17.13±0.25^e	17.16±0.07^e
P-value	0.01	0.01	0.01	0.01	0.01	0.01

PI=pulsatility index, RI=resistance index and PSV=peak systolic velocity. Means with different superscripts within column are significantly different at P<0.05

The decline of both Doppler indices was observed markedly in buck 4 among five bucks followed by buck 2 as shown in Figure 4. Furthermore, testicular peak systolic velocity (PSV; cm/sec) increased slowly till reach week 22 (13.85±0.11) of animal age then showed a marked linear increase at week 23 (16.32±0.12) and afterward, but these parameters does not show any effect between bucks. In order to make a comparison between variables, a correlation was calculated between testicular Doppler parameters and testicular echotexture. SC was positively correlated with ages (weeks) (r=0.68; P<0.05) and testicular peak systolic velocity (r=0.89; P<0.05), peak systolic velocity expressed by PSV; cm/sec, was significantly correlated positively with the colored area in plexus/pixels (r=0.35; P<0.05), moreover, colored pixels% (r=0.60; P<0.05) and bucks age (r = 0.39; P<0.05), both Doppler indices (RI and PI) showed a marked significant negative correlation with the colored area in plexus/pixels (r=-0.54; <0.05), colored pixels % (r=-0.43; P<0.05), SC (r=-0.65; P<0.05), body weight (r=-0.36; P<0.05), and testicular volume. Also, PI and RI were negatively correlated with the peak velocity of the main testicular artery (r= -0.35; P<0.05). There was a positive correlation between both TE and PH (r=0.93; P<0.05) and testicular PI and RI (r=0.92; P<0.05).

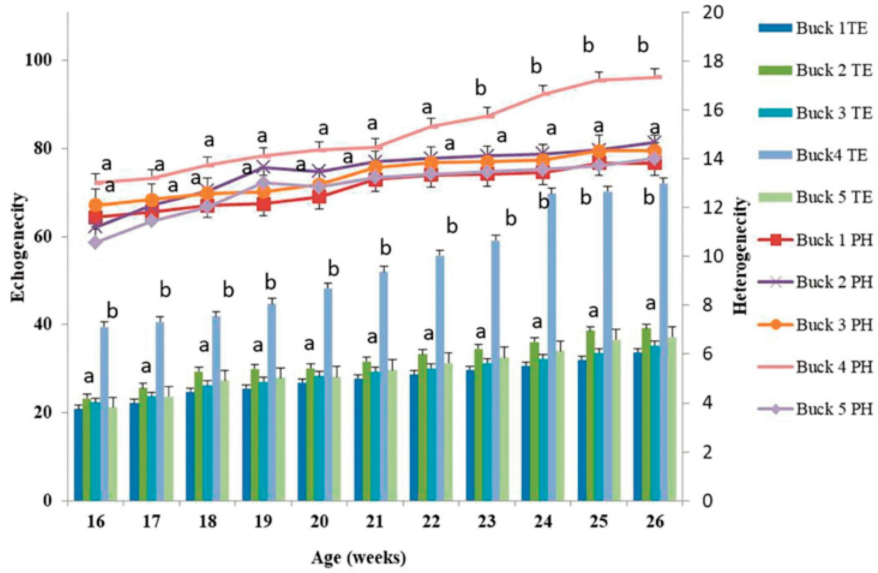

Fig. 2. Bucks total testicular echogenicity (numerical pixel value; Npv; P≤0.001) and total pixel heterogeneity (standard deviation of numerical pixel value; Sdnpv; P≤0.05) expressed by (mean± SEM) in 5 male Baladi goats from week 16 until week 26. Different superscripts above show differences between bucks of P <0.05.

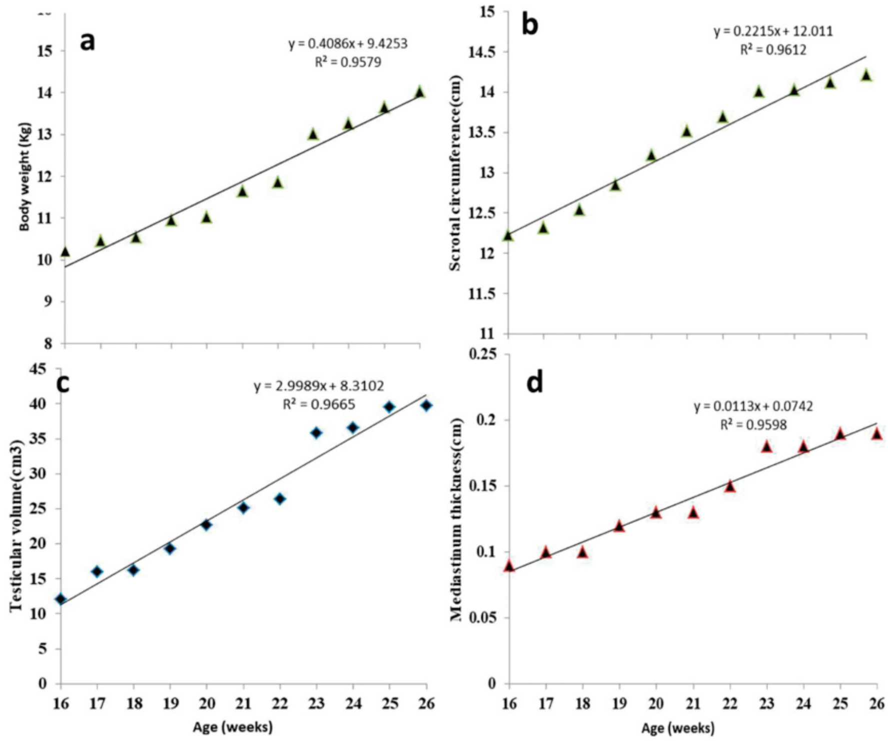

Fig. 3. Regression of scrotal circumference (cm; a), body weight (kg; b), total testicular volume (cm^3; c) and total thickness of mediastinum line inside testes (cm; d) in 5 male Baladi goats from week 16 until week 26 with a linear equation of regression and R2

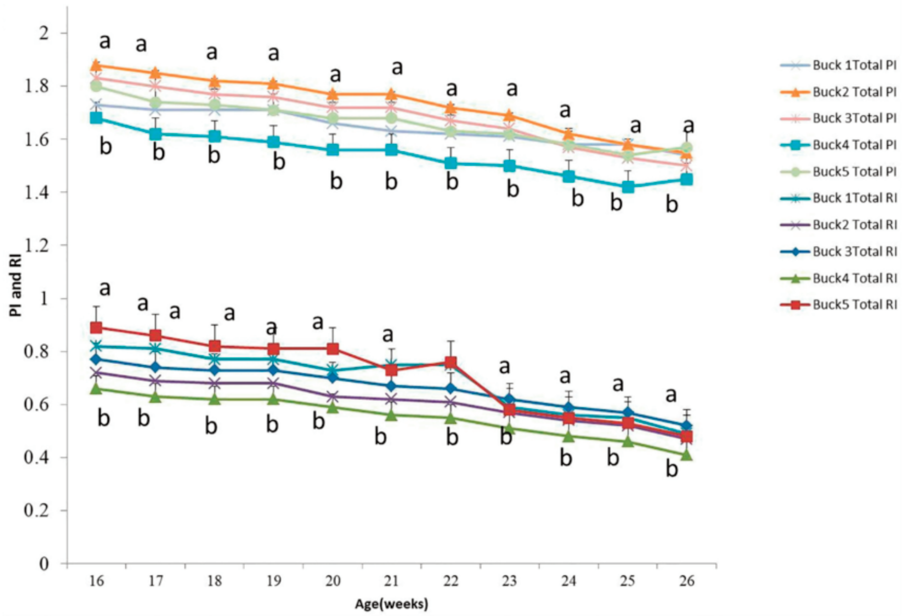

Fig. 4. Bucks main testicular artery Doppler indices (PI; pulstility index) and (RI; resistance index) expressed by (mean± SEM) in 5 male Baladi goats from week 16 until week 26. Different superscripts above show differences between bucks of $P < 0.05$.

Discussion

To the best of the author's knowledge, this is the first study to determine the correlation between testicular vascular indices with testicular echogenicity and pixel heterogeneity in young Baladi bucks at age 4-6.5 months. The Doppler parameters with the studied age in correlation with testicular morphometry were not studied previously. Scrotal circumference is a critical measurement for the future of reproductive performance and animal status (Menegassi *et al*., 2011), as a decline in the SC is considered a good indication of morphologically abnormal sperm elevation in the ejaculate (Mickelsen *et al*., 1982). This study revealed that the scrotal circumference was 12.23±0.39 at week 16 of age and increased linearly with a marked elevation at week 23 (16.22±1.22) then reach 17.33±1.65 at week 26 of age. Similarly, Kulkarni (2003) measured the scrotal circumference at 4 months (11.49±0.55) and the authors concluded that scrotal circumference increased with both body weight and testicular length.

To the best of the authors knowledge, Doppler parameters at age 4.0-4.5 months in correlation with testicular morphometry were not studied previously. The finding of this current study was in accordance with a study reported elevated body weight with age in another goat species (Kumbhar *et al*., 2019). Contrary; another study reported a decline in the body weight at 4 months' age in Indian males (Bilaspuri &Singh, 1992). Scrotal circumference is a critical. Another study reported that the circumference of the entire scrotum was 8.01±0.63 at the fourth month of age in male goat kids (Bilaspuri and Singh, 1992). In animals, testicular dimensions and future sperm production are highly associated as larger testicular volume results in greater production of sperm (Bearden *et al*., 2004). The testicular volume evaluation depends mainly on testicular length, width, and height, therefore; any increase in testicular dimensions would reflect on the testes volume (Sotos and Tokar, 2012). The testicular length in prepubertal bucks in this study was ranged from 3.5 to 4.6 cm. In agreement with the elevation of testicular volume in this period, Nimse *et al*. (2011) found that left and right testes were similar in testicular length (cm) in pre-pubertal rams (5.90±0.12 and 5.89±0.09) when compared to post-pubertal age but both were different in testicular width as they stated the width of the left was 4.17±0.04 and the right testis was 4.71±0.04 cm in rams. Moreover, other report detected that both testes length was the same in adult bucks (Yaseen *et al*., 2010). In contrast, some studies reported that the left testicular length and width was higher than the right one (Mehta *et al*., 1992; Kulkarni, 2003) in goats and others (Kabiraj *et al*., 2011) which reflected on the volume.

In the present study, the thickness of mediastinum line appeared totally as hyper-echoic white line. Similar to this finding, a study reported that the mediastinum testis line appeared in animals with 100% at the center of the testicular parenchyma when visualized by the longitudinal section (Andrade *et al*., 2014). Moreover, Moura *et al*. (2008) found that the mediastinum thickness increased with animal age. This elevation of the thickness line could be associated with important changes in seminiferous tubules (SNT) with became longer with a marked increase in its diameter. In this current study, both testicular echogenicity (TE) and pixel heterogeneity (PH) showed a pattern of moderate increase till week 22 than both parameters showed a marked significant increase in both testes from week 23. The TE and PH increased with animal age, this increase may be due to changes in the testes histomorphology occurring at puberty with clear anatomical changes in SNT (Urt *et al*., 2018), in contrast to the obtained result, Saaed and Zaid (2018) concluded that TE associated with an initial increase followed by a decline. The strong positive correlation ($r=0.93$; $P<0.05$) in this study between TE and PH supported the previous suggestions for use of those two important parameters in the assessment of puberty, sexual maturity and reproductive capacity in bucks as previously stated in rams (Saaed and Zaid, 2018).

The echogenicity of the entire testicle was shown as an ideal indicator for future semen production (Moxon *et al*., 2015; Saaed and Zaid, 2018). This increase in both TE and PH could be related to the effect of gray scale on the composition of tubular cells (Giffin *et al*., 2009), therefore, evaluation of TE and PH could help in expecting the onset of puberty as previously done in rams (Bartlewski *et al*., 2017).

Measurement of pampiniform plexus coloration is very important, as it may use for prediction of future fertility, it was found in the current study that there was a marked elevation of this parameter from week 23 of age. Similarly, it was established recently that animals with high fertility expressed an elevation in the pampiniform plexus-colored pixels, besides semen parameters when compared to animals with a low grade of fertility (Ribeiro *et al*., 2020). In addition, vascular plexus score with pixels percentage could help an indication of the testicular vascularity levels by determining the colored area away from the probe which indicated by the blue color and area toward the probe which indicated by the red color. A similar study found that there was a marked increase in testicular velocity parameters in healthy boys as they got mature, this elevation occurs at pubertal age with marked elevation of plexus coloration (Paltiel *et al*., 1994). Testicular flow is considered the main pathway for transportation of nutrients as well as hormones to and away from the animal testes, therefore this testicular vascular perfusion has a direct effect on spermatogenesis (Bergh and Damber, 1993) as the study showed that the early spermatogenesis process is very sensitive to the vascularization which can be detected through pixel intensity (Bergh *et al*., 2001). The plexus pixels percentage in this study was measured as it ranged from 28-42% from week 16 till week 26 of bucks age which related moderate degree of vascularization with score 2-3 as previously determined by Ribeiro *et al*. (2020).

In this study, a positive significant correlation was measured between both testicular indices resistance (RI) and pulsatility indices (PI) ($r=0.92$; $P<0.05$), while there was a negative correlation between both Doppler indices and testicular peak velocity (PSV cm/sec). The positive correlation may be due to the fact that the main testicular artery showed a relatively lower in both Doppler indices with higher peak velocity (PSV) (Carvalho *et al*., 2008). The testicular vascularization has a direct effect on the tissue functionality (Zelli *et al*., 2013). Consequently, recent studies have revealed that both testicular PI and RI are satisfactory indicators for spermatogenesis as previously reported in humans (Pinggera *et al*., 2008) and dogs (Zelli *et al*., 2013). Conversely, higher Doppler indices indicate a lower peak velocity (PSV) that may lead to declining in the distal testicular vascularization which is related to many testicular affections in men (Schurich *et al*., 2009), and dogs (Bumin *et al*., 2007). The positive significant correlation between SC and PSV ($r=0.89$; $P<0.05$) indicates marked elevation of the blood supply in the testicular artery through sexual development and therefore increased the sperm quality (Brito *et al*., 2002). A similar study reported that the spectral pulsed Doppler can determine the microcirculation testicular status (Semiz *et al*., 2014). In addition, this parameter could be used as a tool to predict the potential of fertility status in bucks

Conclusion

Correlations exist between testicular plexus, scrotal circumference, and testicular volume, as well as between testicular hemodynamics parameters and Baladi bucks' age. The assessment of testicular vascularization and computer assisted analysis has added power to the pre-pubertal bucks' reproductive performance. Bucks with high scrotal circumference and testicular volume values have an elevation in both tissue intensity and heterogeneity.

Conflict of interest

The authors declare that they don't have any conflict of interest

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