OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

Reproductive Hormonal Levels and Nitric Oxide Levels as Guides of Pubertal Reproductive Development in Relation to Testicular Width and Hemodynamics in Baladi Bucks

Khlood G. Abdelkhalek*, Aly B. A. Badawy, Mohamed Fathi, Elshymaa A. Abdelnaby

Theriogenology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

Abstract | Pubertal alterations in plasma levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol 17β (E2), testosterone (T), and serum nitric oxide metabolites (NOMs) were measured in 5 Baladi bucks weighting (13–15 kg; with a body condition score of 3.5 ± 0.01) from 4-months- to 10-months-old in relation to testicular hemodynamics. Testicular morphometry, scrotal circumference, Doppler examinations, image analysis, and blood sampling were performed on a weekly basis. Results showed that FSH level was elevated (P<0.05) firstly from 5-months to 6.5-months-old, and then a second elevation was reported at 9.5-months-old, both E2 and NOMs levels were increased significantly (P<0.05) from 8-months to 10-months-old. Testosterone level showed a significant (P<0.05) increase from 8.5-months to 10-months-old, while LH level was elevated from 6.5-months to 7.5-months-old. Both Doppler indices; resistive index (RI) and pulsatility index (PI) were negatively correlated with PSV (r=-0.52 and -0.68; P<0.05), FSH (r=-0.88 and -0.64), E2 (r=-0.82 and -0.71; P<0.001), LH (r=-0.74 and -0.84), testicular width (r=-0.55 and -0.71), and testicular colored area with blue color (r=-0.85 and -0.75; P<0.001) for both RI and PI, respectively, while there was a positive significant correlation between both indices and T (r=0.66 for RI and 0.85 for PI; P<0.05). NOMs was correlated positively with FSH (r=0.74), E2 (r=0.88), LH (r=0.82; P<0.001) and testicular colored area toward probe before puberty (r=0.61), while after puberty, there was a positive significant correlation between both Doppler indices and testicular colored area toward probe with red color (r=0.74 and 0.66), FSH (r=0.69 and 0.64), LH (r=0.51 and 0.54) for both RI and PI, respectively. In conclusion, pituitary hormones (FSH and LH) and steroids (E2 and T) were changed around age of puberty that likely reflects their roles in regulation of spermatogenesis with the attainment of buck sexual developmental capacity.

Keywords | Estradiol, LH, FSH, Resistive index, Testosterone

Received | October 07, 2021; **Accepted** | November 01, 2021; Published | January 05, 2022
***Correspondence** | Khlood G Abdelkhalek, Theriogenology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt; **Email:** khlooud106@gmail.com
Citation | Abdelkhalek KG, Badawy ABA, Fathi M, Abdelnaby EA (2022). Reproductive hormonal levels and nitric oxide levels as guides of pubertal reproductive development in relation to testicular width and hemodynamics in baladi bucks. Adv. Anim. Vet. Sci. 10(2): 236-243.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.2.236.243
ISSN (Online) | 2307-8316

INTRODUCTION

Goats are considered a valuable source of milk and meat in many countries, so studying the different strategies of reproductive technologies is an area of critical importance in improving goat production (Miller and Lu, 2019). Breeding soundness assay of males is a crucial component in predicting and election of the best performing domestic farm animals, like bucks, as sires (Singla et al., 2017), from this point, it is obligatory to determine the related fertility biomarkers at pre- and post-pubertal age to expand the male animal productivity. The onset of puberty is noticed when the buck firstly exhibits non-ordinary sexual impulses that manifested with releasing ejaculate containing motile sperms (Helbig et al., 2007), variable factors could influence this onset such as breed, nutrition, weight, and

season. These variables have a role in stimulating the hypothalamus-pituitary- gonadal axis to attain their endocrine role (Fabre et al., 2015). The pituitary hormones are of an important key role in modifiable male reproductive functions; such as follicle stimulating hormone (FSH) controls the process of spermatogenesis, while luteinizing hormone (LH) stimulates male sex hormone formation. Both FSH and LH were elevated during first 20 weeks of age then declined coincidently with the elevation of serum testosterone (T); this suggests the negative steroid feedback effects. Additionally, it appeared that T is an important regulator of sperm maturation (Chakraborty et al., 1989). Testosterone assay is useful in selection of young sires, and characterizing their sexual maturity, plasma T concentrations is the potential biomarker that triggers spermatogenic activity (Júnior et al., 2012). Estradiol (E2) has a vasodilatation action on the testicular artery vascularization in humans (Tostes et al., 2003), adult male dogs (Zelli et al., 2013; Abdelnaby et al., 2021a), and bucks (Samir et al., 2018), this vasodilatation is associated with the marked decline in both Doppler indices; resistive index (RI) and pulsatility index (PI). In addition, nitric oxide metabolites (NOMs) have been described to contribute in the mechanism of vasodilatation via the relaxation of cavernous tissue that helps in the erection of the penis (Gur et al., 2015).

A valid testicular hemodynamic architecture is essential for the transport of the previously mentioned endocrine hormones, oxygen and nutrients to the testis (Rawy et al., 2021). Doppler ultrasonography is considered a perfect method to evaluate the testicular blood supply and dynamics of flow parameters (Strina et al., 2016). Recently, it has been suggested as a good predictor of sperms quality.

Testicular blood perfusion (TBP) is essential to the bio function of testis to attain the higher metabolic rate that occurs in the seminiferous tubules with the low concentration of available oxygen (Rawy et al., 2021). Color Doppler ultrasonography aids in calculation of testicular blood supply in various animal species (Rawy et al., 2021; Abdelnaby et al., 2021a; Abdelnaby et al., 2021b). Many studies had assessed the importance of TBP using color Doppler ultrasonography to illuminate its potential role for selection of a sound sire (Samir et al., 2018).

Doppler indices are considered the most sensitive parameters of TBP and penile flow than end diastolic velocity parameters, this could be related to the more sensitive information they send on vascular impedance to blood flow (Ginther, 2007; Serin et al., 2010; Abdelnaby et al., 2021c). With increasing in both Doppler indices values, the vascular resistance of blood flow elevates, and in turn, the blood supply decreases (Bollwein et al., 2016). The aim of our study is to demonstrate the changes of reproductive hormonal values and NO levels as guides of pubertal reproductive development in relation to testicular width and hemodynamics in Baladi bucks.

MATERIALS AND METHODS

Ethical approval number

This current study was ethically approved by the institutional committee for animal use protocol (IACUC-AUP-VET CU 24112020246)

Animal feeding and housing

This study was performed at the Faculty of Veterinary Medicine, Cairo University. Five pre-pubertal Baladi bucks were brought at age of 4 months-old, all bucks were reared till age of 10-months-old). All bucks were regularly vaccinated. And all bucks were undergoing routine clinical examinations, scrotal visualization, and testicular palpation (Kühn et al., 2016). Concentrated feed was created from soybean and corn with 19% crude protein and the feed mixture was supplied in the amount of 500 g / animal. In addition, all animals had unrestricted access to water, mineral salt licks, and unrestricted corn silage.

Experimental design

All bucks were subjected to a weekly B-mode and Doppler testicular ultrasound scanning, as well as blood samplings for further hormonal assay throughout the period from 4-months-old to 10-months-old. The bucks body weight ranged from 13–15 kg and the mean of their body condition scores was 3.5 ± 0.01 (1–5 points scoring system) at 4-months-old (the beginning of the study). The bucks were monitored weekly for penis detachment until they reach puberty by the complete separation of penis (de Souza et al., 2011).

Ultrasonography

Examination by ultrasound was achieved once/week starting with a B-mode ultrasound scanner (EXAGO, France) linked to a 5-7.5 MHz linear array transducer then the Doppler icon was activated to measure the testicular vascular perfusion (Abdelnaby et al., 2021b).

Determination of testicular width, mediastinum thickness, and scrotal circumference: Testicular width (TW; cm) was determined using the transverse section of both testes (separately) with the electronic caliper on the frozen B-mode image. Using a cloth measuring tape (Raji and Ajala, 2015), both testes were pushed downwards, and the circumferences of the scrotum (cm) were measured three times and the means of these measurements were calculated in centimeters. Finally, mediastinum thickness was measured in the frozen image b mode.

Doppler assessment

The device's automatic settings were as follow; 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., 2021c). The measured Doppler parameters included RI, PI, peak systolic velocity (PSV; cm/sec) and end diastolic velocity (EDV; cm/sec) (Ribeiro et al., 2020; Abouelela et al., 2021). Testicular colored area away (blue) and toward probe (red) were determined by the adobe Photoshop magnetic lasso tool as shown in (Figure 1).

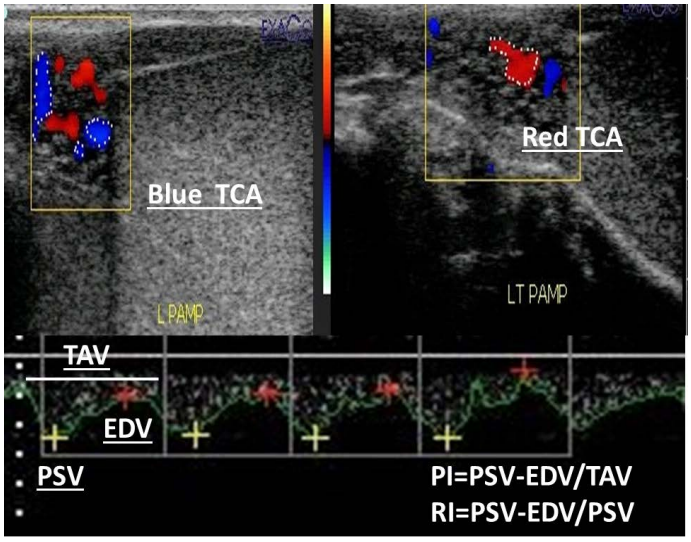

Figure 1: Color mode ultrasonograms showed the measurement of both blue and red testicular colored areas (TCA) either away or toward the probe with the presence of spectral wave graph that determined how to measure both Doppler indices (PI; pulsatility index and RI; resistive index) through different automatic equations using peak systolic velocity (PSV; cm/sec), end diastolic velocity (EDV) and time average to complete the cardiac cycle (TAV; cm/sec).

Blood sampling and serum goat hormonal analysis

Before ultrasound examination, blood samples were collected from the jugular vein in plain and heparinized vacutainer tubes (5-ml). All samples were centrifuged at 3000 rpm for 10 min then serum and plasma samples were stored at -20 °C until further assessment. Species-specific T, E2, FSH, and LH were determined from plasma samples by ELISA (SunLong Biotech Co.,LTD CHINA ,Catalogue Number:SL00116Sp) kits with test sensitivity were (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 measured hormones. For measuring NO, 100 µL of serum samples were mixed with equal volumes of Griess reagent and incubated for 18-20 minutes at room temperature (Abdelnaby et al., 2021b).

Statistical analysis

All data were assessed for normality using the Shapiro-Wilk test. T, E2, FSH, LH, and NOMs were analyzed using one-way ANOVA. The Duncan multiple range test was used to differentiate between significant means ($P<0.05$). Pearson correlation coefficient was measured between testicular blood flow area, Doppler indices (PI and RI) and hormonal levels (T, LH, FSH, E2, and NO) before and after puberty. Correlations that are ≥ 0.55 and 0.66 were significant at $P<0.05$ and $P<0.001$, respectively

RESULTS

FSH and LH changes between 4-months- and 10-months of age

In the present study, at the age period of 4-months to 8-months-old, there was a first significant ($P<0.05$) elevation of FSH which was observed from 5-months- to 6.5-months-old with values (Mean ±SEM) of 35.96 ±0.32 and 46.04 ±0.11, respectively, while after 8-months of age the second significant elevation ($P<0.05$) was observed at 9.5-months-old with value (Mean ±SEM) of 34.25 ±0.33 (Figure 2). While LH levels showed a marked significant elevation from 6.5-months to 7.5-months-old with values (Mean ±SEM) of 7.36 ±0.32 and 8.14 ±0.11, respectively. then the levels of this hormone declined after this age period (Figure 2).

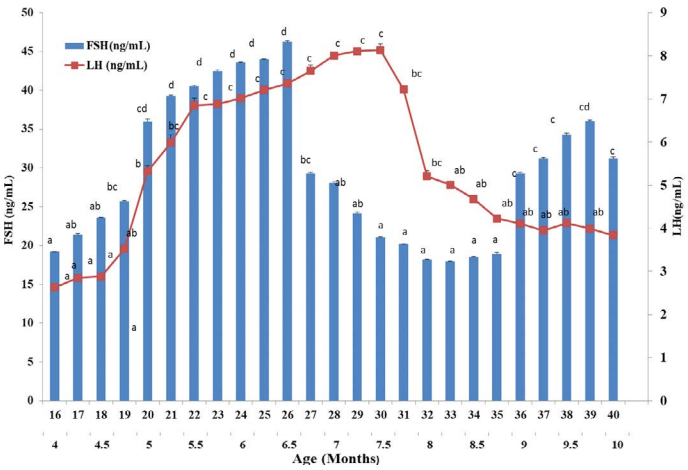

Figure 2: Levels (Mean ±standard error of mean (SEM)) of follicle stimulating hormone (FSH; ng/mL), and o luteinizing hormone (LH; ng/mL) in Baladi bucks between 4- and 10-months-old. Different superscripts indicate significant differences ($P<0.05$).

E2, T, and NO changes between 4-months and 10-months of age

Levels of E2 showed a pattern of significant ($P<0.05$) decline from 4-months- to 7.5-months-old while there was a significant ($P<0.05$) elevation from 8-months- to 10-months-old with values (Mean ±SEM) of 39.34 ±3.21 and 47.66 ±2.15, respectively. While the levels of NOMs

Table 1: Pearson correlation coefficient between testicular blood flow area, Doppler indices, hormonal levels (serum testosterone, luteinizing hormone, follicular stimulating hormone, estradiol), and nitric oxide metabolites before puberty in Baladi bucks.

Parameter	SC	TW	T CA away(-blue)	T CA toward(red)	RI	PI	PSV	T	FSH	E2	LH	NOMs
MT	0.65	0.45	0.96*	-0.58*	-0.53*	-0.41*	0.66*	-0.32	0.93**	0.87**	0.78**	-0.65*
SC		0.57*	0.97**	-0.64	-0.66**	-0.65**	0.45*	-0.54	0.84**	0.83**	0.86**	-0.21
TW			0.41	-0.64**	-0.55*	-0.71**	0.48	-0.68	0.74**	0.71**	0.68**	-0.55*
T CA away(blue)			`	-0.66	-0.85**	-0.75**	0.65	-0.55	0.85**	0.72**	0.66**	-0.52*
TCA toward(red)					0.73**	0.75**	-0.82**	0.85**	-0.92**	-0.63*	-0.65*	0.61*
RI						0.67	-0.52*	0.66*	-0.88**	-0.82**	-0.74**	0.85**
PI							-0.68*	0.85*	-0.64*	-0.71**	-0.84**	0.65*
PSV								-0.25	0.99**	0.96**	0.65*	-0.28
T									0.71**	0.86**	0.96	-0.93**
FSH										0.71**	0.64**	0.74**
E_2											0.25*	0.88**
LH												0.82**

** Means correlation is significant at p<0.001,* means correlation is significant at P<0.05
SC=scrotal circumference, MT=mediastinum thickness, TW= testicular width, TCA=testicular colored area, RI=resistance index, PI=pulsatility index, PSV= peak systolic velocity, T= testosterone, FSH=follicle stimulating hormone, LH= luteinizing hormones, E2=Estradiol, NOMs=nitric oxide metabolites

Table 2: Pearson correlation coefficient between testicular blood flow area, Doppler indices, hormonal levels (serum testosterone, luteinizing hormone, follicular stimulating hormone, estradiol), and nitric oxide metabolites after puberty in Baladi bucks.

Parameter	SC	TW	T CA away(-blue)	T CA toward(red)	RI	PI	PSV	T	FSH	E2	LH	NOMs
MT	0.67	0.49	0.94*	-0.44*	-0.75**	-0.58*	0.56*	-0.45	0.73**	0.88**	0.79**	-0.76*
SC		0.67*	0.55**	-0.47	-0.42	-0.47	0.65*	-0.74	0.54*	0.55*	0.51*	-0.26
TW			0.65	-0.536	-0.65*	-0.68*	0.41	0.63	-0.63*	-0.59*	-0.59*	0.95**
T CA away(-blue)				-0.55	-0.55*	-0.65*	-0.42	0.96	-0.75**	-0.72**	-0.77**	-0.57*
TCA toward(red)					0.74**	0.66*	0.41	-0.66	-0.56*	-0.53*	-0.62*	-0.65*
RI						0.77	-0.99**	-0.32	0.69*	-0.65**	0.51*	-0.78**
PI							-0.92**	-0.51*	0.64*	-0.59*	0.54*	-0.87**
PSV								0.56*	0.55*	0.74**	-0.55*	0.81**
T									0.89**	-0.77**	-0.58*	-0.64*
FSH										-0.81**	0.82**	-0.68**
E_2											-0.65*	0.88**
LH												-0.77**

** Means correlation is significant at P<0.001,* means correlation is significant at P<0.05
SC=scrotal circumference, MT=mediastinum thickness, TW= testicular width, TCA=testicular colored area, RI=resistance index, PI=pulsatility index, PSV= peak systolic velocity, T= testosterone, FSH=follicle stimulating hormone, LH= luteinizing hormones, E2=Estradiol, NOMs=nitric oxide metabolites

were significantly (P<0.05) increased from 8-months- to 10-months-old with values (Mean ±SEM) of 61.05 ±0.65 and 69.55 ±2.12, respectively in comparison to the age from 4-months- to 8-months-old as shown in Figure 3. T levels

showed a pattern of linear elevation from 4-months-old to 10-months-old with values (Mean ±SEM) of 1.56±0.01 and 4.99±0.12, respectively. And a significant (P<0.05) marked increase from 8.5-months- to 10-months-old with values (Mean ±SEM) of 4.68±0.22 and 4.99±0.18, respectively (Figure 3).

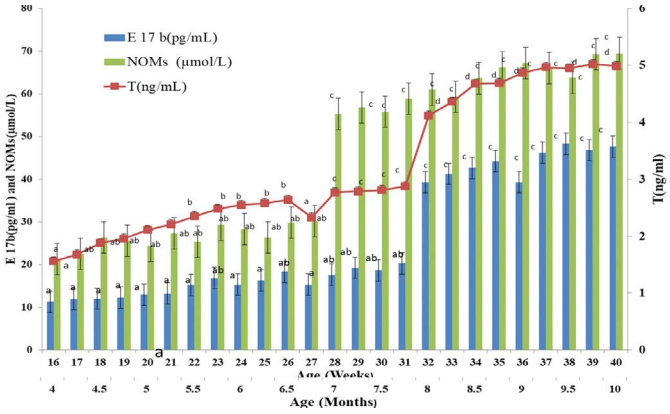

Figure 3: Levels (Mean ± standard error of mean (SEM) of estradiol (E_2 17β; pg/mL), testosterone (T; ng/mL), and nitric oxide metabolites (NOMs; µmol/L) in Baladi bucks between 4-months and 10-months-old. Different superscripts indicate significant differences (P <0.05).

Pearson correlation coefficient between testicular blood flow area, Doppler indices and hormonal levels before and after puberty

Both Doppler indices (RI and PI) were negatively correlated with PSV (r=-0.52 for RI and r=-0.68 for PI; P<0.05), FSH (r=-0.88 for RI and r=-0.64 for PI), E2 (r=-0.82 for RI and r=-0.71 for PI; P<0.001), LH (r=-0.74 for RI and r=-0.84 for PI), mediastinum thickness (r=-0.53 for RI and r=-0.41 for PI; P<0.05), scrotal circumference (r=-0.66 for RI and r=-0.65 for PI), Testicular width (r=-0.55 for RI and r=-0.71 for PI; P<0.05), and testicular colored area away from probe marked by blue color (r=-0.85 for RI and r=-0.75 for PI; P<0.001), while there were positive significant correlations between both indices and T hormone level (r=0.66 for RI and r=0.85 for PI; P<0.05), NOMs (r=0.85 for RI and r=0.65 for PI; P<0.05), and testicular colored area toward probe marked with red color (r=0.73 for RI and r=0.75 for PI; P<0.001) before puberty as shown in Table 1.

FSH, LH and E2 before puberty (4-months- to 6.5-months-old) were positively correlated (P<0.001) with all other measured parameters except both Doppler indices, testicular colored area away probe (r=-0.92 for FSH, r=-0.65 for LH, and r=-0.63 for E2; P<0.05) as shown in Table 1. The levels of NOMs were positively correlated with FSH (r=0.74; P<0.001), E2 (r=0.88; P<0.001), LH (r=0.82; P<0.001) and testicular colored area away probe before puberty (r=0.61; P<0.05), while correlated negatively with mediastinum thickness (r=-0.65; P<0.05), testicular width (r=-0.55; P<0.05), and T levels (r=-0.93; P<0.001) (Table 1).

After puberty, both Doppler indices (RI and PI) were negatively correlated with PSV (r=-0.99 for RI and r=-0.92 for PI), E2 levels (r=-0.65 for RI and r=-0.59 for PI), T levels (r=-0.32 for RI and r=-0.51 for PI), NOMs levels (r=-0.78 for RI and r=-0.87 for PI), mediastinum thickness (r=-0.75 for RI and r=-0.58 for PI), Testicular width (r=-0.65 for RI and r=-0.68 for PI), and testicular colored area away from probe marked by blue color (r=-0.55 for RI and r=-0.65 for PI;) Table 2.

Positive significant correlations were reported between both Doppler indices and testicular colored area toward probe marked with red color (r=0.74 for RI and r=0.66 for PI), FSH (r=0.69 for RI and r=0.64 for PI), LH (r=0.51 for RI and r=0.54 for PI) as shown in Table 2. FSH, LH and E2 were positively correlated (P<0.001) after puberty with all other measured parameters except TW (r=-0.63 for FSH, r=-0.59 for LH, and r=-0.59 for E2) and testicular blood flow away (r=-0.75 for FSH, r=-0.77 for LH, and R=-0.72 for E2) and toward probe (r=-0.56 for FSH, r=-0.62 for LH, and r=-0.53 for E2) as shown in Table 2. The levels of NOMs were negatively correlated with all other measured parameters except TW (r=0.95; P<0.001), PSV (r=0.81; P<0.001), E2 (r=0.88; P<0.001; Table 2).

DISCUSSION

To determine the age of puberty, signs of spermatogenesis achievement were considered in this work. As Baladi bucks' puberty was achieved at 26-29 weeks with an average 27 weeks with the observation of penile separation at the same age. Similar findings have been reported on other breeds of goats and sheep and indicated that the puberty was reached at around 22 weeks (Shaaeldin et al., 2019). However, other breeds of goat and sheep reach puberty between 16 and 21 weeks. The pattern of FSH elevation in this study was in line with others (Walton et al., 1978) and consistent with the view that FSH increases before onset of puberty (Sanford et al., 1984).

The findings revealed that E2 and NO had high levels compared to FSH, which had low levels during the 7.5-months- to 9-months-old, while T and LH had an episodic pulsatile pattern, which could be linked to the progression of puberty age (Renaville et al., 1990). This indicates that hormone levels rise from adolescence to puberty, as such values may peak a few weeks before puberty (Renaville et al., 1990).

Puberty, sexual maturity and succeeding fertility in bucks

are controlled by the hypothalamic– pituitary–gonadal axis, as the axis harmonized by neuroendocrine GnRH in the brain (Marques et al., 2018). The main pituitary hormones (FSH and LH) are critical to maintain the gonadal development via spermatogenesis stimulation. Full spermatogenesis cannot be talented without the effect of LH that enables the T production (Smith and Walker, 2014). This explains the positive correlation of the pituitary hormones; FSH, LH and E2 with all other measured parameters (SC, MT, Doppler indices) before and after puberty except testicular colored area, and TW after puberty.

While NOMs was correlated positively with all parameters except TW and T and testicular colored area before puberty and LH, FSH, testicular colored area, Doppler indices, and Scrotal circumference after puberty. The negative correlation between NOMs and FSH, LH and T could be explained by the advancement of onset of puberty with increase vasodilation mechanism that could also affect estradiol and testosterone levels the increase of nitric oxide could be mediated at first by a marked elevation of estradiol as the mechanism by which estradiol could improve endothelial NOS production is pleiotropic estrogen nature, by suppressing the oxidative stress to increase endothelium-dependent vasodilatation (Levin, 2009; Abdelnaby et al., 2020) as many free radicle could inactivate the activity of NO as a vasodilator (Stirone et al., 2003), therefore estradiol delivers an important impact on NOS function (Strehlow et al., 2003; Miller and Mulvagh, 2007), this could affect for the first time in Baladi bucks on Doppler indices and Doppler velocity parameter as the relation between E2 and testicular hemodynamics is very critical (McNeill et al., 2002; Abdelnaby et al., 2021b), therefore there was a marked negative correlation between E2, and both Doppler indices as there was an inverse relationship between the reduction of Doppler indices and the elevation of PSV (cm/sec) (Abdelnaby et al., 2021b).

The strong negative correlation between T and NOMs before and after puberty could be explained by the direct action of T on vascular tone by activation of thromboxane and ion channel way (Liu et al., 2003), in line with our findings in male pigs coming to puberty, the plasma levels of NO decreased with an increase in plasma level of T and endothelial relations of arteries compared to others (Mendelsohn and Rosano, 2003) that confirms the negative effect of NOMs on T levels (Matsuda et al.,1994), similarly in human female to male transsexuals that administrated higher androgens result in mediated low vasodilation confirming a negative action of NO (McCredie et al., 1998). The higher levels of both E2 and NO could be explained by the vasodilator action induced that could help in the transfer of hormones from vein to artery in the *pampiniform plexus* (Miller et al., 2008).

When comparing hormone levels before and during puberty, the greatest levels of FSH were found at 6.5-months-old, followed by LH peaks at 7.5-months-old, and a significant drop in T value. This supports the findings of Renaville et al. (1990), and Courot et al. (1979) who found that hormone levels rose steadily from birth until puberty and peaked around the age of puberty. This suggests that the onset of reproductive activity in bucks is linked to a rise in T, E2 (Schulster et al., 2016), and NOMs (Duckles and Miller, 2010) levels as they get older.

The result is indicative of a highly significant negative correlation ($P<0.01$) between FSH and T levels before and after puberty, The puberty could reached from week of elevation of T with a decline in FSH levels (week 27) with special references to scrotal circumference, testicular width and mediastinum thickness in line with other study reported that the maximum age of puberty was at 24 week, which is similar to bucks (Shaaeldin et al., 2019), also some studies reveled that there was a strong relation between amount of nutrients and body measurements that could effect of testicular dimensions in small domestic animals.

Various LH-characteristics and T production before puberty, as well as FSH secretion and scrotal circumference before and after puberty, showed positive relationships. Given the reliance of testicular functions on gonadotropins, these positive associations were not surprising (Sanford et al., 1978; Courot et al., 1979).

CONCLUSION

Pituitary hormones (FSH and LH) and steroid hormones (E2 and T) were changed around age of puberty that likely reflect their roles in regulation of spermatogenesis with the attainment of buck sexual developmental capacity, as pituitary hormones are correlated with testicular Doppler indices (PI and RI) with testicular width (TW) before and after puberty. In addition, NO likely play a role in the vasodilatation with the aid of E2.

ACKNOWLEDGEMENTS

The authors thank Dr. Mustafa Abo Ahmed, professor of Theriogenology Cairo university for his help all over the study.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

Funding

The authors' research included into this article did not receive any specific grant from funding agencies in the pub-

lic, commercial, or not-for-profit sectors.

AUTHORS CONTRIBUTION

KhloodG. Abdelkhalek performed the practical work. Ali Badawy involved in supervision and protocol assessment. Mohamed fathi involved in semen collection, supervision, writing original draft and editing. Elshymaa A. abdelnaby involved in doppler assessment, supervision, statistical analysis and writing final paper.

REFERENCES

- Abdelnaby EA, Abdelkhalek KG, Emam IA (2021a). The beneficial effects of enriched diet on testicular blood flow and seminal parameters using colour and pulsed doppler ultrasound in dogs. Bulg. J. Vet. Med., Accepted manuscript, online first.
- Abdelnaby EA, Abouelela YS, Yasin NAE (2021c). Evaluation of penile blood flow in dogs with TVT before and after chemotherapeutic treatment with special reference to its angioarchitecture. Adv. Anim. Vet. Sci., 9 (8): 1159-1168. https://doi.org/10.17582/journal.aavs/2021/9.8.1159.1168
- Abdelnaby EA, Emam IA, Fadl AM (2021b). Assessment of the accuracy of testicular dysfunction detection in male donkey (Equus asinus) with the aid of colour-spectral Doppler in relation to plasma testosterone and serum nitric oxide levels. Reprod. Dom. Anim., 56: 764–774. https://doi.org/10.1111/rda.13916
- Abdelnaby EA, Emam IA, Salem NY, Ramadan ES, Khattab MS, Farghali HA, Abd El Kader NA (2020). Uterine hemodynamic patterns, oxidative stress, and chromoendoscopy in mares with endometritis. Theriogenology, 158: 112-120 https://doi.org/10.1016/j.theriogenology.2020.09.012
- Abouelela YS, Yasin NAE, El karmoty AF, Khattab MA, El-Shahat KH, Abdelnaby EA (2021). Ovarian, uterine and luteal hemodynamic variations between pregnant and non-pregnant pluriparous Egyptian buffalos with special reference to their anatomical and histological. Theriogenology, 173 :173-182 https://doi.org/10.1016/j.theriogenology.2021.06.022
- Bollwein H, Heppelmann M, Lüttgenau J (2016). Ultrasonographic Doppler use for female reproduction management. Vet. Clin. North Am. Food Anim. Pract., 32(1): 149-164. https://doi.org/10.1016/j.cvfa.2015.09.005
- Chakraborty PK, Stuart LD, Brown JL (1989). Puberty in the male Nubian Goat: Serum concentrations of LH, FSH and testosterone from birth through puberty and semen characteristics at sexual maturity. Anim. Reprod. Sci., 20: 91-101. https://doi.org/10.1016/0378-4320(89)90067-5
- Courot M, llochereau-de Reviers MT, Monet-Kuntz C, Locatelli A, Pisselet C, Blanc MR, Dacheux JL (1979). Endocrinology of spermatogenesis in the hypophys tomized ram. J. Reprod. Fertil., 26: 165-173.
- de Souza LEB, da Cruz JF, Neto MRT, Nunes RDS, Coelho CMH (2011). Puberty and sexual maturity in Anglo-Nubian male goats raised in semi-intensive system R. Bras. Zootec., 40 :7. https://doi.org/10.1590/S1516-35982011000700019
- Duckles SP, Miller VM (2010). Hormonal modulation of endothelial NO production. Pflugers Arch., 459(6): 841–851. https://doi.org/10.1007/s00424-010-0797-1
- Fabre-N C, Kendrick KM, Scaramuzzi RJ (2015). The "ram effect": new insights into neural modulation of the gonadotropic axis by male odors and socio-sexual interactions. Front. Neurosci., 9: 111 https://doi.org/10.3389/fnins.2015.00111
- Ginther OJ (2007). Ultrasound imaging and animal reproduction: Color-Doppler ultrasonography. Cross Plains: Equiservices publishing. 258p.
- Gur S, Kadowitz PJ, Sikka SC, Peak TC, Hellstrom WJ (2015). Overview of potential molecular targets for hydrogen sulfide: A new strategy for treating erectile dysfunction. Nitric oxide: Biology and Chemistry/official. J. Nitric Oxide Soci., 50: 65–78. https://doi.org/10.1016/j.niox.2015.08.005
- Helbig L, Woodbury MR, Haigh JC, Barth AD (2007). The onset of puberty in North American bison (Bison bison) bulls. Anim. Reprod. Sci., 97(1-2): 12-24. https://doi.org/10.1016/j.anireprosci.2006.01.007
- Júnior AA, Oliveira LS, Neto AC, Alves FR, Miglino MA, Carvalho MA (2012). Spermatogenesis in goats with and without scrotum bipartition. Anim. Reprod. Sci., 130(1-2): 42-50. https://doi.org/10.1016/j.anireprosci.2011.12.012
- Kühn AL, Scortegagna E, Nowitzki KM, Kim YH (2016). Ultrasonography of the scrotum in adults. Ultrasonography, 35: 180-197. https://doi.org/10.14366/usg.15075
- Levin ER (2009). Plasma membrane estrogen receptors. Trends Endocrinol. Metab., 20: 477–482. https://doi.org/10.1016/j.tem.2009.06.009
- Liu PY, Death AK, Handelsman DJ (2003). Androgens and cardiovascular disease. Endocr. Rev., 24: 313–340. https://doi.org/10.1210/er.2003-0005
- Marques P, Skorupskaite K, George JT, Anderson RA (2018). Physiology of GNRH and Gonadotropin Secretion. Jun 19.
- Matsuda K, Ruff A, Morinelli TA (1994). Testosterone increases thromboxane A2 receptor density and responsiveness in rat aortas and platelets. Am. J. Physiol., 267: 887–893. https://doi.org/10.1152/ajpheart.1994.267.3.H887
- McCredie RJ, McCrohon JA, Turner L (1998). Vascular reactivity is impaired in genetic females taking high-dose androgens. J. Am. Coll. Cardiol., 32: 1331–1335. https://doi.org/10.1016/S0735-1097(98)00416-1
- McNeill AM, Zhang C, Stanczyk FZ, Duckles SP, Krause DN (2002). Estrogen increases endothelial nitric oxide synthase via estrogen receptors in rat cerebral blood vessels: effect preserved after concurrent treatment with medroxyprogesterone acetate or progesterone. Stroke, 33(6): 1685-1691. https://doi.org/10.1161/01.STR.0000016325.54374.93
- Mendelsohn ME, Rosano GM (2003). Hormonal regulation of normal vascular tone in males. Circ. Res., 93: 1142–1145. https://doi.org/10.1161/01.RES.0000108694.68635.1C
- Miller B A, Lu C D (2019). Current status of global dairy goat production: an overview. Asian-australas. J. Anim. Sci., 32(8): 1219–1232. https://doi.org/10.5713/ajas.19.0253
- Miller VM, Mulvagh SL (2007). Sex steroids and endothelial function: translating basic science to clinical practice. Trends Pharmacol. Sci., 28: 263–270. https://doi.org/10.1016/j.tips.2007.04.004
- Miller, Virginia M, Sue P Duckles (2008). Vascular actions of estrogens: functional implications. Pharmacological reviews, 2(60): 210-241. https://doi.org/10.1124/pr.107.08002

- Oduwole OO, Peltoketo H, Huhtaniemin T (2018). Role of Follicle-Stimulating Hormone in Spermatogenesis. Front. Endocrinol., 9: 763. https://doi.org/10.3389/fendo.2018.00763
- Raji LO, Ajala OO (2015). Scrotal Circumference as a parameter of breeding age for West African dwarf bucks. TURJAF, 3: 668-671. https://doi.org/10.24925/turjaf.v3i8.668-671.453
- Rawy MS, Megahed GA, Abdelbaset AE, Hassaneen AS (2021). Evaluation of testicular hemodynamics following gonadotropin-releasing hormone administration with the aid of pulse wave Doppler in rams and their relation to hormonal response. SVU-IJVS, 4(2): 60- 75. https://doi.org/10.21608/svu.2021.70099.1117
- Renaville R, Berkans X, Portetelle D, Sneyers M A (1990). Plasma testosterone and insulin like growth factor I evolution around puberty in hepatic cannulated cattle. Annales de Recherches Vétérinaires, 21187-21194
- Ribeiro DL, dos Santos LS, de França IG, Pereira HG, Lima J, Miranda BDB, Carvalhoneta AV, Torres-júnior JR (2020). The use of Doppler ultrasound as a potential fertility predictor in male goats Turk. J. Vet. Anim. Sci., 44: 1115-1124. https://doi.org/10.3906/vet-2003-135
- Samir H, Nyametease P, Nagaoka K, Watanabe G (2018). Effect of seasonality on testicular blood flow as determined by color Doppler ultrasonography and hormonal profiles in Shiba goats. Anim. Reprod. Sci., 197: 185–192. https://doi.org/10.1016/j.anireprosci.2018.08.027
- Sanford L M, Beaton D B, Palmer W M (1978). Photoperiod induced changes in luteinizing hormone, follicle-stimulating hormone. prolactin and testosterone secretion in the ram. Can. J. Anim. Sci., 58: 123-128. https://doi.org/10.4141/cjas78-018
- Sanford LM, Holvland BE, Palmer WM (1984). Seasonal changes in endocrine responsiveness of the pituitary and testes of male sheep in relation to their patterns of gonadotropic hormone and testosterone secretion. Can. J. Physiol. Pharmacol., 62: 827-833. https://doi.org/10.1139/y84-136
- Schulster M, Bernie AM, Ramasamy R (2016). The role of estradiol in male reproductive function. Asian J. Androl., 18(3): 435–440. https://doi.org/10.4103/1008-682X.173932
- Serin G, Gokdal O, Tarimcilar T, Atay O (2010). Umbilical artery Doppler sonography in Saanen goat fetuses during singleton and multiple pregnancies. Theriogenology, 74(6): 1082-1087. https://doi.org/10.1016/j.theriogenology.2010.05.005
- Shaaeldin SA, Makawi S A, Tingari M D, Eltayeb AE (2019). Development of hormonal profiles of Nubian bucks at puberty in Sudan. J. Vet. Med. Anim. Health, 11(6): 106-114. https://doi.org/10.5897/JVMAH2018.0728
- Singla M, Saini AL, Kumar A, Kaswan S, Brar PS (2017). Breeding soundness evaluation of stall-fed bucks using ultrasonography. Indian J. Anim. Sci., 87: 584–586.
- Smith LB, Walker WH (2014). The regulation of spermatogenesis by androgens. Semin Cell Dev. Biol., 30: 2-13. https://doi.org/10.1016/j.semcdb.2014.02.012
- Stirone C, Chu Y, Sunday L (2003). 17β-estradiol increases endothelial nitric oxide synthase mRNA copy number in cerebral blood vessels: quantification by real-time polymerase chain reaction. Eur. J. Pharmacol., 478: 35–38. https://doi.org/10.1016/j.ejphar.2003.08.037
- Strehlow K, Rotter S, Wassmann S (2003). Modulation of antioxidant enzyme expression and function by estrogen. Circ. Res., 93: 170–177. https://doi.org/10.1161/01.RES.0000082334.17947.11
- Strina A, Corda A, Nieddu S, Solinas G, Lilliu M, Zedda MT, Pau S, Ledda S (2016). Annual variations in resistive index (RI) of testicular artery, volume measurements and testosterone levels in bucks. Comp. Clin. Pathol., 25: 409-413. https://doi.org/10.1007/s00580-015-2199-4
- Tostes RC, Nigro D, Fortes ZB, Carvalho MHC (2003). Effects of estrogen on the vascular system. Braz. J. Med. Biol. Res., 3(9): 1143-1158. https://doi.org/10.1590/S0100-879X2003000900002
- Walton J S, Evins JD, Waites GMH (1978). Feedback control of follicle-stimulating hormone in pre- and postpubertal rams as revealed by hemicastration. J. Endocrinol., 77: 75-84. https://doi.org/10.1677/joe.0.0770075
- Zelli R, Troisi A, Ngonput EA, Cardinali L, Polisca A (2013). Evaluation of testicular artery blood flow by Doppler ultrasonography as a predictor of spermatogenesis in the dog. Res. Vet. Sci., 95: 632-637. https://doi.org/10.1016/j.rvsc.2013.04.023