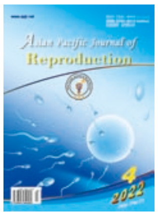

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Testicular vascularization at two locations in relation to hormonal levels, and pixel echotexture in bulls at different ages

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ABSTRACT

Objective: To assess the hemodynamic changes that occurred in the main testicular artery at two different locations such as supra and marginal ones in bulls at three different ages.

Methods: Eighteen Baladi bulls were divided according to their age into three different age groups: 1 year [*n*=6; group Ⅰ with bodyweight (380±10) kg], 3 years [*n*=6; group Ⅱ with bodyweight (570±10) kg], and 6 years old [*n*=6; group Ⅲ with bodyweight (650±10) kg] Baladi bulls. Circumference of the scrotum, thickness of the mediastinum, testicular dimensions, vascularity, and hormonal levels were measured. Testicular hemodynamics were assessed by Doppler ultrasound scanning.

Results: Testicular width, length, and volume were significantly different among the three age groups, with the highest in bulls of group Ⅲ (P<0.05). The bulls in group Ⅲ had significantly higher mediastinal line thickness and scrotal circumference than group Ⅰ (P<0.05), but there was no significant difference between group Ⅱ and group Ⅲ. Bulls in group Ⅱ showed a decline in Doppler indices (resistance and pulsatility indexes) at both testicular branches compared to the other two groups (P<0.05). Supra and marginal end diastolic velocities in the three age groups were significantly different, with the highest in group Ⅱ (P<0.05). Time average velocity (cm/s) was significantly elevated in group Ⅱ compared to the other two groups (P<0.05). Both testicular echotextures were significantly decreased in group Ⅱ (P<0.05). Estradiol and nitric oxide metabolites were significantly high in group Ⅱ compared to the other two groups (P<0.05). Meanwhile, the level of plasma testosterone was maximum in group Ⅲ (P<0.05). Positive correlations were found between supratesticular artery resistance and pulsatility indexes (r=0.81; P<0.001), while both resistance and pulsatility indexes had correlated negative with estradiol (r=-0.71 and r=-0.91; P<0.001), and nitric oxide metabolites (r=-0.92 and r=-0.72; P<0.001).

Conclusions: Three-year old Baladi bull has the lesser Doppler indices with lesser echotexture and greater nitric oxide with estradiol concentrations, which directs a significant ability for the thermoregulation process. These parameters will help in breeding selection.

KEYWORDS: Bull; Doppler indices; End diastolic velocity; Estradiol; Testicular blood flow; Resistance index; Pulsatility index

1. Introduction

Testicular parameters are recommended in fertile farm animals used for natural or in the artificial insemination breeding centers to help in their selection and to record their decrease in response to any health problems that could affect their fertility[1]. Male breeding soundness examination is an important factor in the

Significance

Male breeding soundness examination is an important factor in the selection of males for breeding reasons, as the known based on testicular volume and scrotal circumferences. This study adds the application of Doppler technology in assessing testicular functionality in bulls at different ages that could help in breeding selection.

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selection of males for breeding reasons[2,3] because testicular size is still the most important factor in determining the scrotum format along with animal weight[4]. Breed differences that used variation in development rates and expected fertility are influenced by the measurement of the scrotal circumference[5]. As a consequence of male selection for breeding purposes that focused on improving scrotal circumference, bulls' testicles have become more oval[6]. In addition, the testicular volume could be utilized as a procedure to increase the accuracy of the bull examination[7].

Ultrasonographic technology has been utilized in a variety of data to measure the normal testicular function or to diagnose many pathological disorders[8]. The ultrasonographic research examined tissue echotexture in beef bulls, which can be useful for tracking the testicular growth[9] and assessing the acute testicular injury[10] but not in mature bulls[9]. The principal testicular artery[11] supplies the testicular parenchyma with presence of capillaries. Testosterone level elevation is associated with a marked elevation in both anterior pituitary hormones[12]. In adult male dogs[13] and bucks[14], both estrogen and nitric oxide in form of its metabolites have an action in the testicular vasodilatation process, which are linked with declined levels of vascular resistive and pulsatile factors in the circulation[15]. Testicular hemodynamics has been recently reported *via* Doppler ultrasonographic technique. In human medicine, color Doppler has been utilized to measure testicular blood flow in abnormal situations[16]. Although the pulsed wave Doppler ultrasound approach in dogs[13] and horses has received little attention in veterinary medicine[17], Doppler indices have been used in rams throughout the year. This study used spectral Doppler to assess the changes in the two branches of testicular artery of bulls at different ages, and to determine the changes in testicular echotexture pattern that affect the testicular vascularization pattern in bulls.

2. Materials and methods

2.1. Animals, location and housing

The current investigation was done at large animal partitions in the Department of Theriogenology at Cairo University at Giza square with an average temperature of 36 ℃. Eighteen Baladi bovine Egyptian bulls were used and subdivided according to their age into three different age groups: 1 year [n=6; group Ⅰ with bodyweight (380±10) kg and body condition scoring was 4.0±0.5], 3 years [n=6; group Ⅱ with bodyweight (570±10) kg and body condition scoring was 3.5±0.5], and 6 years old [n=6; group Ⅲ with bodyweight (650±10) kg and body condition scoring was 4.0±0.5] Baladi bulls. Bulls were normally regularly vaccinated against infectious disease with no abnormalities in their reproductive system. A pasture was used to keep the animals with a temperature about 36 ℃ and relative humidity about 56%. The dietary needs of a bull included a commercial ration and hay with free access to water[18].

2.2. Scrotal circumference determination, and B mode ultrasound measurement

Prior to ultrasonography, all bulls were detained without painkillers. Bull was secured by tying a rope around the bull with passing the rope over the transverse process of bull sacrum. The scrotum was determined in cm. All examinations were done in bulls with a standing position[19]. The testis dimension was determined in cm and the testis volume was calculated according to Andrade *et al*[20]. The white hyperechoic line, also known as the mediastinal line, was also evaluated in the vertical view of the testicles based on its thickness measured in millimeters. Echotexture was measured by the Image J program as previously described by Brito *et al*[9].

2.3. Testicular hemodynamics at two locations

Examinations were done by the one operator using both B and Doppler modes ultrasonic scanner (SonoAce; Medison; Samsung, South Korea) equipped with 12 MHz linear array transducer. The liner transducer was located with an angle of 45° at the site of the spermatic cord distally[21] as depicted in Figure 1. On the other hand, the marginal testicular artery is the branch from the supratesticular artery[22], which can be superficially felt at the level of the parietal testis (Figure 2). Blood flow factors were estimated such as resistance index and pulsatility index, peak systolic and end diastolic velocities in cm/s (Figure 1, Figure 2). Resistance index and pulsatility index were expressed by those equations[23] as below: Resistance index = PSV-EDV/PSV; Pulsatility index = PSV-EDV/TAMV where PSV (peak systolic velocity; cm/s), EDV (end diastolic velocity; cm/s) and TAMV (time average velocity; cm/s).

2.4. Blood collection and hormonal analysis

For harvesting sera, blood samples were collected from the tail vein in blank vacuum tubes, centrifuged at 488×g for 20 min, and stored at -18 ℃. Testosterone (ng/mL), estradiol (pg/mL), follicle stimulating (FSH; ng/mL), and luteinizing (LH; ng/mL) hormones were determined with intra-assay and inter-assay coefficients <10% and <14% (Sunlong Biotech Co.) enzyme linked immunosorbent assay kit. Nitricoxide (μmol/L) measurement was performed as previously reported[24].

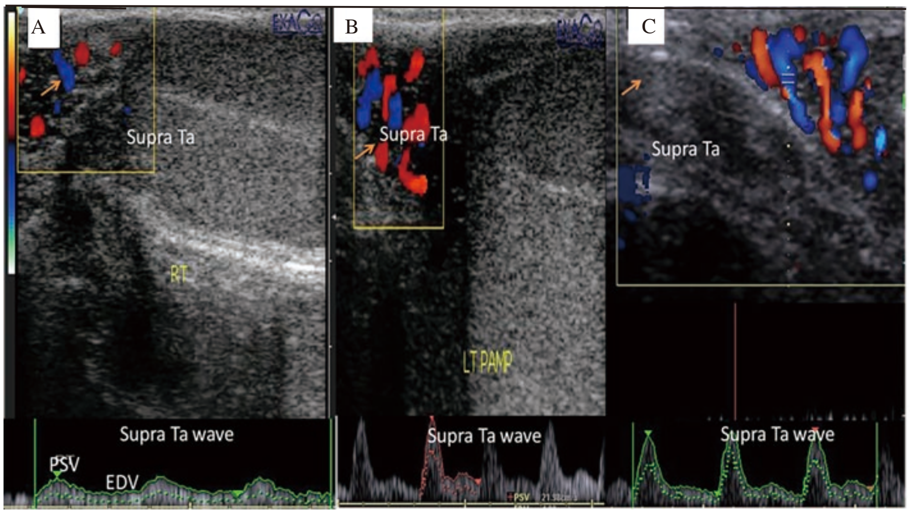

Figure 1. Color mode ultrasonograms show the measurement of supratesticular artery (Supra Ta; brown arrow) with pulsed wave Doppler spectral graph with placement of linear probe with angle of insonation 45° in order to measure Doppler indices from peak systolic and end diastolic velocity (PSV and EDV) in the bulls aged 1 year (A), 3 years (B), and 6 years (C).

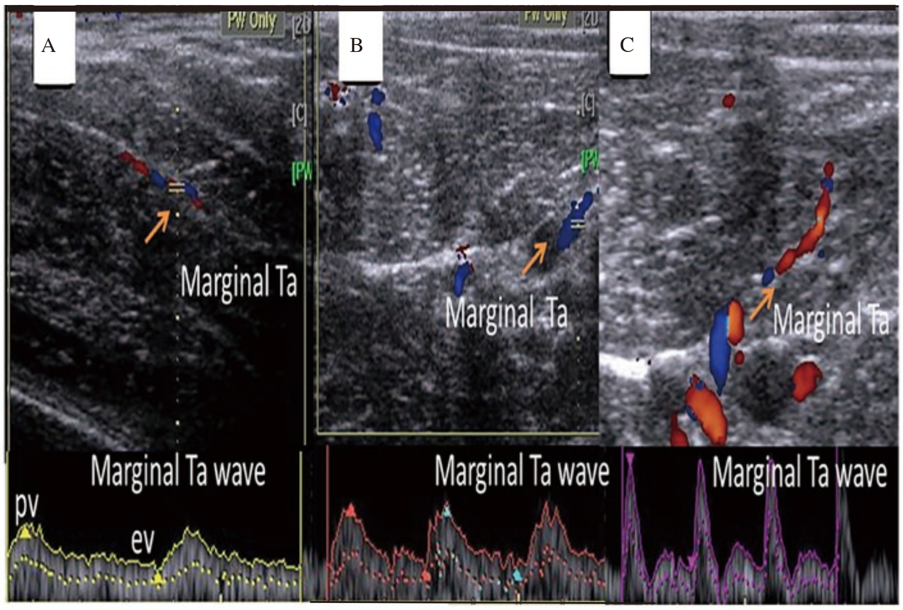

Figure 2. Color mode ultrasonograms show the measurement of marginal testicular artery (Marginal Ta; brown arrow) with pulsed wave Doppler spectral graph in order to measure Doppler indices from PSV and EDV in the bulls aged 1 year (A), 3 years (B), and 6 years (C).

2.5. Statistical analysis

Data were analyzed by SPSS version 20 (IBM). After confirming data normality, analysis of variance was used to measure the comparisons between the three different age groups. Afterwards, *post-hoc* test (Tukey's HSD) was used to identify the significant difference between three groups with pairwise comparison (Group Ⅰ *versus* Group Ⅱ, Group Ⅰ *versus* Group Ⅲ, and Group Ⅱ *versus* Group Ⅲ). Data were expressed as mean±standard deviation (mean±SD). We also performed a Pearson correlation between testicular width, mediastinal thickness, hemodynamics of the supratesticular branch, and hormonal levels in Baladi bulls. $P<0.05$ was considered to demonstrate statistically significant differences.

2.6. Ethics statement

This study was approved by the Veterinary Medical Committee for Animal Use (VET-CU- 24112021456) at the Faculty of Veterinary Medicine Cairo University.

3. Results

3.1. Dimensions of the testes, circumference of the scrotum and mediastinal line thickness

There was no significant difference in testicular dimensions between the right and left testicles of bulls, so data of both testicles were merged. Testicle length, width, and volume increased significantly in group Ⅲ compared to group Ⅱ and group Ⅰ [for length: (5.23±0.02) cm, (4.99±0.01) cm, and (4.25±0.04) cm in group Ⅲ to Ⅰ, respectively; for width: (4.47±0.24) cm, (4.05±0.03) cm and (3.18±0.11) cm in group Ⅲ to Ⅰ, respectively; for volume: (341.21±11.21) cm^3, (337.71±3.29) cm^3, and (265.11±11.74) cm^3 in group Ⅲ to Ⅰ, respectively] (P<0.05). The bulls in group Ⅲ had significantly higher mediastinum thickness (mm) and scrotal circumference (cm) than group Ⅰ (P<0.05) but there was no significant difference between group Ⅱ and group Ⅲ (Table 1).

3.2. Testicular artery Doppler analysis

Bulls of group Ⅱ showed a significant lower Doppler resistance and pulsatility indexes compared to group Ⅰ and group Ⅲ (0.47±0.01 *vs.* 0.78±0.02 and 0.64±0.01; 0.84±0.01 *vs.* 1.52±0.02 and 1.22±0.01) (P<0.05). Supra and marginal end diastolic velocities (cm/s) were significantly elevated in bulls of group Ⅱ compared to the other two groups (Supra: 9.99±0.02 *vs.* 5.87±0.01 and 7.89±0.01; Marginal: 9.65±0.04 *vs.* 6.02±0.01 and 6.89±0.01) (P<0.05). Time average velocity (cm/s) was significantly elevated in group Ⅱ compared to the other two groups (P<0.05). Testicular echogenicity and pixel heterogeneity were significantly lower in bulls of group Ⅱ compared to group Ⅰ and group Ⅲ (P<0.05) (Table 1).

Table 1. Testicular dimensions, computer assisted analysis, hormonal concentrations, and testicular vascularity in different locations in Baladi bulls aged 1, 3, and 6 years.

Items	Group Ⅰ (*n*=7; 1 year old)	Group Ⅱ (*n*=7; 3 year old)	Group Ⅲ (*n*=7; 6 year old)	*P*-value
Testicular morphometry				
Length, cm	4.25±0.04[c]	4.99±0.01[b]	5.23±0.02[a]	0.011
Width, cm	3.81±0.11[c]	4.05±0.03[b]	4.47±0.24[a]	0.011
Depth, cm	4.11±0.01	3.99±0.01	3.38±0.01	0.251
Volume, cm^3	265.11±11.74[c]	337.71±3.29[b]	341.21±11.21[a]	0.011
Mediastinum thickness, mm	2.56±0.01[b]	2.97±0.04[a]	3.01±0.10[a]	0.030
Scrotal circumference, cm	23.74±0.32[b]	31.14±2.01[a]	33.61±2.04[a]	0.030
Testicular artery hemodynamics				
Supratesticular artery PSV, cm/s	16.88±1.12	16.98±0.01	17.05±0.11	0.161
Supratesticular artery EDV, cm/s	5.87±0.01[c]	9.99±0.02[a]	7.89±0.01[b]	0.001
Supratesticular artery PI	1.52±0.02[a]	0.84±0.01[c]	1.22±0.01[b]	0.001
Supratesticular artery RI	0.78±0.02[a]	0.47±0.01[b]	0.64±0.01[a]	0.001
Supratesticular artery TAMV, cm/s	2.64±0.02[b]	3.55±0.02[a]	2.87±0.02[b]	0.020
Marginal testicular artery PSV, cm/s	15.98±1.13	16.62±0.08	17.01±0.01	0.231
Marginal testicular artery EDV, cm/s	6.02±0.01[c]	9.65±0.04[a]	6.89±0.01[b]	0.001
Marginal testicular artery PI	1.42±0.02[a]	0.79±0.01[c]	1.03±0.01[b]	0.001
Marginal testicular artery RI	0.77±0.02[a]	0.38±0.01[b]	0.66±0.01[a]	0.001
Marginal testicular artery TAMV, cm/s	2.12±0.02[b]	2.99±0.02[a]	2.31±0.02[b]	0.020
Pixel further evaluation				
Echogenicity, NPvs	111.14±0.01[a]	61.21±0.04[c]	77.82±1.05[b]	0.030
Heterogeneity of pixel, Sd of NPvs	31.11±2.81[a]	19.31±0.25[c]	25.54±0.98[b]	0.030
Hormonal concentrations				
Testosterone, ng /mL	0.95 ± 0.01[c]	1.96±0.08[b]	3.33±0.11[a]	0.040
Estradiol, pg /mL	22.17±0.01[c]	46.23±0.05[a]	39.52±4.26[b]	0.001
Testosterone/Estradiol ratio	0.15±0.11[b]	0.79±0.02[ab]	0.91±0.01[a]	0.040
FSH, ng/mL	29.06±0.22	31.02±0.02	30.71±2.12	0.113
LH, ng/mL	5.75±0.15	5.21±0.51	4.55±0.01	0.090
Nitric oxide metabolites, μmol /L	29.11±2.05[c]	67.74±0.04[a]	43.14±1.11[b]	0.001

Tukey's HSD is used to identify the significant difference between three groups with pairwise comparison. Data are expressed as mean±SD. The different superscripts (a, b, c) within a row are significantly different (P<0.05). PSV: peak systolic velocity, EDV: end diastolic velocity, PI: pulsatility index, RI: resistance index, TAMV: time average maximum velocity, NPvs: numerical pixel value, Sd: standard deviation of pixel, FSH: follicle stimulating hormone, LH: luteinizing hormone.

Table 2. Pearson correlation coefficient (*r*) between scrotal circumference, testicular width, mediastinum thickness, supratesticular artery hemodynamic, and hormonal levels in Baladi bulls.

Variables	Scrotal circumference	Testicular width	RI	PI	PSV	FSH	LH	Estradiol	Testosterone	Nitric oxide metabolites
Mediastinum thickness	0.68**	0.79**	-0.91**	-0.84**	0.55*	0.56*	0.85**	0.71**	NS	0.88**
Scrotal circumference		0.58*	-0.71**	-0.91**	0.56*	0.57*	0.66*	0.59*	-0.64*	0.77**
Testicular width			NS	NS	0.87**	NS	NS	0.55*	NS	0.68**
RI				0.81**	-0.61**	NS	NS	-0.71**	-0.81**	-0.92**
PI					-0.81**	NS	NS	-0.91**	-0.81**	-0.71**
PSV						NS	NS	0.78**	-0.57*	0.59*
FSH							-0.61**	0.77**	NS	0.65*
LH								NS	-0.66*	0.58*
Estradiol									0.77**	0.88**
Testosterone										-0.78**

*Means correlation is significant at $P<0.05$; **Means correlation is significant at $P<0.001$. RI: resistance index, PI: pulsatility index, PSV: peak systolic velocity, FSH: follicle stimulating hormone, LH: luteinizing hormones, NS: none.

3.3. Hormone levels

Estradiol and nitric oxide metabolites were significantly increased in bulls of group Ⅱ compared to group Ⅰ and group Ⅲ [(46.23±0.05) pg/mL *vs.* (22.17±0.01) pg/mL and (39.52±4.26) pg/mL for estradiol, and (67.74±0.04) μmol/L *vs.* (29.11±2.05) μmol/L and (43.14±1.11) μmol/L for nitric oxide metabolites] ($P<0.05$), respectively as shown in Table 1. On the other hand, testosterone level was significantly increased in the bulls of group Ⅲ [(3.33±0.11) ng/mL] when compared to the other two groups ($P<0.05$) (Table 1).

3.4. Correlation between scrotal circumference, testicular width, mediastinum thickness, supratesticular artery hemodynamic, and hormonal levels in Baladi bulls

Doppler indices (resistance and pulsatility indexes) of supratesticular branch positively correlated ($r=0.81$; $P<0.001$), but both were negatively correlated with peak systolic velocity (for resistance $r=-0.61$, for pulsatility $r=-0.81$; $P<0.001$), estradiol (for resistance $r=-0.71$, for pulsatility $r=-0.91$; $P<0.001$), testosterone (for resistance $r=-0.81$, for pulsatility $r=-0.81$; $P<0.001$), nitric oxide metabolites (for resistance $r=-0.92$, for pulsatility $r=-0.71$; $P<0.001$), and scrotal circumference (for resistance $r=-0.71$ and for pulsatility $r= -0.91$; $P<0.001$). All the Pearson's correlations are showed in the Table 2.

4. Discussion

This current study revealed that both color and pulsed wave Doppler modes could help in determining the testicular usefulness in Baladi bulls at distinctive ages as studied in canine[25], stallions[17], and jacks[24]. As there was no information between the tissue inside and cleared outside as detailed in numerous species[26]. Considering this, testicular volume, length, and width were essentially determined in the age groups of 3 and 6 years old. Camela *et al*[27] also revealed the checked increase in postpubertal rams' testicular length and width only with respect to the testicular depth, and the decay in depth with age progression might be correlated with the testicular size elongation in the seminiferous tubules in arrange to uncover a more noteworthy surface for a thermoregulatory component in bulls[28,29]. In expansion, the scrotal circumference assessment is certainly a valuable marker of testicular measure, sperm generation, and age at adolescence, which may influence sperm production[30]. The thickness of mediastinum is essentially raised in adult compared to young ages. A study[31] showed that mediastinum line thickness was classified into lower, moderate, and highest echogenic, as any height within the line thickness was specifically related to age, which has been already discussed in sheep[20].

In this study, no difference in peak systolic velocity of contraction was observed between different age groups because the distribution of blood flow systemically helps in the control of animal temperature within the body. This suggests a decrease in testicular blood flow. Both Doppler resistance and pulsatility indices were increased throughout the arterial vasoconstriction[24,25] because systolic peaks decrease or become constant at moderate rates[25,32]. When there was an increase in peak systolic velocity, this means that both Doppler indices are declined as there was an inversely relation between both indices and blood flow velocities such as peak systolic and end diastolic points. Another study reported that both average and maximum velocities may be slower in animals[33]. Moreover, in bulls, lower resistance index values at higher velocity could be involved in achievement of spermatogenesis mechanism in addition to their role in thermoregulation[29,34].

The anatomical locations of testicular artery with different blood supply had been confirmatory that those arteries such as the supratesticular artery and marginal branch contained different blood flow velocity pattern as the blood flow velocity was elevated in the supra branch compared to the marginal one. The bull supratesticular artery is tortuous like camels[26]. In this investigation, both arteries resistance and pulsatility indices were declined in group Ⅱ bulls with three years old. Similarly, a study[21] concluded that all male bulls with 1 year old showed an elevation in resistance and pulsatility indexes than other ages. The decline in the resistance index at the stage of puberty could be associated with the vasodilatation process[18,35–38]. The decrease in pixel evaluation of echogenicity and heterogeneity was additionally determined in three years old bulls; this may be associated with many histological occasions in the puberty achievement with growing the cell density and germ cells as formerly stated in bucks[39] and rams[40]; in addition, a report confirmed a decline in each echotexture parameters[9].

The lower in resistance and pulsatility indexes corresponds to improved vascular perfusion and testosterone concentrations, in addition to the improved testicular volume[41]. In addition, higher degrees of testosterone could lead to increase the diameter of the vascular lumen, therefore leading to the marked discount in the resistive vascularization[17,35]. In addition, the hemodynamic value is essential for new research in Baladi bull. Gonadotropin-releasing hormone could be important in the growth achievement[42], which reflects on the spermatogenesis technique and gonadal improvement by the action of both FSH and LH as the whole spermatogenesis cannot be executed without each testosterone and LH presentation[23]. This explains the relation among pituitary hormones (FSH and LH) and steroids (estradiol). Scrotal circumference and thickness of mediastinum, besides the estrogen and nitric oxide metabolites, are negatively correlated with each Doppler indices (resistance and pulsatility indexes). While nitric oxide became correlated undoubtedly with scrotal circumference, thickness of mediastinum, testicular width, and all hormones beside each Doppler indice of the supratesticular artery, with a marked widespread elevation of nitric oxide with estradiol in three-year old bulls in comparison to other different ages. This can be defined *via* way of means of the development of puberty with an increase in vasodilator mechanisms that also can have an effect on estradiol and testosterone levels. Estrogen production is multifaceted in that it suppresses oxidative pressure and will increase endothelium-established vasodilation[24], with many loose radicals of nitric oxide as vasodilators. Because it can inactivate activity[43], estradiol therefore has a vital impact on nitric oxide metabolites[44], which may, for the primary time, have an effect on male Doppler indices and motor parameters Doppler speed as a dating among estradiol and testicular hemodynamics is vital[23], so there may be a marked bad correlation among estradiol and both Doppler indices due to the fact there may be an inverse relation among a lower in Doppler indices and an increase in peak systolic velocity[23]. The shortage of correlation among testosterone and testicular width with thickness of mediastinum that replicate the loss of relationships with testicular echogenicity can be related to the quantity of Leydig cells in bull[45]. The synergistic movement among estradiol and nitric oxide is because of nitric oxide metabolites involvement in the initiation of erection manner of the corpus cavernous[15]. Also, an endothelial shape of nitric oxide is particularly located in the frame of the penis[24].

The main vital limitation of this study is the workability, as the usage of Doppler mainly in Baladi bull is still limited because of the dearth of anatomical arterial supply; there are numerous limitations as the motion of animals that supply a completely awful photograph with hazy dimension because of the presence of many colors acquiring all through motion, and incorrect Doppler shift, and consequently we need to use a widespread 45° attitude for insonation. Finally, it is hard to differentiate among artery and vein besides *via* way of means of the spectral graph because the arterial graph resembles the cardiac cycle; however, the venous graph supplies the best blood flow speed with no systolic and diastolic points[23].

In conclusion, bulls with three years old reflect an excellent flair for thermoregulation by the declination in both Doppler indices and an elevation of estradiol and nitric oxide metabolites compared to other ages, as these parameters can be taken into recognition while appearing the animal choice for breeding as the parameters of the one provide a wonderful check out on testicular hemodynamic and good reproductive circulation.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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The study received no extramural funding.

Authors' contributions

Elshymaa A. Abdelnaby made data curation and Doppler scanning, and carried out the writing, reviewing and editing of the manuscript. Ibrahim A. Emam carried out data curation, methodology, writing and reviewing of the manuscript, and conducted Doppler examination and animal avaliabiliy.

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