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Inter-Relationship Between Testicular and Accessory Sex Glands Biometry and Circulating Steroid Hormones in Jacks

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Abstract: To perform biometric analysis of Egyptian indigenous donkeys´ reproductive organs using ultrasound and correlate the findings with serum hormonal levels, 25 apparent healthy jacks aged between 2-12 years were used. The ultrasonographic measurements of internal (bulbourethral, prostate, vesicular glands, colliculus seminalis and pelvic urethra) as well as external (testes, epididymis and scrotum) organs were measured over three-weeks period for each animal and for each structure. The scrotal length, width and thickness were measured with a caliper (mm). An impact of testicular size in normal donkeys was noticed on the measures (length and width) of the prostate gland, vesicular gland, ampulla ductus deference and colliculus seminalis. Testes measures were highly ($p<0.01$) correlated with vesicular gland length ($r=0.713$) and ampulla ductus deference width ($r=0.743$). A substantial increase in epididymal and scrotal measures was observed in donkeys with mean testicular volume > 100 mm^3. Moreover, there was a close positive association between testicular volume and scrotal thickness ($r=0.939$, $p<0.001$). Serum testosterone and estradiol were negatively correlated ($r=-0.802$, $p<0.001$). Yet, testosterone hormone varied significantly ($P<0.05$) between donkeys of different testicular biometry. A significant positive correlation was found between serum estradiol and testicular length ($r=0.41$, $p<0.05$), width ($r=0.37$, $p=0.08$) and mean and total testicular volume ($r=0.41$, $p<0.05$). In conclusion, the ultrasound aided biometry of accessory sex glands, testes and epididymis with the testosterone levels are promising non-invasive tools for the breeding soundness evaluation and prediction of the sexual activity in male donkeys.

Key words: Accessory sex glands · Biometry · Donkey · Epididymis · Scrotum · Testes · Testosterone hormone · Ultrasound

INTRODUCTION

The necessity to advance the reproductive capabilities of indigenous donkey's breeds has not got a considerable attention. In spite of the fact that there is increasing concern in the use of donkeys as draft animals, there is little published data on their biology [1]. Jack or jackass (male donkey) like the jenny (she-donkey) has many reproductive similarities to the horse, being that they both belong to the same genus Equus. However, some differences do exist, particularly when discussing reproduction. Donkeys tend to be more fertile than horses, having a higher conception rate (78%) compared to mares (65%) [2]. The testes and penis of the jack seem to be larger than that of comparably sized horses [3]. Although the jack and stallion are having the same accessory sex glands, the ampulla is larger in the jack than the stallion [1].

Donkeys come in many sizes, from the miniature (=36 inch at the withers) to the mammoth, which may be in excess of 56 inches tall. These size differences may alter the practitioner’s ability to adopt some diagnostic procedures, e.g. rectal palpation [1]. This upsurges the significance of the use of non-invasive diagnostic approach for the evaluation of the animals’ fertility potential.

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Ultrasonographic examination of the testes, epididymis and accessory sex glands has proven to be a valuable, non-invasive technique for the assessment of genital macroscopic morphology and pathology in several mammalian species of veterinary interest [4]. Ultrasonography has been used to demonstrate the ultrasonic morphology of bulls' testes [5] and accessory sex glands [6] by means of trans-cutaneous and trans-rectal techniques, respectively. For the stallion, ultrasound guided testicular measurements have been an integral part of the physical examination of the breeding status. It can be valuable for the fertility evaluation and as a management tool of stallions [7].

Testosterone serves several functions in males, including the initiation and maintenance of spermatogenesis as well as the maintenance of sexual behavior. The concentration of testosterone in adult male donkeys appeared to be lower than in other domestic species, but fluctuated considerably, as in other species [8].

The current work was designed to nominate the correlation between ultrasonographic findings and biometry of accessory sex glands, testes and hormonal levels in donkeys.

MATERIALS AND METHODS

Twenty five healthy male donkeys (*Equius asinus*), aged between 2-12 years, weighed 100-200 kg, owned by private farmers at Quweisna city, Monufia Governorate, Egypt, were used in the current study during the period between September to November 2015. Animals´ age was dictated by the appearance of specific dental features [9]. Animals were categorized into 3 groups according to the mean testicular tissue volume / testes (MTV); group I (MTV< 50 mm^3, n=6), group II (50 mm^3< MTV < 100 mm^3, n=12) and group III (MTV> 100 mm^3, n=7).

Trans-Rectal and Trans-Abdominal Ultrasonography: Most examinations were performed in standing un-sedated haltered donkeys tied to a solid object as described by Contri *et al*. [10] with little modifications using Magic 2200 Vet Scanner (Eickemeyer Veterinary Equipment Inc., Germany) equipped with transrectal 4-6 MHz linear transducer. An ultrasound probe was fixed to a fiberglass extension rod of two cm width and 40 cm length. This was necessary due to size limitations constrain hindering introducing hand and arm fully into the rectum as is possible in the full-size equine.

A well-lubricated transducer was placed against the ventral wall of the rectum and was oriented to produce ultrasonographic images of the bulbourethral glands (at the dorso-lateral aspect of the pelvic urethra), the prostatic gland (dorsal to the neck of the bladder), vesicular glands (lateral to the neck of the urinary bladder and cranial to the prostate gland) and ampulla ductus deferens (dorsal to the bladder with excretory ducts on dorsal wall of the urethral lumen. The size of each of the glands was evaluated in the dorso-ventral (width) and cranio-caudal (length) directions. The ultrasound settings (focus, gains, brightness and contrast) were standardized and the same settings were used in all the examinations.

Ultrasonographic examinations of the testes were done by placing the transducer vertically (parallel to the longitudinal axis of the testis) on the caudal surface of the scrotum as described by Love *et al.* [11]. An imagining of the mediastinum was the guide to capture a frozen image across the middle of the testis. The testicular morphometric assessment was achieved for each testis, measuring length, height and width. The volume of the ellipsoid ($4/3\pi$ abc, a= length/2, b=height/2, c= width/2) was used for calculation of the testicular volume.

Blood Sampling and Hormonal Analysis: Blood samples (10 ml) were harvested from the jugular vein into plain tubes and centrifuged at 3000 rpm for 20 min. Serum samples were labeled and stored at -20 °C until assayed. Testosterone (Cat. No. BC-1115, BioCheck Inc.,CA, USA) and 17-β estradiol (Cat. No. DNOV003, NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany) levels were estimated in sera with the use of the enzyme immunoassay test kit according to the manufacturer's instructions. The least measurable concentrations were 0.05 ng/ml and 8.68 pg/ml for testosterone and estradiol, respectively.

Statistical Analysis: The data of each accessory sex glands, testes and epididymis measure (length, width... etc) are presented as mean (±SEM) and used in the statistical analysis using SPSS statistical package version 16 (SPSS, Chicago, Illinois, USA). One-way ANOVA with LSD multi-comparison test was used to compare between the mean values of three reproductive groups. The associations between biometry of reproductive organs (internal and external) were computed with Pearson correlation co-efficient. P value was set at < 0.05 to define the significant differences.

RESULTS

Ultrasonographic Biometry of Male Donkeys Internal Reproductive Organs

Bulbourethral: The mean length and width of the bulbourethral gland in donkeys of the studied groups categorized according to their reproductive activity depending on testicular volume were 25.90±1.22 and 17.29±1.47, 29.30±1.70 and 18.33±1.21, 28.52±2.25 and 18.70±1.24 mm, respectively. Statistically, the bulbourethral gland measures were consistent among animals of different testicular volume (Table 1).

Analysis of the correlation between bulbourethral gland and testicular biometry measures indicated the presence of positive correlation between the bulbourethral gland width and testicular thickness ($r=0.40$, $p=0.05$), width ($r=0.43$, $p<0.05$) and volume ($r=41$, $p<0.05$). Nevertheless, the bulbourethral gland length had no correlation with testicular measures (Table 2).

Prostate Gland: Investigating accessory sex glands by means of ultrasonography disclosed that each lobe mean length, width and diameter of the prostate gland significantly ($p<0.001$) varied between donkeys having diverse testicular volume (Table 1).

The prostate gland length and width clearly correlated with testicular measures in donkeys as shown in Table 2.

Vesicular Glands: The mean length and width of vesicular gland measured by means of ultrasonography was 30.68±3.30 and 22.38±2.65, 39.31±1.83 and 23.68±0.74, 55.88±2.72 and 35.09±4.24 mm in the three donkey groups, respectively. An impact of testicular activity judged by its dimensions in normal donkeys was noticed on substantial differences in right and left vesicular gland dimensions as demonstrated in Table 1.

The vesicular gland length and width were highly correlated with testicular gland biometrical measures as seen in Table 2.

Ampulla Ductus Deference: The width of the right and left ampulla ductus deference was significantly ($p<0.001$) different between animal groups of different testicular sizes (Table 1), being larger in sexually active donkeys had MTV > 50 mm^3 and 100 mm^3 (24.50±0.64 & 27.38±1.73 mm) than those with MTV < 50 mm^3 i.e. lower activity (16.21±2.58 mm).

The thickness of ampulla ductus deference significantly ($p<0.001$) correlated with testicular length ($r=0.819$), thickness ($r=0.766$), width ($r=0.744$) and volume ($r=0.743$) as shown in Table 2.

Colliculus Seminalis and Pelvic Urethral Diameter: The colliculus seminalis length and width and urethral diameter were significantly different in group second and third (sexually active) than those of the first group (MTV < 50 mm^3) as seen in Table 1.

The significant correlations between testicular measures (length, thickness, width and volume) and colliculus seminalis dimensions and urethral diameter were statistically verified as shown in Table 2.

Ultrasonographic Biometry of Male Donkeys External Reproductive Organs

Testes: The overall mean length, height, width and volume of the testes were significantly higher in group 3 (61.63±4.12 mm, 48.18±1.34 mm, 45.21±1.43 mm, 579.99±62.82 mm^3) than other examined groups and this reflected on the expected daily sperm output ($26.83\pm1.63\times10^9$) as shown in Table 3.

Epididymis: Records of the length and width of the right and left epididymis indicated a significant difference between studied animal groups, with the eminent higher values were noticed in the third group (MTV > 100 mm^3). In the meantime, epididymal measures of the first two groups were not significantly different. A significant ($p<0.001$) positive correlation between epididymal measures and testicular dimensions was statistically proven (Table 2).

Scrotum: The mean length, width and thickness was 55.13±4.43, 60.38±6.02 and 35.19±3.06 mm in the first group, 64.25±2.77, 72.25±2.03 and 38.83±0.88 mm in the second group and 78.75±2.48, 89.33±2.81 and 48.67±0.61 mm in the third group of donkeys, respectively (Table 3). Data herein showed a highly significant ($p<0.001$) impact of sexual activity on scrotal measures. Moreover, there was a close positive correlation between testicles and scrotal measures as shown in Table 2.

Serum Testosterone and Estradiol Concentrations: Data presented in Fig. 1 showed that the serum testosterone levels significantly ($P<0.05$) increased in the third group of higher testicular activity (MTV> 100 mm^3)

Table 1: Ultrasonographic biometry of male donkeys internal reproductive organs

Accessory sex gland		Item (mm)	Group I	Group II	Group III	Significance
Bulburethral gland	Right	Length	29.97±4.47	30.48±1.68	29.97±1.78	ns
		Width	20.40±3.38	19.44±0.87	21.55±1.18	ns
	Left	Length	28.60±2.57	33.23±1.39	32.92±1.39	ns
		Width	18.14±2.13	19.86±1.15	23.00±0.90	ns
	Overall mean	Length	25.90±1.22	29.30±1.70	28.52±2.25	ns
		Width	17.29±1.47	18.33±1.21	18.70±1.24	ns
		Diameter	21.59±1.14	23.81±1.36	23.61±1.21	ns
Prostate gland	Right	Length	38.21±2.98	41.83±1.65	46.00±1.85*	ns
		Width	22.35±2.03	23.23±1.33	26.38±1.24	ns
	Left	Length	38.96±3.15^{b}	43.05±1.10^{ab}	49.03±1.95^{a}	<0.05
		Width	22.55±2.28	23.53±1.06	25.05±2.04	ns
	Overall mean	Length	38.59±2.10^{c}	42.44±0.98^{b}	48.38±1.02^{a}	<0.001
		Width	22.45±1.47^{b}	23.38±0.83^{b}	28.12±1.41^{a}	<0.001
		Diameter	30.52±1.67$_{b}$	32.91±0.74^{b}	38.25±1.05^{a}	<0.001
Vesicular gland	Right	Length	31.54±5.29^{b}	38.58±2.49^{b}	57.47±3.32^{a}	<0.001
		Width	22.41±3.86^{b}	23.64±1.08^{b}	34.53±6.40^{a}	= 0.05
	Left	Length	29.83±4.30^{c}	40.04±2.76^{b}	54.28±4.51^{a}	<0.05
		Width	22.35±3.91^{b}	23.73±1.06^{b}	35.65±6.17^{a}	<0.001
	Overall mean	Length	30.68±3.30^{c}	39.31±1.83^{b}	55.88±2.72^{a}	<0.001
		Width	22.38±2.65^{c}	23.68±0.74^{b}	35.09±4.24^{a}	<0.001
		Diameter	26.53±2.93^{b}	31.49±1.16^{b}	45.48±3.33^{a}	<0.001
Ampulla Ductus Deferense	Right	Width	16.21±2.51^{b}	24.79±0.46^{a}	26.38±1.57^{a}	<0.001
	Left	Width	16.23±2.67^{b}	24.22±0.88^{a}	28.37±1.99^{a}	<0.001
			16.21±2.58^{b}	24.50±0.64^{a}	27.38±1.73^{a}	<0.001
Colliculis seminalis		Length	15.97±2.33^{b}	22.73±1.39^{a}	24.02±2.19^{a}	<0.05
		Width	11.60±1.51^{b}	16.25±0.95^{ab}	17.83±2.79^{a}	<0.05
			13.79±1.87^{b}	19.49±1.05^{a}	20.93±2.41^{a}	<0.05
Urethra diameter			2.92±0.32^{b}	3.66±0.21^{b}	5.11±0.85^{a}	<0.01

Values (Mean±SEM) with different superscript letters within the same raw were significantly different.* p=0.06 between G1 and G3

Table 2: Correlation between ultrasonographic biometry of male donkeys reproductive organs

	Testicular length		Testicular thickness		Testicular width		Mean testicular volume		Total testicular volume	
	r value	P value	r value	P value	r value	P value	r value	P value	r value	P value
Bulbourethral gland length	0.17	0.41	0.12	0.55	0.17	0.41	0.13	0.54	0.13	0.54
Bulbourethral gland width	0.37	0.06	.396*	0.05	.431*	0.03	.414*	0.04	.414*	0.04
Prostate length	.684**	0.00	.631**	0.00	.537**	0.01	.633**	0.00	.633**	0.00
Prostate width	.619**	0.00	.454*	0.02	.445*	0.02	.596**	0.00	.596**	0.00
Vesicular gland length	.630**	0.00	.698**	0.00	.727**	0.00	.713**	0.00	.713**	0.00
Vesicular gland width	.547**	0.00	.525**	0.01	.584**	0.00	.629**	0.00	.629**	0.00
Ampulla thickness	.819**	0.00	.766**	0.00	.744**	0.00	.743**	0.00	.743**	0.00
Colliculis seminalis length	.517**	0.01	.414*	0.04	.440*	0.02	.435*	0.03	.435*	0.03
Colliculis seminalis width	.453*	0.02	0.36	0.07	.437*	0.03	.408*	0.04	.408*	0.04
Urethra diameter	.579**	0.00	.542**	0.00	.522**	0.01	.597**	0.00	.597**	0.00
Epididymal length	.621**	0.00	.584**	0.00	.552**	0.00	.594**	0.00	.594**	0.00
Epididymal width	.620**	0.00	.521**	0.01	.435*	0.03	.566**	0.00	.566**	0.00
Scrotal length	.921**	0.00	.702**	0.00	.615**	0.00	.775**	0.00	.775**	0.00
Scrotal width	.825**	0.00	.941**	0.00	.979**	0.00	.924**	0.00	.924**	0.00
Scrotal thickness	.897**	0.00	.941**	0.00	.913**	0.00	.939**	0.00	.939**	0.00
Testosterone	-0.21	0.34	-0.26	0.21	-0.20	0.36	-0.32	0.13	-0.32	0.13
Estradiol	0.41*	0.05	0.37	0.08	0.28	0.19	0.41*	0.04	0.41*	0.04

Table 3: Ultrasonographic biometry of male donkeys external reproductive organs

Item			Group I	Group II	Group III	Significance
Testis	Right	Length	34.30±3.50^{b}	39.45±1.13^{b}	48.93±1.86^{a}	<0.001
		Height	31.20±3.03^{c}	36.70±1.03^{b}	44.56±1.16^{a}	<0.001
		Width	29.63±2.81^{c}	35.08±1.21^{b}	43.17±1.01^{a}	<0.001
		Volume	157.62±44.97^{b}	217.13±17.86^{b}	396.23±27.85^{a}	<0.001
	Left	Length	54.13±4.40^{b}	61.17±2.27^{b}	74.33±2.62^{a}	<0.005
		Height	37.91±2.86^{b}	40.57±0.94^{b}	51.79±1.15^{a}	<0.001
		Width	35.12±3.08^{b}	38.23±1.35^{b}	47.25±2.50^{a}	<0.01
		Volume	342.54±92.52^{b}	396.95±22.76^{b}	763.76±55.51^{a}	<0.001
	Overall mean	Length	44.21±3.73^{b}	50.31±2.58^{b}	61.63±4.12^{a}	<0.01
		Height	34.55±2.19^{c}	38.61±0.79^{b}	48.18±1.34^{a}	<0.001
		Width	32.37±2.13^{c}	36.65±0.94^{b}	45.21±1.43^{a}	<0.001
		Volume	250.08±55.13^{b}	307.04±23.49^{b}	579.99±62.82^{a}	<0.001
	Total testicular volume		500.16±134.04^{b}	614.08±38.04^{b}	1160.00±68.07^{a}	<0.001
Expected daily sperm output ($\times 10^{9}$)			10.99±3.22^{b}	13.73±0.91^{b}	26.83±1.63^{a}	<0.001
Epididymis	Right	Length	23.29±0.56^{b}	26.90±1.25^{ab}	27.82±1.55^{a}	<0.05
		Width	20.83±1.10^{b}	21.10±0.94^{b}	25.20±0.92^{a}	<0.05
	Left	Length	23.99±1.56^{b}	26.08±1.14^{b}	31.70±0.90^{a}	<0.001
		Width	19.59±1.26^{b}	21.80±1.02^{b}	26.28±1.60^{a}	<0.01
	Overall mean	Length	23.64±0.81^{c}	26.49±0.83^{b}	29.76±1.04^{a}	<0.001
		Width	20.21±0.82^{b}	21.45±0.68^{b}	25.74±0.90^{a}	<0.001
Scrotum		Length	55.13±4.43^{b}	64.25±2.77^{b}	78.75±2.48^{a}	<0.001
		Width	60.38±6.02^{c}	72.25±2.03^{b}	89.33±2.81^{a}	<0.001
		Thickness	35.19±3.06^{c}	38.83±0.88^{b}	48.67±0.61^{a}	<0.001

Values (Mean±SEM) with different superscript letters within the same raw were significantly different

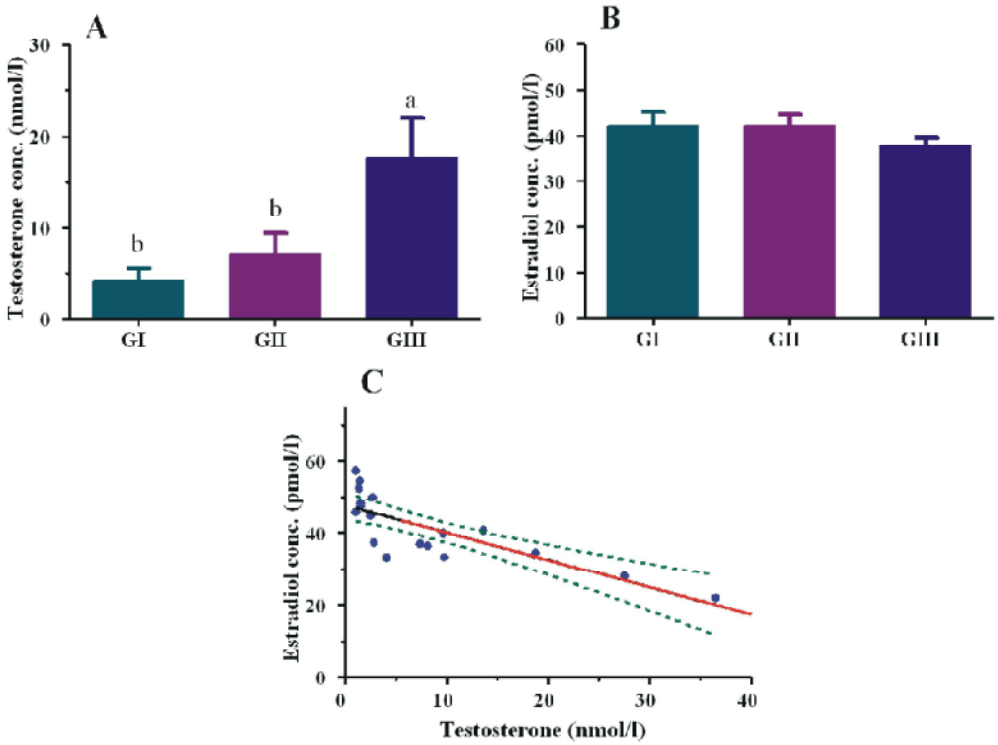

Fig. 1: Hormonal levels in male donkey (jacks) in groups of different testicular biometry. A. Serum testosterone level (ng/ml). B. Serum estradiol-17β level. C. Correlation between serum testosterone and estradiol levels in jacks. Columns with different letters were significantly different at $p<0.05$.

as compared with the groups of smaller testicular volume (< 100 mm^3). Nevertheless, non-significant differences in serum estradiol concentration have been found between the donkey groups.

A significant positive correlation was found between serum estradiol and testicular length ($r=0.41$, $p<0.05$), width ($r=0.37$, $p=0.08$) and mean and total testicular volume ($r=0.41$, $p<0.05$). A clear ($p<0.001$, $r=-0.802$). A negative correlation ($p<0.001$, $r=-0.802$) was perceived between the testosterone and estradiol hormones in male donkeys.

DISCUSSIONS

An understanding of the morphometric characterization of the reproductive organs is desirable when developing a management strategy to maximize male breeding competence. The application of real-time ultrasonography to study animal reproduction represents a technological invention that has updated the background of reproductive biology. It could be used to assess the scrotal contents for testicular volume and morphology as well as patency and functional activity of accessory sex glands.

Bulbourethral gland (also called Cowper's gland) function in equines is mainly to produce a clear fluid that flushed the urethra from urine, bacteria before ejaculation [12]. Current study data presented that the dimensions (length and width) of bulbourethral gland lobes even in the sexually active donkeys (28.52±2.25 & 18.70±1.24 mm, respectively) were smaller than that reported formerly [13]. These authors found that the measures of the bulbourethral gland were 4.4 ± 0.25 cm long and 2.5 ± 0.25 cm wide. Bulbourethral gland length and height in normal geldings was 22.4 ± 0.66 and 12.3 ± 0.32 mm, with the corresponding range in stallions of 35 (30–44) and 22 (16–33) mm [14]. The difference in the recorded measures is probably a reflection of the diverse testicular activity or the breed. Nevertheless, current data did not show differences between bulbourethral gland measures between the studied animal groups of variable testicular activity. Former studies in horse [15] and jackass [10] showed that diameters and ultrasound appearances of sexual glands, e.g. bulbourethral glands, do not seem to be correlated to the semen features, particularly total and gel-free volume and sperm concentration. In the meantime, present findings verified a significant positive correlation between the bulbourethral gland width and testicular thickness ($r=0.40$, $p=0.05$), width ($r=0.43$, $p<0.05$) and volume ($r=41$, $p<0.05$). One factor that may obviously affect the accessory sex gland size and contents is the interval from ejaculation at the time of examination [16]. Moreover, studies addressing age, breeding history and sedation for inspection and other factors would add to our understanding to the current observation. It has been found that accessory sex gland size and content varied with the socio-sexual environment, where the glands were bigger in stallion with harem status as compared with those with bachelor status [17].

Prostate gland secretion significantly contributes to pre-sperm friction of semen in equines. Investigating prostate gland with ultrasound in this work revealed that the mean length, width and diameter significantly ($p<0.001$) varied between the studied donkeys of variable testicular activity. Moreover, statistically, the prostate gland and testicular measures were noticeably interrelated. This indicated a putative role of testes and/or testosterone hormone in controlling the donkeys' reproductive organs function with peculiarity to the prostate gland. Nuclear immunostaining of androgen receptor was found in the secretory cells of the prostate in stallions [18]. This immunoreactivity is maintained even in cryptorchid stallions, but at weaker expression [19]. From the beginning of embryogenesis to puberty, maturation and beyond androgens are a prerequisite for the normal development and physiological control of the prostate [20]. Likewise, testis, epididymis and prostate are highly androgen-dependent tissues in the stallion [21].

Seminal vesicle secretory activity adds nutrients, volume and buffer to the semen. In the present study, assessment of vesicular gland measured by means of ultrasonography in normal donkeys verified an impact of testicular activity (judged by its dimensions) on the differences in vesicular gland mean length and width. Also, the vesicular gland dimensions were highly correlated with testicular gland biometric measures. This indicated the implication of testicular secretory activity on the functional morphology of vesicular gland in donkeys. Measures of vesicular glands for heavy horses were greater than for those miniature Horses, ponies and light horses. In the meantime, measures were similar for miniature horses and ponies and for light horses and heavy horses [16]. Geldings treated with testosterone with or without estradiol increased the weights of the seminal vesicles, ampullae and prostate greater than in estradiol-treated or control geldings [22].

The ampullae are enlargements of the terminal part of the vas deferens and they have been presumed to function as a storage site of spermatozoa, but also in the transport of spermatozoa through the urethra during

ejaculation [23]. The current results demonstrated that the width of ampulla ductus deference significantly varied between animal of different testicular volume, being larger in sexually active donkeys (MTV > 100 mm^3) than those of lower activity (MTV < 50 mm^3). Furthermore, the thickness of ampulla ductus deference was highly correlated with testicular measures. This assumed the role of testes and/or androgen hormone in adjusting the functional activity of accessory genital glands of the jackasses with eccentricity to ampulla ductus deference. Testosterone supplementation to castrated stallions increased the weight of ampullae, compared with control [22].

Pelvic urethra is generally quite narrow, except in the region of the colliculus seminalis just caudal to the prostate [7]. In this study, the colliculus seminalis dimensions as well as urethral diameter increased with sexual activity and this was addressed by the substantial relation between testicular biometry and colliculus seminalis and urethral measures. The erectile tissue of colliculus seminalis consists of erectile stratum cavernosum and the striated urethralis muscle surrounds the pelvic urethra [7].

External male genital organs are scrotum with its contents (testes, epididymis and vas deference) and penis. In addition to sperm maturation, the epididymis plays an important role in sperm transport, concentration, protection and storage. In the present study, epididymal tail and scrotal biometry were testicular activity-dependent, with statistical significance was noticed in the third group (MTV > 100 mm^3). In stallions, the total scrotal width showed a linear increase with testicular growth [24]. The primary factor regulating epididymal function is androgens, but there is a rising proof that estrogens, retinoids and other factors coming directly into the epididymis from the testis [25]. Former findings suggested that the stallion epididymal function is regulated by both androgens and estrogens and that estradiol is more important in the stallion [26].

Many hormones are involved in the regulation of sexual behavior, sexual desire, erection onset and ejaculation and the post-ejaculatory detumescence [27]. In the current data, serum testosterone levels were noticeably different between donkeys of different testicular biometry. Nevertheless, a substantial positive correlation was found between serum estradiol and testicular length ($r=0.41$, $p<0.05$), width ($r=0.37$, $p=0.08$) and volume ($r=0.41$, $p<0.05$). In the meantime, a clear negative correlation ($p<0.001$, $r=-0.802$) has been found between the testosterone and estradiol hormones in male donkeys. Veronesi *et al.* [27] indicated the significant high levels of testosterone in jackass at erection, ejaculation and dismounting, probably due to its impact on these events and on sexual behavior.

CONCLUSION

The demonstration of normal ultrasonographic anatomy of male genitalia as well as the influence of testicular activity on the reproductive organs biometry and steroid hormones can be used to enhance the routine breeding soundness evaluation and aid the clinician in differentiating abnormalities of these structures in donkeys.

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